Artificial ecosystem selection reveals relationships between microbiome composition and ecosystem function

Andrew H. Morris1\* and Brendan J. M. Bohannan1

1 Institute of Ecology and Evolution, University of Oregon, Eugene, OR, USA

\* Correspondence: Andrew H. Morris, [amorris3@uoregon.edu](mailto:amorris3@uoregon.edu)

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# Abstract

Microbiomes mediate important ecosystem functions, yet it has proven difficult to determine the relationship between microbiome composition and the rate of any ecosystem function. This challenge remains because microbiomes are often complex, it is difficult to know a priori which microbiome members limit the rate of an ecosystem function, and it is usually challenging to manipulate microbiome composition directly. We propose that artificial ecosystem selection can overcome these challenges. Artificial ecosystem selection involves creating replicate ecosystems, selecting replicates with a desired ecosystem-level trait, and using the microbiomes from the selected ecosystems to inoculate a new set of ecosystems. We performed artificial ecosystem selection on replicate soil microcosms to select for increased soil methane oxidation rate. We observed a strong response of methane oxidation rate to artificial ecosystem selection with a 51% increase in methane oxidation rate per round over five rounds of selection. This suggests that a large portion of the variation in methane oxidation rate in these soils can be attributed to microbiome variation. We also found that selection did not enrich for known methane oxidizers; rather microbes that were enriched after selection included 24 families such as Puniceicoccaceae, Elsteraceae, and Cytophagaceae. This result is in contrast to the typical assumption that the rate of an ecosystem function is limited by the final step in the associated microbial pathway. Our study demonstrates that artificial ecosystem selection is a useful strategy for revealing relationships between microbiome composition and ecosystem function.

# Introduction

Microbiomes mediate a variety of important functions in ecosystems, and there is great interest in understanding how attributes of microbiomes may influence variation in ecosystem functions (1). Biodiversity-ecosystem function relationships have been described for a variety of macroorganismal communities. For example, plant species richness correlates with productivity and marine community diversity correlates with functions such as production, consumption, and nutrient release (2–4). While there is evidence that variation in the microbiome contributes to variation in ecosystem functions, describing this relationship has been elusive (5,6).

One reason for this difficulty is that it is challenging to directly manipulate microbiome composition and diversity the way we can for macroorganismal communities. Instead, we must use genetics through marker gene or metagenomic sequencing to characterize the microbiome. However, studies that measure the taxonomic or functional diversity of a microbiome through these approaches rarely find a correlation between diversity and function (6). Another approach is to quantify cells or genes using quantitative PCR. Studies such as this still rarely find a correlation between the abundance of a functional group and the rate of the corresponding process (5). As a result, it remains unclear how and when microbiome variation contributes to variation in the rate of ecosystem functions.

Another likely reason that microbiome-ecosystem function relationships have been difficult to document is that the most common approaches for looking for a relationship requires some prior knowledge of the likely causal links between microbiomes and ecosystem functions. For example, many studies have looked for relationships with the abundance or diversity of a “marker gene” for a particular function. Marker genes are microbial genes that code for an enzyme or enzyme-subunit known to be involved in a particular function. But this approach assumes that one can identify the “right” marker gene a priori which, given how little is understood about microbial diversity, seems highly unlikely. What is needed are approaches to microbiome-ecosystem function mapping that do not require this degree of prior knowledge.

Yet another likely reason that microbiome-ecosystem function relationships have been difficult to document is that microbiomes can be related to ecosystem functions in two related but distinct ways that historically have been difficult to separate. One way is that microbiomes may simply be conduits through which the environment drives function. That is to say that the environmental conditions completely regulate the attributes of the microbiome and a shift in the environment causes a shift in microbiome attributes, such as the abundance of a microbial functional group. This shift results in a change in the rate of an ecosystem function. In this case, microbiome attributes would be statistically associated with variation in ecosystem function, but also strongly covary with environmental conditions. Therefore, there would be no association between microbiomes and ecosystem functions independent of the environmental conditions. Under this scenario, knowing anything about the microbiome would not necessarily provide you with information regarding the causes of the change in ecosystem function or improve one’s ability to predict changes in ecosystem function. Furthermore, attempting to alter ecosystem function by altering the microbiome without changing the underlying environmental conditions would likely be fruitless.

Another possibility is that the microbiome itself alters the rate of ecosystem function independent of the environment. In this case, one cannot predict the rate of ecosystem function without understanding changes in the microbiome. In addition, managing the microbiome through inoculation or selective antibiotics would potentially be an effective approach for enhancing or mitigating certain changes to ecosystem functions. These two scenarios are not mutually exclusive, nor are they likely unique to microbes, but methods to determine the relative important of these two scenarios have not until recently been employed.

