Title: Microbial contributions to soil carbon storage during simulated range shifts of plant-fungal symbionts

Primary Investigator (PI): Michael Mann

Focus area: New Investigator

Selected area(s) of DOE mission relevance: carbon cycling

Sequencing Request

Estimated number of samples to be sent for each product:
Metatranscriptomes: 92

Metagenome/Metatranscriptome samples

Number of samples	Description
92	Soil samples from Rocky Mountain Biological Laboratory in Crested Butte, CO.

Proposal File Attachments

Filename	Description
Taylor_NSF_Biosketch_Feb_2018.pdf	Taylor_CV
Mann_biosketch.docx	Mann_CV
Moore_biosketch_2018_02_19.doc	Moore_CV
Rudgers_Biosketch2018.docx	Rudgers_CV
Kivlin_biosketch_2018_02_13.doc	Kivlin_CV

	Filename	Description	Delete File
Download	Figures_JGI_Mann.docx	Figures	Delete
Download	References_JGI_Mann.docx	References	Delete

Summary

Description:

We propose to submit mRNA preparations from 92 soil samples for metatranscriptome sequencing (~ 28 million reads each; 485 GB total). These soils have already been collected, stored at -20°C within 12 hours and archived at -80°C. The soil samples originate from an experiment that examined the climate-induced disruption of symbiosis between C3 grass species and root-associated fungi (arbuscular mycorrhizal (AM) fungi and other root endophytes).

By the end of the 21st century, predicted climate changes include a 2–4.5°C increase in mean annual temperature and major shifts in precipitation patterns (IPCC, 2012). Divergent responses of species to climate change can disrupt species interactions (Tylianakis et al., 2008), decrease species' overlap in space or time (mismatch), and create communities that lack contemporary analogs (Putten, 2012). Furthermore, novel species interactions may feed back to the rate of climate change itself by altering processes that influence pools and fluxes of carbon (C) at ecosystem scales (Denman, 2007).

Plant-fungal interactions provide tractable models for exploring general questions on symbiont responses to climate change. Belowground symbiotic fungi play critical roles in terrestrial ecosystems, including decomposition, nutrient cycling, and C sequestration (Langley et al., 2003; Lemons et al., 2005; Van Der Heijden et al., 2008; Wilson et al., 2009). Roots and associated fungi can account for 50-70% of soil carbon (Clemmensen et al., 2013). While differences in soil C and nutrient cycling have been observed between the two large functional groups of belowground mycorrhizal fungi (i.e., arbuscular mycorrhizal (AM) and ectomycorrhizal fungi (Averill et al., 2014; Averill et al., 2016)), shifts in the composition of taxa within a fungal guild may also alter soil C dynamics. In particular, shifts in soil C and nutrient storage that may occur as plant shift their distributions and encounter novel fungal symbionts have not been examined. In an NSF-funded grant, we manipulated plant-fungal symbiosis by performing a reciprocal transplant experiment of plants and root-associated fungal symbionts between replicated high and low elevations in the Colorado Rocky Mountains (Fig. 1). In this experiment, we observed that when plant-fungal pairs were mismatched (e.g., low elevation plants with high elevation fungi) soil C degrading enzymes decreased and soil C storage increased, suggesting a breakdown of function that cascaded to the ecosystem level. However, the microbial mechanisms leading to these shifts in soil C cycling remain unidentified. The power of our experiment is the field-based manipulation of microbial community composition - we know the manipulations had functional consequences for soil C, plant mortality, and plant biomass production, but we do not know which fungal taxa/genes were associated with these changes in function.

Justification:

Plant species are moving up mountainsides in response to global climate change (Chen et al., 2011; Rumpf et al., 2018; Savage et al., 2015). As plants move, ecologically important interactions with symbionts may be disrupted. Mycorrhizal symbioses occur in > 80% of plant species (Smith & Read, 2010) and can have large impacts on belowground carbon (C) storage (Averill et al., 2014; Averill et al., 2016). Given the potential for symbionts to influence plant resilience to climate variability (Kivlin et al., 2013), the potential for climate change to decouple host and symbiont distributions deserves careful attention. Plant-fungal symbiosis may be decoupled in future climates if (1) plants shift their distributions at different rates than their fungal symbionts or (2) the magnitude or direction of the outcome of plant-fungal symbiosis

(e.g., from mutualist to parasite) depends on environmental conditions, among other mechanisms. Shifts in plant-fungal symbiosis with simulated climate change have been observed in a variety of ecosystems (Deveautour et al., in press; Rudgers et al., 2014). However, there are currently no direct tests of how disrupted plant-fungal symbioses affects soil C storage.

