title

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# Abstract

# Introduction

## Outline

* Broad intro to the question of microbial BEF and motivation behind that question:
  + Ecolgoy wants to understand BEF
  + This is important for prediction and management
  + Decades of research has revealed diversity-function correlations for macrobes and microbes
* Challenge/call-to-action/gap-in-knowledge:
  + But for microbes the mechanism behind that correlation remains unknown

## Text

Biodiversity-ecosystem function (BEF) research has the central goal of understanding whether and how biodiversity - the diversity and relative abundance of species in a community - determines the magnitude of ecosystem functions (cite Hooper, Naeem, Tilman, …). Ecosystem functions are a broad range of processes, including pollination, water filtration, carbon storage, and nutrient cycling, that are driven by both abiotic and biotic factors. However, typically the BEF literature has focused on the size of elemental pools (e.g., carbon storage) and the rate of elemental fluxes (e.g., greenhouse gas emissions, photosynthesis) in an ecosystem. The fundamental question for this research is what is the nature of the “map” between elements of biodiversity and elements of ecosystem function? And generally the intention is to be able to predict how changes in biodiversity due to climate change or land use change will alter ecosystem function and, in turn, understand how we should protect biodiversity in order to preserve essential ecosystem functions.

One could hypothesize a relatively simple map in which a single taxon determines the rate of an ecosystem function based entirely on its abundance. (e.g. of this?) Alternatively, instead of defining biodiversity based on a taxonomic unit, biodiversity could be defined by functional groups or guilds. For example, photosynthesis could be determined by the abundace of photosynthesizers (of any taxonomic group) in a community. (Example of abundance of photosynthesizers?). A somewhat more complex map could define biodiversity based on the diversity (i.e., richness or number of species) of photosynthesizers. Using this mapping, an abundance of literature has demonstrated that productivity increases asymptotically with the richness of producers (Tilman 1997).

When microbial ecologists have applied this model to ecosystem functions performed by microorganisms, these simple models rarely hold.

By performing selection on communities, we reduce the number of elements contributing to processes other than methane oxidation. We reduce the variation due to other selective factors. (need some lit on artificial selection)

With this study, we ask what is the “map” between microbial biodiversity and ecosystem functions? And how do we reveal the mapping betwee microbial biodiversity and ecosystem function? To address the first question, we perform an artificial ecosystem selection experiment (*sensu* Swenson et al. 2000).

Microbial biodiversity-ecosystem function research (cite Hall, Rocca, )

Questions:

1. What is the “map”? And how do we get to that map? What is the nature of the map? Taxonomy? Or something other than taxonomy? Simple – taxon. Diffuse – something other than taxonomy. Complicated interactions among organisms leads to “hitchhikers.”
2. Can we isolate 1 or a few strains and add them to the N community? Or does it take a whole complex community? The
3. Lewontin posited that higher levels of selection (species, community, ecosystem) would have weaker heritability. Do we observe this?
4. Does soil methane oxidation rate respond to selection at the ecosystem level?
5. Does a community that has been selected on for high methane oxidation rate differ in diversity or composition from a community that has been passaged without selection for methane oxidation rate?
6. What aspects of community composition differ between the positive and neutral lines? Changes in alpha diversity, beta diversity? Presence/absence of specific microbial taxa? Relative abundance of microbial taxa?
7. Does selection lead to greater differences in the bacterial/archaeal community (inferred from 16S sequences) or in the methanotroph community (inferred from pmoA sequences)?
8. Does a community with high methane oxidation rate dominate coalescence with a natural community compared to a community with average methane oxidation rate?
9. Many-to-one mapping
10. Crossing/mixing lines or competing lines or applying to natural soils for the application of the tool.

Hypotheses:

1. Soil methane oxidation rate responds to selection at the ecosystem level

Questions to address in the Intro:

Is this “just enrichment” or is it community selection? i.e., is it dilution to extinction?

How do people enrich for methanotrophs?