The problem of connecting microbiome attributes to the rate of an ecosystem function is analogous to the problem of connecting genomic variation to phenotypic traits in organisms, and solutions to this problem may be found by exploring this analogy (7). For example, one approach to the problem of genotype-phenotype mapping is to perform artificial selection on a trait of interest and identify genes that respond to selection. Such genes are likely to be causally linked to the trait under selection. An analogous approach for exploring microbiome-ecosystem function relationships is gaining acceptance among microbiologists (8,9). Microbiomes have been shown to respond to selection at the ecosystem-trait level (8), and some ecosystem-scale microbiome traits have been shown to be transferable through microbiome inoculations (9). Applying artificial selection to whole microbiomes could be a novel approach for quantifying how much of the variation in the rate of an ecosystem function could be attributed independently to microbiome variation (10). In addition, this approach could be used to identify microbiome attributes, such as genes or taxa, associated with the rate of an ecosystem function, although, to my knowledge this has not previously been accomplished (7,11). Such an approach has the potential to expand our ability to model microbial ecosystem-function relationships more accurately and allow us to manage ecosystems for particular outcomes (12,13). Here I use this approach to estimate how much of the variation in ecosystem function (the flux of CH4 gas between the soil and atmosphere) can be attributed to variation in the microbiome and to identify microbial markers associated with this ecosystem function.

Soil CH4 oxidation is a suitable function for this study because there is evidence that it may vary with microbiome attributes. For example, variation in CH4 emissions in arctic permafrost is correlated with the transcriptional activity of certain methanogens (14,15). In addition, soil microbiome phylogenetic variation is a strong predictor of CH4 oxidation in forests and pastures of the Brazilian Amazon (16). Finally, methanogenesis and methanotrophy are two of the most deeply conserved microbial physiologies and are represented in a narrow range of taxa and so the taxonomic composition of the microbiome is more likely to be associated with the rate of CH4 flux than other broader or more shallowly conserved functions (13,17). This suggests that microbiome variation might drive variation in CH4 oxidation in soils.

For the current project, we applied artificial ecosystem selection on soil microbiomes by selecting on the ecosystem-scale CH4 oxidation rate in order to estimate the amount of microbiome variation underlying variation in soil CH4 oxidation rate (8). To identify which microorganisms could be markers of CH4 oxidation rate, we compared the composition of the artificially selected microbiomes to a control set of microbiomes without selection. Artificial ecosystem selection has a similar effect to enrichment culturing by amplifying the population of interest (8). This will reduce the diversity of the soil microbiome and allow for greater power in detecting significant markers of CH4 oxidation rate. We then evaluated whether these markers meet our underlying assumptions about which taxa limit the rate of ecosystem function.

# Materials and Methods

## Experimental design

We performed an artificial ecosystem selection experiment (8) by passaging replicate soil microbiomes. The trait we selected on was CH4 oxidation rate. This experiment had two selection lines with twelve jars each for a total of twenty-four jars per passage. 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 positive jars. The other line was a neutral selection line where an equal number of jars as the positive line were chosen at random to inoculate the next set of neutral jars. 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 CH4 oxidation rates based on visual inspection of histograms. The experiment was carried out over five passages until a significant divergence in functional rates was observed between the two selection treatments.

The initial soil microbiome was sampled from the top 10 cm of an upland mineral soil under a deciduous forest ecosystem near the University of Oregon campus in Eugene, OR, USA. Incubations were performed in 500 mL mason jars with rubber septa installed in the lids. Each jar was sterilized with 70% ethanol to which was added 45 g of autoclaved potting mix, 5 g of living soil, and 3.5 mL of sterile deionized water to bring them to 60% of field capacity. The potting mix was Lane Potting Mix from Lane Forest Products (Eugene, OR, USA). 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. To create the two treatment groups, twenty-four jars were created in an identical manner and then randomly assigned to either the positive or neutral selection line. Jars were flushed and respiked twice per week to maintain aerobic conditions and elevated CH4 concentrations and were incubated at ambient temperature for approximately three weeks. Methane oxidation rates were determined at the end of the incubation period. For the positive treatment, the three jars with the greatest CH4 oxidation rates were chosen to inoculate the next generation. For the neutral treatment, three jars were randomly selected to inoculate the next generation. For each treatment, these three jars were homogenized and 5 g of this homogenized soil was used to inoculate the next set of jars and which represents a 10% subsampling. The next set of jars were created in an identical manner to the first generation with fresh autoclaved potting mix and the same moisture and CH4 content.

## Methane oxidation rate

Methane oxidation rates were determined after flushing and spiking jars to approximately 1000 ppm CH4. Headspace 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 SRI model 8610C gas chromatograph equipped with a flame ionization detector (SRI Instruments, Torrance, CA, USA) to determine the headspace CH4 concentration. Fluxes were calculated from a first-order exponential decay function as decay constant with units day-1. Oxidation rates are presented as the additive inverse of (i.e., ) so that a more positive value represents a greater oxidation rate. The jars selected for the positive treatment in passage 2 had the lowest CH4 oxidation rate of the twelve jars due to a calculation error in the CH4 oxidation rate. All other passages correctly used the top three jars.