To test how symbiont disruption may affect C cycling, we manipulated plant-fungal symbioses using reciprocal transplants of fungi along three replicated elevational gradients. Seeds of two abundant C3 grass species (Festuca saximontana and Festuca thurberi) were collected from a low elevation site and either planted in sterilized soil from that site or at ~430m higher in elevation to simulate range shifts expected with ~3°C warming. Plants were either inoculated with fungal spores extracted from the low elevation site "Original fungi", the high elevation site "Novel fungi", or had no added spores ("No fungi") in a fully-factorial design (Fig. 1). We utilized 0.45-μm mesh to construct the fungal exclusion cylinders (McGuire, 2007); the mesh excludes fungal colonization from the local soil environment. This design allowed us to understand consequences of (1) plants shifting upwards in elevational distribution in response to warming without co-dispersing with their associated root microbiome (Novel fungi at High elevations), (2) plants shifting their distributions upslope, and co-dispersing with their root microbiome (Original fungi moved up to High elevations), and (3) fungi native to high elevations experiencing warming (Novel fungi moved down to warmer, Low elevations). We also included the controls of plants at low elevations with low elevation fungi (Original fungi at Low elevations).

We grew plant-fungal symbioses in the field for two years, then assessed how disruption of symbiosis affected plant survival, biomass, and local belowground C and nutrient dynamics. Plant mortality was lowest and growth was highest when fungal symbionts were inoculated into their home elevation; plants inoculated with low elevation (Original) fungi did best at low elevations and vice versa for plants with high elevation source fungi (Novel; Fig. 2 a,b). Moreover, the activity of soil C-degrading enzymes increased when fungal origin was matched with elevation (Fig. 2c), with associated decreases in soil C (Fig. 2d). Effects on soil C were largest at low, warm elevations, indicating that plant-fungal mismatches could become increasingly relevant in future, warmer climates.

Because the initial fungal composition was the same within each fungal inoculum treatment, these findings suggest that functional gene expression differs among treatments and may correlate with differences in C storage. By sequencing the functional metatranscriptome of soils in each treatment, we aim to evaluate how much shifts in genes involved in soil C cycling may underlie these patterns. This step is essential to predicting how future shifts in plant and fungal distributions may alter soil C storage under a warmer climate.

Utilization:

Our research team has been studying plant-fungal interactions at these same research sites since 2011 (Kivlin et al., 2017; Ranelli et al., 2015; Rudgers et al., 2014). We have collected extensive background on plant (plant growth rate, mortality, biomass, specific leaf area, and phenology), soil metrics (soil nutrients, microbial biomass), as well as fungal abundance in both soils (extraradical hyphal extractions) and in roots (root colonization). The fungal composition of the soil samples in this JGI proposal will be analyzed via sequencing the ITS2 region. We have

obtained a large fungal culture collection of root endophytes (135 isolates from Festuca spp.) from roots collected from low and high elevations and identified them by Sanger sequencing and will be analyzed for trait data. The soil metatranscriptome is the final step that will allow us to mechanistically determine how functional potential of soil C-degrading genes may shift with climate-change induced decoupling of plant-fungal symbionts.

Bioinformatics and statistical analysis

The mRNA in the soil transcriptome will be analyzed using standard JGI pipelines including MG-RAST (Meyer et al., 2008) with functional gene classification against the COG, KOG, Pfam, and KEGG databases. Read abundances will be normalized to reads per kilobase per million mapped reads (RPKM) with the edgeR package (Robinson et al., 2010). We will especially focus on genes implicated in soil C cycling and storage (e.g., glucose, chitin, cellulose, lignin decomposition). The normalized read counts for each treatment will be compared in an ANOSIM model in Primer. Given the mRNA will include all eukaryotes, we will run the analysis once with all the annotated genes found and again with only fungal-specific genes.