Community-level selection? Paul Rainey, Martin Polz

# Materials & Methods

## Selection experiment

We performed an artificial ecosystem selection experiment similar to Swenson et al. (2000) by passaging replicate soil microbial communities. The trait we selected on was CH4 oxidation rate. Our experiment had two selection lines with twelve jars each for a total of twenty-four jars. One line was a positive selection line where the two or three jars with the highest CH4 oxidation rate were chosen to inoculate the next set of jars. The other line was a neutral selection where an equal number of jars as the positive line were chosen at random among the twelve. The number of jars chosen was based on the distribution of fluxes among the positive jars, i.e., we chose the top three jars unless only two jars had considerably greater oxidation rates based on visual inspection of histograms.

The initial soil microbial community was sampled from the top 10 cm of an upland mineral soil under a deciduous forest ecosystem near the University of Oregon campus. Incubations were performed in 500 mL mason jars (Ball Corporation, Broomfield, Colorado, USA) with rubber septa installed in the lids. Jars were incubated at ambient temperature on the benchtop. To create the first set of jars, twenty-four jars were created in an identical manner and then randomly assigned to either the positive or negative selection line. Each jar was sterilized with 70% ethanol to which was added 45 g of autoclaved potting mix, 5 g of live soil, and 3.5 mL of sterile deionized water to bring them to 60%??? of field capacity . The jars were then homogenized, capped, and injected with 4.3 mL of 99% CH4 to bring the headspace concentration to approximately 1000 ppm CH4. Jars were flushed and respiked twice per week to maintain elevated CH4 concentrations until travel, holidays, and COVID-19 got in the way and then we just did it as often as we could. Jars were incubated for approximately three weeks and then flux measurements were taken.

Once CH4 oxidation rates were determined, the top two or three jars were chosen for selection in the positive line and an equal number of jars were randomly chosen from the neutral line. The selected jars for each line were homogenized and used as the 5 g live soil for the subsequent passage. Twelve new jars were created for each line in the same manner as above using the inoculum for that line. This procedure continued until a divergence was observed in the mean flux of each line for two consecutive generations. Jars in passage number 2 and 5 were homogenized and a sample was collected and stored at -80C for later DNA extraction.

## Methane flux measurements

Methane oxidation rates were determined after flushing and spiking jars to approximately 1000 ppm CH4. Head-space samples of 1 mL were collected from each jar immediately after spiking and then at time points 3, 6, 24, and 48 hours for a 5-point curve. Samples were immediately injected into a ??? gas chromatograph fitted with an electron capture detector (company, city, state, USA) to determine the headspace CH4 concentration. Fluxes were calculated from a first-order exponential decay function as decay constant k with units???. Oxidation rates are presented as the additive inverse of k (i.e., -k) so that a more positive value represents a faster oxidation rate.

## Soil DNA extraction and sequencing

Samples to extract:

* generation 2
* generation 5
* starting soil
* autoclaved potting mix negative control
* sterile water negative control

Soil DNA was extracted from a subsample of soil from the starting inoculum and from each jar in passage 2 and 5 after CH4 flux measurements. Negative controls were extracted from autoclaved potting mix and autoclaved, deinoized water. Extractions were performed using the DNeasy PowerSoil kit (QIAGEN, Hilden, Germany). Find example extraction and sequencing text. In order to estimate the diversity and relative abundance of the bacterial and archaeal community in our soil ecosystems, we sequenced the V3-V4 region of 16S rRNA gene using the Earth Microbiome Project protocol and primers???. In addition, to estimate the diversity of CH4 oxidizers, we sequenced the particulate methane monooxygenase subunit A gene (*pmoA*) using primers???.

## Bioinformatics

Bioinformatic analyses were conducted using the QIIME2 bioinformatics platform (Bolyen et al. 2019). Raw sequencing reads were demultiplexed using ??? (citation), denoised using DADA2 (citation). Taxonomic assignment for the 16S reads was performed using ??? based on the Silva database. All subsequence statistical analyses were performed in the R statistical programming environment (R Core Team 2020). Richness estimates were made using the breakaway package (Willis et al. ???).

## Statistical Analysis

### Response to selection

To test whether there was a significant change in CH4 oxidation rate (k) over multiple passages as a response to selection, we fit a multiple linear regression model using ordinary least squares:

where was the predicted CH4 oxidation rate in jar as a function of treatment (positive or neutral selection), passage number , and the interaction between treatment and passage number . We fit the model in R version 3.6.3 using the lm and anova functions (R Core Team 2020). Oxidation rates were highly skewed and residuals did not meet the assumptions of normality and heterogeneity of variance, therefore, fluxes were rank-transformed prior to analysis.