## Soil DNA extraction and sequencing

A subsample of soil from the starting inoculum and from every jar in passages 2 and 5 was collected and stored at C. Soil DNA was extracted from 0.25 g soil. Negative controls were extracted from autoclaved potting mix and DNase-free water. Extractions were performed using the DNeasy PowerSoil kit (QIAGEN, Düsseldorf, Germany) and quantified using Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). To estimate the diversity and relative abundance of the bacterial and archaeal taxa in our soil ecosystems, we sequenced the V4 region of the 16S rRNA gene using the using the 515F - 806R primer combination (18). PCR mixtures were: 10 l NEBNext Q5 Hot Start HiFi PCR master mix, 9.2 l primer mixture (1.09 M concentration), and 0.8 l of DNA template. Reaction conditions were: C for 30 s (initialization); 35 cycles of C for 10 s (denaturation), C for 20 s (annealing), and C for 20 s (extension); and C for 2 m (final extension). Reactions were performed in triplicate and then combined. Amplicons were purified twice using 0.8x ratio Mag-Bind RxnPure Plus isolation beads (Omega Bio-Tek, Norcross, GA, USA). Sequencing libraries were prepared using a dual-indexing approach (19,20). Amplicon concentrations were quantified using Qubit and multiplexed at equimolar concentration. Sequencing was performed at the University of Oregon Genomics Core Facility on the Illumina NovaSeq 6000 with paired-end 150 bp reads (Illumina, Inc., San Diego, CA, USA).

## Bioinformatics

Bioinformatics processing was performed in ‘R’ (21). Demultiplexed sequencing reads were denoised using ‘DADA2’ to generate a table of amplicon sequence variants (ASVs) (22). Taxonomic assignment for the 16S reads was performed using the Ribosomal Database Project naive Bayesian classifier (23). The presence of contaminants was evaluated using both the prevalence and frequency methods from ‘DECONTAM’ by comparing samples to negative controls (24). We did not identify any obvious contamination using these methods.

## Statistical Analysis

Statistical analyses were performed in ‘R’ (21). To test whether there was a significant change in CH4 oxidation rate () as a response to selection, we tested a difference in slopes between the positive and neutral selection lines. The CH4 oxidation rates were strongly right-skewed with most values close to zero and few large, positive values. This resulted in residuals that did not meet the assumptions of constant variance and normal distribution. Therefore, CH4 oxidation rates were log10 transformed to better meet the assumptions of a linear model and to make figures easier to interpret. First, we tested the effect of treatment by fitting two nested models with and without treatment using ‘lm’. We compared these models using the likelihood ratio test with the ‘anova’ function. We then fit the full model to estimate the slope of the positive line, which represents the change in CH4 oxidation rate per passage as a response to selection.

To estimate the proportion of variance in ecosystem function due to variation in the microbiome, we estimated narrow-sense heritability () as the regression of mid-offspring on mid-parent (25). The mid-parent was the mean for all three selected jars and the mid-offspring was the mean for all jars produced by those parents. First, we tested if there was an effect of treatment on the heritability estimate. We compared these models using the likelihood ratio test with the ‘anova’ function. We then fit the full model to estimate the heritability of the Positive and Neutral treatments.

Richness estimates and tests were performed using ‘breakaway’ (26). We tested a difference in richness between Passages 2 and 5 with both treatments combined. Then we subset the samples from Passage 5 and compared richness between the Positive and Neutral treatment. Next, we compared beta-diversity as both Aitchison dissimilarity, which is the Euclidean distance after center-log ratio transformation, and the binary Jaccard distance for presence or absence (27). We tested a difference in centroid and dispersion of beta diversity between the Positive and Neutral treatment and Passage 2 and 5 with permutational analysis of variance (PERMANOVA) using the ‘adonis2’ function from ‘vegan’ (28,29).

To identify taxa that responded to selection on CH4 oxidation rate, we tested differential abundance using a beta-binomial model (30). We first grouped ASVs at the family level keeping ASVs that lacked a family-level taxonomic assignment. We then subset the samples in Passage 5 and removed all families with a prevalence of less than 10%. We compared families in passage 5 between the Positive and Neutral treatment to identify taxa that were enriched or depleted as a response to selection. we used the likelihood ratio test to estimate p-values with a significance threshold of false-discovery rate < 0.05 (30). Estimates are presented as the expected relative abundance in the positive treatment relative to the neutral treatment.

# Results

We observed a response to selection on whole-ecosystem soil CH4 oxidation rate (Figure 1). In addition, the response to selection varied with treatment (LRT of nested models with and without treatment: df = 2, ss = 1.86, p = 0.02). At the start of the experiment, the Positive treatment had a mean CH4 oxidation rate that was 24% lower than the Neutral treatment (difference of y-intercepts = -0.34, SE = 0.16, t = -2.14, p = 0.03). There was no change in CH4 oxidation rate of the Neutral treatment over the five passages (slope = -0.01, SE = 0.05, t = -0.26, p = 0.80). By contrast, the Positive treatment had a 51% increase in CH4 oxidation rate per passage (slope = 0.18, SE = 0.06, t = 2.76, p = 0.01).

