

CALL FOR COLLABORATION

A call to characterize functional mycobiome responses to experimental climate change**Mark A. Anthony*** and **Arthur Gessler**

Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Zürcherstrasse 111, 8903 Birmensdorf, Switzerland

* Corresponding author, Email: mark.anthony@wsl.ch

Received 27 October 2022 | Accepted 17 November 2022

Published online at www.soil-organisms.de 1 December 2022 | Printed version 15 December 2022

DOI 10.25674/so94iss3id300

Abstract

The impacts of climate change are increasingly threatening terrestrial ecosystems. Understanding how this will influence fungal communities (i.e. the mycobiome) is one of the most consequential domains of climate change research because fungal functions, like decomposition and mycorrhizal symbiosis, feedback to influence climate change. Efforts to study fungal functioning have been stymied by technological limitations and the complexity of fungal biology compared to prokaryotes, but recent molecular advances now enable us to study their functional responses to climate change in greater detail. Here, we announce an open invitation for collaboration with researchers across the globe studying terrestrial ecosystem responses to climate change. In particular, we invite submissions of soil or DNA extracts isolated from climate change field experiments for detailed characterization of fungal community structure using full-length ITS DNA metabarcoding and a novel probe capture and enrichment next generation sequencing technique to quantify fungal functional genes involved in oxidative and hydrolytic enzyme biosynthesis, carbohydrate metabolism, organic and inorganic nitrogen cycling, phosphorus acquisition, stress tolerance, and mycorrhizal symbiosis. By contributing samples, supporting analyses, and helping to draft manuscripts, co-authorship will be offered to all collaborators. We will also freely provide the unique datasets we generate from samples collected at your experiment for downstream analyses. We hope that by crowd-sourcing collaborations, we will be able to establish consistent ecological principles for how fungi respond to climate change, enabling us to more accurately forecast the impacts of future global changes on ecosystem function.

Keywords Climate change | fungi | genomics | global change | metagenomics

The impacts of climate change are becoming increasingly salient. By 2100, Earth is projected to warm 2–5°C above pre-industrial temperatures (Pörtner et al. 2022). Droughts, which have already affected many local communities and ecosystems, are forecasted to worsen across vast regions of the globe (Hao et al. 2014). How this will impact soil microbiology is one of the most consequential aspects of climate change because even modest changes to soil microbial functioning dramatically shift the balance of carbon storage on land versus the atmosphere via respiratory release of CO₂ (Raich & Schlesinger 1992). This is especially evident for fungal communities (i.e. the mycobiome), which have the highest soil microbial

biomass on Earth (He et al. 2020) and occupy multiple distinct niches in terrestrial ecosystems. Fungi are the main decomposers of plant litter (Schneider et al. 2012) and, alongside bacteria and other soil life, are some of the only organisms to decompose plant cell wall components like lignin (Floudas et al. 2012). They also interact with >90% of all land plants via mycorrhizal symbioses (Brundrett & Tedersoo 2018). And, when fungi grow and die, their exudates and necromass form soil organic matter that can be sequestered long-term (Cotrufo et al. 2013, Sokol et al. 2022). Structural and functional changes in the soil mycobiome in response to climate change will therefore uniquely influence ecosystem functioning in the future.

Understanding how the functioning of fungal communities will shift with climate change has been difficult to disentangle from other components of the soil microbiome. This has hindered development of mechanistic frameworks and microbial ecological theory. Widely used soil biogeochemical techniques capture emerging processes, such as soil respiration and extracellular enzyme activities. Common molecular approaches like metagenomics (Donovan et al. 2018) or microarrays (e.g. GeoChip) (Brempong 2012), as well as bioinformatic tools used to predict functional genomic information from taxonomic assignments such as PICRUSt (Langille et al. 2013), poorly capture fungi compared to prokaryotes. From a technical point of view, this has stymied efforts to quantify fungal functioning and sensitivities to global change.