### Ecosystem heritability

To test the heritability of CH4 oxidation rate as an ecosystem property, we calculated narrow-sense heritability as the slope of the relationship between the mid-parent CH4 oxidation rate and the mid-offspring CH4 oxidation rate (Goodnight 2000). For example, the mid-parent of passage 1 was calculated as the mean of the three jars selected to inoculate passage 2 and the mid-offspring was calculated as the mean of all 12 jars produced from those parental jars. To estimate heritability, we fit the following model using ordinary least squares:

where was the parental mean, was the offspring mean, and was heritability.

In addition, we calculated the inbreeding coefficient, , as:

where is the population size and is the number of generations (Goodnight 2000).

## Taxa differentially abundant in the positive selection treatment

To identify taxa that responded to selection on CH4 oxidation rate, we tested differential abundance using a beta-binomial model (Martin et al. 2020). We compared taxa in passage 5 between the positive and neutral treatment to identify taxa that

Other experiments:

* Continue selection for longer
* Start over and actually collect soil from every jar in every generation and actually flush and respike 2x per week. Maybe get soil from a particular source. (Maybe get an undergrad if I earn it by being consistent.)
* Use the positive and neutral lines to perform a community coalescence experiment with a natural soil and see if inoculating these selected lines into an already established community has any effect on community composition or function. Do communities with higher rates of EF due to selection exhibit greater dominance than other communities?
* Pick out specific crobes that respond to selection and are differentially abundant and try to isolate and culture them. Either on media or in soil??
* Add individual crobes back to a lab soil community to “overexpress” and see if the same “phenotype” is achieved. Compared that to an extraction of the full community. Use water as a control.

# Results

Methane oxidation rates increased over the course of the experiment in the positive selection treatment and did not change in the neutral selection treatment (Figure 1). This can be seen in the significant interaction between treatment and passage number for the rank of CH4 oxidation rate (F1,113 = 9.90, p = 0.002). While there was no difference in the overall mean of the two treatments (F1,113 = 0.12, p = 0.73), the mean rank CH4 oxidation rate increased by 13.3 per passage for the positive selection treatment (t = 3.15, p = 0.002), but the neutral treatment did not change ( = -1.9, t = -0.63, p = 0.53).

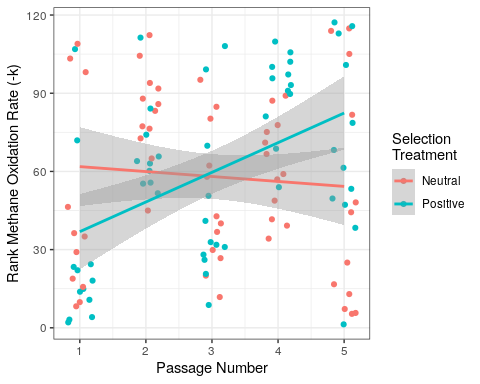


Figure 1: Rank CH4 oxidation rate for each jar in each passage.

Methane oxidation rate was heritable between consecutive generations (Figure 2). Specifically, the narrow-sense heritability (), calculated as the slope of the mid-parent and mid-offspring, was 0.70.

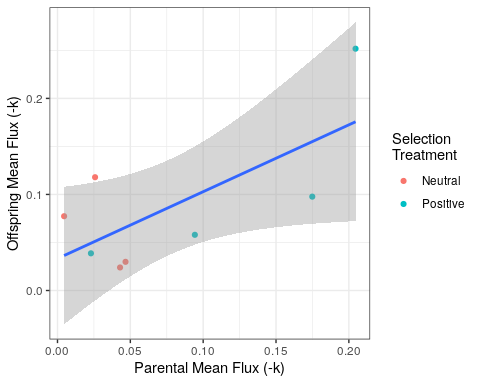


Figure 2: Correlation between mid-parent CH4 flux and mid-offspring CH4 flux.

## [1] 0.1916807

# Discussion

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# Competing Interests

We declare we have no competing interests.

# References