We estimated heritability as the regression of mid-offspring on mid-parent (Figure 2). Offspring CH4 oxidation rates were correlated with parental CH4 oxidation rates in both the Positive treatment (slope = 1.08, SE = 0.24, t = 4.43, p = 0.01) and the Neutral treatment (slope = -0.58, SE = 0.20, t = -2.89, p = 0.04). Notably, the sign of the effect was reversed with a positive heritability for the Positive treatment and a negative heritability for the Neutral treatment.

Sequencing of 16S rRNA amplicons revealed that there were 9717 unique ASVs across all 46 jars sampled. Richness of ASVs decreased among all of the jars between Passage 2 and 5 (Richness difference between Passage 5 and Passage 2 = -2451.8, SE = 285.19, p < 0.001). However, there was no difference in richness between the Positive and the Neutral treatment in Passage 5 (Difference = 6.1, SE = 36.04, p = 0.866). In addition, there was no correlation between richness and CH4 oxidation rate across all passages and treatments. (slope = -18.3, SE = 55.41, p = 0.742).

Taxonomic dissimilarity of the soil microbiome varied strongly by passage and weakly by treatment (Figure 3). Permutational analysis of variance demonstrated an effect of passage, treatment, and their interaction on Aitchison dissimilarity (Table 1). Passage explained 41.9% of the variation in Aitchison dissimilarity and 47.4% in Jaccard dissimilarity. Treatment explains 4.7% for Aitchison and 3.4% for Jaccard. Variation in microbiome composition decreased as a result of passaging in jars (F = 80.5, p < 0.001), but did not differ between selection treatments (F = 0.40, p = 0.54).

To investigate which taxa responded to selection on soil CH4 oxidation rate, we first looked at which taxa were unique to the Positive and Neutral treatments aggregated at the family level. Several taxa were present in the Positive treatment and absent in the Neutral treatment. Most of these families had low prevalence (present in 3 or fewer samples) and low abundance (median < 5 reads). There were two families unique to the Positive treatment with relatively high prevalence. This included an ASV that was a member of the Bacteroidia Class with no lower taxonomic designation. This ASV had a prevalence of 10/12 and a median abundance of ~2 reads. The other prevalent family was a member of the Silvanigrellaceae, a newly described family placed in its own order. This family was present in all 12 samples and had a median abundance of 8 reads. Silvanigrella is the only cultivated member of Silvanigrellaceae and was isolated from a temperate fresh water lake (31). Of the families unique to the Neutral treatment, only one had a prevalence greater than 2/12. This family, Armatimonadaceae, was present in half of the Neutral samples (prevalence = 6/12). The type strain for Armatimonadaceae was isolated from the rhizosphere of *Phragmites australis* (32).

The remaining families were shared between the Positive and Neutral treatment, but some differed in their relative abundance. To identify taxa that responded to selection on soil CH4 oxidation rate, we tested the differential relative abundance of families in the Positive jars relative to the Neutral jars within Passage 5 using a beta-binomial model. We identified 37 families that were enriched or depleted in the Positive treatmeant relative to the Neutral treatment (Figure 4).

Overall, none of the families enriched in the Positive selection treatment are known methanotrophs. Several taxa identified had a higher taxonomic designation that contains methanotrophs, for example, the Gammaproteobacteria class had a large effect size. This class is one of the groups containing methanotrophic families such as Methylococcaceae. However, gammaproteobacteria is among the most diverse groups in the Prokaryotes, so this is not strong evidence for a selection response by methanotrophs (33). The next family was Puniceicoccaceae, which is a member of the phylum Verrucomicrobia and contains marine microbes. The Verrucomicrobia is a diverse group that contain known methanotrophs as well as ammonia-oxidizing bacteria (34). An ASV from the order Armatimonadales was also enriched in the Positive treatment. This order also contains the family Armatimonadaceae, which was found to be unique to the Neutral treatment. Cytophagaceae was also enriched in the Positive treatment and contains a number of mainly aerobic heterotrophs that can digest a variety of macromolecules (35). Notably, the family Gemmatimonadaceae has a single bacterial bin, Candidatus ‘*Methylotropicum kingii*’, that contains methanotrophy genes, but this family was depleted in the Positive selection treatment (36).

# Discussion

We observed a response to selection on whole-ecosystem soil CH4 oxidation rate. This type of response has been observed for other functions, such as chloroaniline degradation in water (8), but this is the first instance, to our knowledge, of selection being performed directly on soil biogeochemical cycling. With this experimental design, we attempted to create multiple ecosystems that were very similar with respect to the type and quantity of soil, soil moisture content, and the headspace concentration of CH4. we further controlled for environmental variation across replicates by having twelve replicates per treatment, using controlled laboratory conditions, and by having a random selection treatment as a control. Therefore, the observed response to selection on CH4 oxidation rate is likely due to changes in the microbiome.