To connect molecular mechanisms to shifts in in soil C pools, these processes will be modeled using separate structural equation models (SEM) for C cycling. SEMs will directionally connect microbial composition (e.g., NMS axes) and estimates of microbial biomass and fungal abundance -> microbial metagenomic potential -> proximate soil enzyme activities (Wang et al., 2007) -> the soil C pool, as the end response variable. We will use multi-group SEM, with a separate group representing each of the four manipulations of plant-fungal symbiosis (low elevation sites and Original fungi, low elevation sites and Novel fungi, high elevation sites and Novel fungi, high elevation sites and Original fungi). Multi-group SEM models use maximum likelihood estimation to determine whether each path in the path diagram is better fit separately for each group or as a single path estimate in common for all groups. This method will therefore identify how pathways of interaction, from microbial abundance and composition to potential microbial gene expression to enzymatic functions that drive soil C, are each affected (or not) by mismatches between fungi and their home elevation. We will build separate models for the two focal plant species. Models will be compared using log-likelihood ratio tests that constrain paths to be the same or vary among treatments.

Finally, we will correlate shifts in belowground microbial gene expression of C and nutrient-acquiring genes with changes in plant growth and mortality (Fig. 2). Determining these plant-soil feedbacks can aid predicting when plant-fungal disruption will negatively or positively affect plant fitness.

Community interest:

While mycorrhizal fungi can have large impacts on terrestrial C cycling (Averill et al., 2014), predicting how interactions among plants and their diverse fungal partners may shift in future climates remains challenging. Here we have implemented a reciprocal transplant experiment that does exactly this. The next step is to characterize the ecosystem-level ramifications of plant-fungal symbiont disruption. Our preliminary data suggest that ecosystem-level shifts in soil C may be realized when fungi are mismatched to their native climate conditions along sharp

gradients in elevation. The proposed work here has the potential to elucidate genetic mechanisms behind observed shifts in soil C.

Current model projections remain uncertain as to whether the Earth will be a future C source or sink from the atmosphere (Friedlingstein et al., 2006) and are solely based on abiotic parameters. Our work will elucidate one type of biotic interaction (fungal symbiosis) that may have large effects on soil C storage and should be included in future global C projections. We focus on these associations because more than 80% of plant species form associations with AM fungi (Smith & Read, 2010) and most plants also associate with a number of other root endophytic taxa (mainly Ascomycota). Predicting the disruption of plant-fungal symbiosis at the global scale is a research priority. We already have empirical (Chen et al., 2011; Rumpf et al., 2018; Savage et al., 2015) data that plant species have or will shift their ranges in future climates, and we have projections of where these shifts will occur (Gottfried et al., 2012). Similar models of fungal distributions are under development by Kivlin's group. However, all of these models lack direct links between shifts in fungal symbiosis and changes to ecosystem C cycling. By understanding if fungi themselves are responding to range shifts, or affecting the organisms around them, we will know which parts of the microbial community may be the most functionally variable under future climates. The scientific community can then focus empirical and modeling efforts on these microbial groups.

DOE mission:

This research will address a key element of the DOE environmental mission: the carbon cycle. Our study will be the first to mechanistically understand how disruption of plant-fungal symbiosis under climate change affects microbial genomic expression linked to changes in to soil C over time. We propose to analyze these patterns in two plant species, but our overall experimental design includes eight plant species manipulated at six sites. Therefore, this dataset has the potential to be scaled up to include effects on all dominant grasses critical for forage and C fixation in Rocky Mountain subalpine grasslands. Moreover, by understanding how functional gene expression is linked to plant performance, our research can also elucidate how plant-fungal symbiosis disruption will impact the size of the plant C pool as well.

Sample preparation:

Our research team has extensive training in handling, extracting and preparing metatranscriptomic RNA libraries. Kivlin and Rudgers have received $98K in funding from the National Institutes of Health to sequence naturally occurring microbial metatranscriptomes of our focal grasses at the same research sites, providing a highly useful context for evaluating the experimental data. Kivlin has also prepared hundreds of soil metagenomes for functional analyses across precipitation gradients in Texas grasslands and Neotropical rainforests (data published on MG-RAST server). Along with Kivlin, other members of our research team (Mann, Moore) have extensive experience in microbial community analysis via Sanger and next-generation sequencing. In addition, our team has experience incorporating microbial processes in to soil C models using SEM and other modeling frameworks (Moore, Kivlin).

Samples have already been collected and stored at -80°C since 2016. Upon approval of this application, we will begin extracting total RNA via the Qiagen RNeasy PowerSoil Total RNA kit and enrich for mRNA using poly(A) selection with the Qiagen Oligotex mRNA kit. In our experience, extractions for 92 metatranscriptomes take 4 weeks. These expected timeframes include time needed to measure product quality and re-extract if necessary (we have ~20g of soil per sample). Kivlin has extensive experience using the Qubit system and bioanalyzer to determine DNA/RNA quality. We anticipate a tentative shipment date of August 2018.