The goal of this project therefore asks an important question: how does mycobiome functioning shift with climate change? While this remains an open question, there is indirect evidence to suggest fungal functioning will be sensitive to climate change. Culture-based studies show that fungal growth and decomposition are highly responsive to experimental global change conditions (e.g., Alberton et al. 2007, Romero-Olivares et al. 2015, van Diepen et al. 2017, Finestone et al. 2022). These results are limiting since they are based on individual fungi versus entire communities, but they are supported by community analyses using DNA metabarcoding (Zhou et al. 2020). Relative abundances and diversity of saprotrophic, mycorrhizal, and pathogenic fungi often shift in opposing directions in response to experimental climate manipulations (e.g., Geml et al. 2015, Treseder et al. 2016, Jasse et al. 2018, Morrison et al. 2019, Anthony et al. 2021, see review by Baldrian et al. 2022). While DNA metabarcoding is not a direct measure of fungal functioning, it signals that there may be parallel changes in fungal functional activities. In a handful of studies, researchers have selectively targeted fungal functional genes to measure their responses to climate change, but these studies are rare. Notably, fungal genes encoding cellulolytic versus oxidative enzyme biosynthesis tripled in response to ten years of soil warming (Anthony et al. 2021), while genes related to nitrogen acquisition and cellulolytic enzyme biosynthesis more than doubled in response to simulated drought (Treseder et al. 2018). When we consider all available evidence together, it raises the hypothesis that fungal functioning will shift to favor the metabolism of particular soil organic matter compounds and nutrients in response to climate change.

In order to test this and explore fungal functional sensitivities to climate change, we announce an open invitation for collaboration and offer an opportunity to characterize mycobiomes from individual climate

manipulation experiments across the globe. Supported by a recently funded Swiss National Science Foundation Ambizione proposal, we invite researchers to submit soil samples or DNA extracts for molecular characterization of soil mycobiomes. We will perform full-length ITS DNA metabarcoding to characterize fungal community structure (sensu Tedersoo & Anslan 2019), and we will employ a novel probe capture and enrichment next generation sequencing technique to quantify fungal functional genes involved in oxidative and hydrolytic enzyme biosynthesis, carbohydrate metabolism, organic and inorganic nitrogen cycling, phosphorus acquisition, stress tolerance, and mycorrhizal symbiosis. We have already deployed this technique to study fungal genes involved in hydrolytic and oxidative enzyme biosynthesis and abiotic stress-tolerance in response to soil warming, nitrogen deposition, and invasive species (Anthony et al. 2020, Moore et al. 2021). The principle scientific goal of this project is to generate a global dataset of fungal community and functional gene responses to simulated climate change in order to identify mycobiome responses to climate change at an unprecedented scale.

To that end, we wish to invite researchers to submit samples for molecular analyses of soil fungi to be conducted free-of-charge at the Swiss Federal Research Institute WSL. We specifically request the following:

1. Contributed samples should come from climate manipulation experiments in the field versus from natural gradients or lab/greenhouse studies. At the time of sampling, treatment duration must be at least one year and include any of the following treatments: warming (soil or air via active or passive warming), drought, irrigation, and elevated CO₂, either alone or in combination. At least three replicate treatment and control plots must be included.
2. Submit already archived mineral soil samples or genomic DNA extracts (collected within the last five years and stored at -20°C or colder) or collect new soil samples for us to analyze. Ideally, soil will be collected at a 0–10 cm depth, but all depth increment variations between 0–20 cm will be accepted. Organic horizon samples from forests can also be included.
3. Meta-data about the field site and sampling (i.e., experimental treatment, treatment methodology, treatment duration at the time of sampling, date of sampling, ecosystem type, latitude, longitude, elevation, mean annual temperature and precipitation), vegetation (i.e., dominant species and functional group, dominant mycorrhizal type) and soil (i.e., soil type, depth of soil sample, bulk density, soil carbon and nitrogen content) should be included.

The project is open for contributions starting immediately until October, 2023. Contributions of samples alongside support analyzing and writing manuscripts will merit co-authorship, and the data generated from this project will be freely shared with each collaborator as it is generated. If you are interested in contributing, please contact Mark Anthony at mark.anthony@wsl.ch.

It is our ultimate goal that by crowd-sourcing collaborations, we will be able to establish consistent principals for how fungi respond to climate change at the functional level, enabling us to more accurately forecast the impacts of climate change on global biogeochemistry. With the support of collaborators around the world, we can move above and beyond the capacities of any single research group.

Acknowledgements

This work was funded by an Ambizione grant from the Swiss National Science Foundation awarded to MA (PZ00P3_208648). We would like to thank two anonymous reviewers for their helpful feedback.

Author contributions

Mark A. Anthony conceived the study. Mark A. Anthony and Arthur Gessler contributed resources and wrote the manuscript.

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