To investigate the relationship between microbiome structure and ecosystem function, we wanted to determine how much of the variation in soil CH4 oxidation was attributable to variation in the microbiome. This ecological question is analogous to the problem of estimating heritability in quantitative genetics (7). Variation in an organismal trait is determined by the sum of genetic variation, environmental variation, and the interaction between the two (25). One goal of quantitative genetics is to determine the proportion of phenotypic variation attributable to genetic variation. This is commonly estimated as the narrow-sense heritability defined as the additive genetic variance. This goal can be achieved through artificial selection experiments with selection on the character of interest (25).

The heritability we observed in the Positive treatment was large ( = 1.08) relative to other studies of biological heritability (37). However, given the small sample size and wide confidence intervals (95% CI: 0.40, 1.75) it is likely that a larger study could more precisely estimate the true heritability. We intentionally eliminated much of the environmental variation that would be present in natural ecosystems, but it is unclear how much jar-to-jar variation there is in the composition of the soil substrate. Given these data, though, we conclude that variation within the soil microbiome has a very strong effect on soil CH4 oxidation rates independent of any environmental variation.

The negative heritability observed in the Neutral treatment could indicate a negative genetic correlation between CH4 oxidation rate and traits associated with persistence in a jar (38). The conditions in the jar favor organisms that can grow in potting mix, reproduce within a three week incubation, and survive passaging between jars. These traits might be negatively correlated with a community’s ability to oxidize CH4 indicating a potential trade-off (38). This trade-off would result in a negative heritability of CH4 oxidation rate in the Neutral treatment, which did not experience selection on CH4 oxidation rate.

Since there was a response to selection, we wanted to identify which taxa responded to selection. That is, the taxa that were enriched in the Positive treatment relative to the Neutral treatment. Studies of microbiome structure-function relationships typically quantify marker genes for the final enzyme in a pathway to try to predict the rate of flux through that pathway. However, of the 37 taxa identified by the differential abundance test, none were members of a family known to oxidize CH4. This suggests that, in this system, the taxa that perform the final step in the CH4 oxidation pathway do not limit the rate of flux through the pathway. Traits that we assume are important for regulating function (such as the diversity or relative abundance of methanotrophs estimated from phylogenetic marker genes) may not be important for determining variation in function. Perhaps once a sufficient number of methanotrophs are present, the process itself is limited by other metabolic processes in the ecosystem, such as nitrogen cycling mediated by non-methanotrophic organisms.

We found that 24 taxa were enriched in the Positive treatment, which might indicate that the soil CH4 oxidation rate in these jars is controlled by multiple taxa. This conclusion is analogous to the conclusion in quantitative genetics that most traits at the organismal level are the result of the interaction between many independent genes. Indeed, very few traits or genetic diseases are the result of a single gene or mutation (39,40). This results in the use of polygenic risk scores for predicting phenotype or disease risk in humans (41,42). An analogous conclusion can be drawn for predicting the rate of ecosystem function from the presence or relative abundance of microbial taxa. The rate of an ecosystem function at the whole-ecosystem level is the result of interactions among a variety of disparate taxa with different traits. To better understand microbiome structure-function relationships, our results suggest that we should move away from single marker genes and instead investigate the role of multiple functional groups in determining the function of ecosystems whether it is CH4 flux from soil or host-microbiome health.

Taxonomic richness dropped precipitously between Passage 2 and Passage 5. There are several explanations for this drop in diversity. The initial community was a diverse microbiome sampled from a natural soil. With the initial inoculation and at each subsequent passaging, the ecosystem was subsampled to 10% (5 g of living soil was combined with 45 g of sterilized substrate). This subsampling likely explains a large part of the drop in diversity. In addition, beyond the imposed selection regime based on CH4 oxidation rate, these soil microbiomes were under a variety of selection pressures such as persistence in a jar, survival during the transfer from one jar to the next, and the ability to colonize a new jar in a couple of weeks, to name a few. These selection pressures would have further filtered the microbiome for taxa that could persist in this laboratory environment.

In addition to the drop in richness, there was also a decrease in beta diversity among the jars as a result of passaging. This could be due to the homogenization step between each passage. Once the jars were selected to generate the next set of jars, the soil from those jars was homogenized and this homogenate was used to inoculate the next set of jars. The goal of this step was to “shuffle” membership in the microbiomes among the jars in order to test different combinations and relative abundances of taxa in terms of their effect on CH4 oxidation rate. This likely also made the communities more similar in their membership by increasing the likelihood that each taxon was represented in each jar. Another explanation for this biotic homogenization is the aforementioned selection for persistence in a jar. These selection pressures would have favored the subset of taxa suited to these laboratory conditions regardless of whether they were in the Positive or Neutral selection line. This can be seen by the relative similarity of the Positive and Neutral jars in Passage 5. Despite the Positive treatment undergoing selection on CH4 oxidation rate, both the Positive and Neutral jars were experiencing selection on a variety of other traits that made the overall soil microbiomes appear similar.

One caveat that is important to keep in mind is that 16S rRNA genes are not markers of functional characteristics of microorganisms. They are simply phylogenetic markers useful for determining the relative position of taxa in a phylogenetic tree. In addition, assigning functions to taxa based solely on their taxonomy is a fraught exercise because many microbial functions are not very deeply conserved, are spread across disparate lineages of the tree of life, or are easily transferred between distantly related taxa. However, methanotrophy and methanogenesis are two of the most deeply conserved microbial traits (17). This results in part from the fact that these functions require multiple genes to perform and are therefore not easy to evolve independently or to transfer between lineages through horizontal gene transfer. However, in order to be confident that the response we observed was not simply due to a relative increase in methanotrophs, we would need to apply a functional approach to characterizing the microbiome, for example, by sequencing whole metagenomes.

Future research in this area should use methods that are able to detect functions and metabolic pathways within the community that respond to selection. For example, genome-resolved metagenomics could identify pathways that are enriched in the Positive selection treatment. This would further advance our understanding of the exact traits that are enriched in high-flux ecosystems and therefore could be markers of ecosystem function. In addition, future studies could build on experimental results such as these to sample a natural gradient of ecosystem CH4 fluxes to determine whether the taxa identified in our selection experiment could be useful indicators of CH4 flux in the field. These studies would allow us to build a picture of the multiple traits that drive variation in ecosystem function at the whole-ecosystem level.

# Conclusion

We performed an artificial ecosystem selection experiment on whole-ecosystem CH4 oxidation rate to determine whether variation in microbiome composition can contribute to variation in ecosystem function. We observed a significant response to selection on CH4 oxidation rate - the first example of an experiment performing selection on soil biogeochemical cycling. In addition, we observed strong heritability of CH4 flux between passages, suggesting that variation in microbiome composition could be a major source of variation in CH4 flux in ecosystems. Surprisingly, the taxonomic groups that experienced selection on CH4 oxidation rate were not enriched in methanotrophs. This suggests that understanding a single functional group is insufficient for predicting the effect of microbiome composition on the rate of ecosystem function. Instead, we may need to investigate alternative functional groups or multiple interacting taxa to understand the role of microbiomes in ecosystem function. While it has often been assumed that microorganisms play a major role in determining variation in ecosystem function, this has not previously been shown empirically. This experiment demonstrates that variation in microbiome composition can contribute to considerable variation in ecosystem function.

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# Competing Interests

We declare we have no competing interests.

# Data Availability Statement

The 16S rRNA sequencing data generated during the current study are available in the NCBI Sequence Read Archive (SRA).

# Supplement

# References

1. Crowther TW, Hoogen J van den, Wan J, Mayes MA, Keiser AD, Mo L, et al. The global soil community and its influence on biogeochemistry. Science. 2019 Aug;365(6455).

2. Cardinale BJ, Srivastava DS, Emmett Duffy J, Wright JP, Downing AL, Sankaran M, et al. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature. 2006 Oct;443(7114):989–92.

3. Hooper DU, Adair EC, Cardinale BJ, Byrnes JEK, Hungate BA, Matulich KL, et al. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. Nature. 2012 Jun;486(7401):105–8.

4. Gamfeldt L, Lefcheck JS, Byrnes JEK, Cardinale BJ, Duffy JE, Griffin JN. Marine biodiversity and ecosystem functioning: What’s known and what’s next? Oikos. 2015;124(3):252–65.

5. Rocca JD, Hall EK, Lennon JT, Evans SE, Waldrop MP, Cotner JB, et al. Relationships between protein-encoding gene abundance and corresponding process are commonly assumed yet rarely observed. The ISME Journal. 2015 Aug;9(8):1693–9.

6. Graham EB, Knelman JE, Schindlbacher A, Siciliano S, Breulmann M, Yannarell A, et al. Microbes as Engines of Ecosystem Function: When Does Community Structure Enhance Predictions of Ecosystem Processes? Frontiers in Microbiology. 2016;0.

7. Morris A, Meyer K, Bohannan B. Linking microbial communities to ecosystem functions: What we can learn from genotypePhenotype mapping in organisms. Philosophical Transactions of the Royal Society B: Biological Sciences. 2020 May;375(1798):20190244.

8. Swenson W, Wilson DS, Elias R. Artificial ecosystem selection. Proceedings of the National Academy of Sciences. 2000 Aug;97(16):9110–4.

9. Panke-Buisse K, Poole AC, Goodrich JK, Ley RE, Kao-Kniffin J. Selection on soil microbiomes reveals reproducible impacts on plant function. The ISME journal. 2015 Mar;9(4):980–9.

10. Goodnight CJ. Heritability at the ecosystem level. Proceedings of the National Academy of Sciences. 2000 Aug;97(17):9365–6.

11. Arias-Sánchez FI, Vessman B, Mitri S. Artificially selecting microbial communities: If we can breed dogs, why not microbiomes? PLOS Biology. 2019 Aug;17(8):e3000356.

12. Fuhrman JA. Microbial community structure and its functional implications. Nature. 2009 May;459(7244):193–9.

13. Schimel JP, Gulledge J. Microbial community structure and global trace gases. Global Change Biology. 1998;4(7):745–58.

14. Freitag TE, Prosser JI. Correlation of Methane Production and Functional Gene Transcriptional Activity in a Peat Soil. Applied and Environmental Microbiology. 2009 Nov;75(21):6679–87.

15. Freitag TE, Toet S, Ineson P, Prosser JI. Links between methane flux and transcriptional activities of methanogens and methane oxidizers in a blanket peat bog. FEMS Microbiology Ecology. 2010 Jul;73(1):157–65.

16. Meyer KM, Morris AH, Webster K, Klein AM, Kroeger ME, Meredith LK, et al. Belowground changes to community structure alter methane-cycling dynamics in Amazonia. Environment International. 2020 Dec;145:106131.

17. Martiny AC, Treseder K, Pusch G. Phylogenetic conservatism of functional traits in microorganisms. The ISME Journal. 2013 Apr;7(4):830–8.

18. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, et al. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences. 2011 Mar;108(Supplement 1):4516–22.

19. Fadrosh DW, Ma B, Gajer P, Sengamalay N, Ott S, Brotman RM, et al. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. Microbiome. 2014 Feb;2(1):6.

20. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. Applied and Environmental Microbiology. 2013 Sep;

21. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2018.

22. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods. 2016 Jul;13(7):581–3.

23. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. Applied and Environmental Microbiology. 2007 Aug;73(16):5261–7.

24. Davis NM, Proctor DM, Holmes SP, Relman DA, Callahan BJ. Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome. 2018 Dec;6(1):226.

25. Falconer DS(S, MacKay TFC. Introduction to quantitative genetics. Harlow : Prentice Hall; 1996.

26. Willis A, Bunge J. Estimating diversity via frequency ratios. Biometrics. 2015;71(4):1042–9.

27. Aitchison J. The Statistical Analysis of Compositional Data. Journal of the Royal Statistical Society: Series B (Methodological). 1982;44(2):139–60.

28. McArdle BH, Anderson MJ. Fitting Multivariate Models to Community Data: A Comment on Distance-Based Redundancy Analysis. Ecology. 2001;82(1):290–7.

29. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. Vegan: Community ecology package. 2019.

30. Martin BD, Witten D, Willis AD. Modeling microbial abundances and dysbiosis with beta-binomial regression. The Annals of Applied Statistics. 2020 Mar;14(1):94–115.

31. Hahn MW, Schmidt J, Koll U, Rohde M, Verbarg S, Pitt A, et al. Silvanigrella aquatica gen. Nov., sp. Nov., isolated from a freshwater lake, description of Silvanigrellaceae fam. Nov. And Silvanigrellales ord. Nov., reclassification of the order Bdellovibrionales in the class Oligoflexia, reclassification of the families Bacteriovoracaceae and Halobacteriovoraceae in the new order Bacteriovoracales ord. Nov., and reclassification of the family Pseudobacteriovoracaceae in the order Oligoflexales. International Journal of Systematic and Evolutionary Microbiology. 2017;67(8):2555–68.

32. Tamaki H, Tanaka Y, Matsuzawa H, Muramatsu M, Meng X-Y, Hanada S, et al. Armatimonas rosea gen. Nov., sp. Nov., of a novel bacterial phylum, Armatimonadetes phyl. Nov., formally called the candidate phylum OP10. International Journal of Systematic and Evolutionary Microbiology. 2011;61(6):1442–7.

33. Garrity GM, Bell JA, Lilburn TG. Class III. Gammaproteobacteria class. Nov., p. 1. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM, editors. Bergey’s manual of systematic bacteriology. Second. New York, NY: Springer; 2005.

34. Freitag TE, Prosser JI. Community Structure of Ammonia-Oxidizing Bacteria within Anoxic Marine Sediments. Applied and Environmental Microbiology. 2003 Mar;

35. McBride MJ, Liu W, Lu X, Zhu Y, Zhang W. The Family Cytophagaceae. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F, editors. The Prokaryotes: Other Major Lineages of Bacteria and The Archaea. Berlin, Heidelberg: Springer; 2014. pp. 577–93.

36. Bay SK, Dong X, Bradley JA, Leung PM, Grinter R, Jirapanjawat T, et al. Trace gas oxidizers are widespread and active members of soil microbial communities. Nature Microbiology. 2021 Feb;6(2):246–56.

37. Visscher PM, Hill WG, Wray NR. Heritability in the genomics era concepts and misconceptions. Nature Reviews Genetics. 2008 Apr;9(4):255–66.

38. Conner JK. Artificial Selection: A Powerful Tool for Ecologists. Ecology. 2003;84(7):1650–60.

39. Kerem B, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, et al. Identification of the cystic fibrosis gene: Genetic analysis. Science. 1989 Sep;245(4922):1073–80.

40. MacDonald ME, Novelletto A, Lin C, Tagle D, Barnes G, Bates G, et al. The Huntington’s disease candidate region exhibits many different haplotypes. Nature Genetics. 1992 May;1(2):99–103.

41. Reich DE, Lander ES. On the allelic spectrum of human disease. Trends in Genetics. 2001 Sep;17(9):502–10.

42. Hindorff LA, Sethupathy P, Junkins HA, Ramos EM, Mehta JP, Collins FS, et al. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. Proceedings of the National Academy of Sciences. 2009 Jun;106(23):9362–7.

# Figures

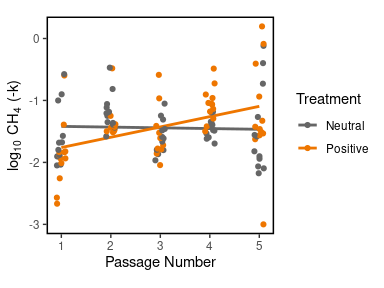


Figure 1: Response to selection on soil CH4 oxidation rate fit by ordinary least squares regression. The y-axis is the log10 of the additive inverse of the first-order exponential decay constant (i.e., ) with units day-1. The x-axis is the passage number. The orange line and points are the Positive treatment and the gray line and points are the Neutral treatment.

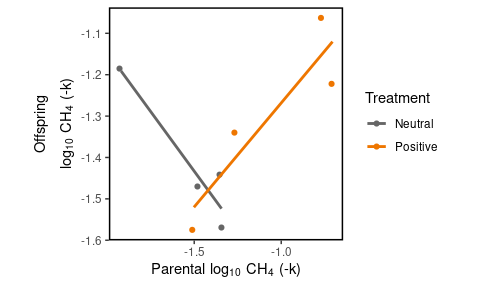


Figure 2: Ordinary least squares regression of mid-offspring CH4 flux on mid-parent CH4 flux ( with units day-1). Mid-parent is the mean of the jars selected to inoculate the next passage. Mid-offspring is the mean of all twelve jars produced in one passage. The orange lines and points are the Positive treatment and the gray lines and points are the Neutral treatment

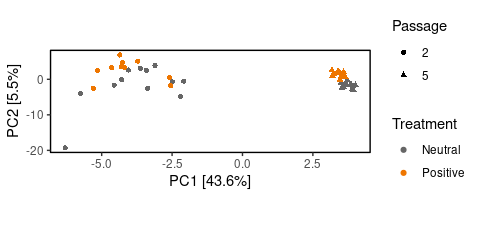


Figure 3: Principal component analysis plot of beta diversity for all jars. Dissimilarities are based on Aitchison dissimilarity, which is Euclidean distance after center-log ratio transformation. Colors represent treatment and shapes represent passage number. The ratio of the axes is proportional to the variance explained by each principal component in order to accurately represent the distance between the points.

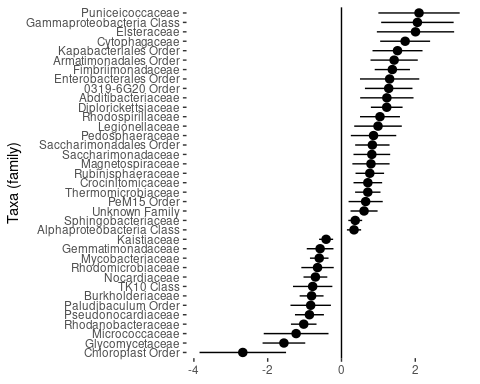


Figure 4: Differentially abundant family-level taxa based on a beta-binomial model. Values on the x-axis are estimates and standard errors of the expected difference in the logit-transformed relative abundance between the two treatments. Positive values are enriched in the Positive treatment and negative values are enriched in the Neutral treatment. Taxa presented here are significant at FDR < 0.5

Table 1: PERMANOVA on Aitchison and Jaccard dissimilarities.

| metric | term | df | SumOfSqs | R2 | statistic | p.value |
| --- | --- | --- | --- | --- | --- | --- |
| aitchison | Passage | 1 | 192,687.5912905 | 0.41932912 | 34.957336 | 0.001 |
| aitchison | Treatment | 1 | 21,430.5165026 | 0.04663735 | 3.887919 | 0.004 |
| aitchison | Pass:Treat | 1 | 13,888.5630478 | 0.03022446 | 2.519660 | 0.031 |
| aitchison | Residual | 42 | 231,507.3101917 | 0.50380907 |  |  |
| aitchison | Total | 45 | 459,513.9810326 | 1.00000000 |  |  |
| jaccard | Passage | 1 | 3.6801921 | 0.47353513 | 42.509232 | 0.001 |
| jaccard | Treatment | 1 | 0.2634443 | 0.03389772 | 3.042997 | 0.025 |
| jaccard | Pass:Treat | 1 | 0.1919981 | 0.02470464 | 2.217735 | 0.075 |
| jaccard | Residual | 42 | 3.6361059 | 0.46786251 |  |  |
| jaccard | Total | 45 | 7.7717403 | 1.00000000 |  |  |