PARTICIPANTS:

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Tayte Campbell, Metagenomics and metatranscriptomics, ORCID 0000-0001-8806-1534

PROJECT DESCRIPTION

Title: Characterization of simplified soil communities with high and low carbon use efficiency across differing moisture treatments

Relevant Categories: Bacteria/archaea, Fungi, Metagenome/metatranscriptome, Metabolomics

Specific Aims:

This project is directed under the "Terrestrial Microbial Carbon Cycling" SFA project awarded to Los Alamos National Laboratory. The goal of the SFA is to interrogate how soil microbial community variation contributes to the fate of carbon in the environment across differing moisture treatments. Here, we propose a set of integrated experimental approaches to characterize simplified microbial communities we have identified that have high or low carbon use efficiency (CUE). We posit that microbial communities with high CUE will favor metabolic pathways to create recalcitrant dissolved organic carbon (DOC) metabolites. By characterizing the metabolic pathways and metabolites present in high and low CUE communities, we will be able to better understand which microbial pathways lead to carbon sequestration versus CO_2 emission leading to better C cycle modeling.

Aim 1. Identify metabolic pathways that are present and active in high CUE communities

Aim 2. Identify metabolites that are enriched in high CUE communities

Mission Relevance:

Our proposed project addresses important BERAC grand challenges and action items:

> **Grand Challenge:** Integrate molecular and process data to improve the ability to define ecologically significant traits of individual taxa and communities and use trait-based models to develop predictive links between community dynamics and ecosystem processes.
>
> **Action Item:** Promote integrated studies that explicitly test predicted microbial network Interactions and attempt to assess membership and species-specific and collective functional capabilities within ecologically coherent microbial communities.

Our vision directly addresses these challenges by using data collected from an incubation of nearly 200 different soil inocula to 1) resolve the microbial pathway and interaction traits that are linked with carbon emission and sequestration, 2) assess the metabolites produced by these ecologically coherent microbial communities, and ultimately, 3) determine if metagenomics and metatranscriptomic methods are good predictors of the resulting metabolite pools. The FICUS proposal is key to achieving goals 1 and 2.

Introduction:

Soil microbial communities are responsible for releasing 60-75 Pg C through CO_2 emissions annually [1]. Since soil is such a large source of carbon emission it is important to understand the underlying carbon transformations that take place in the soil from initial inputs of degradation to C fate through efflux or sequestration. A better understanding of how specific microbes, microbial communities, and metabolic functions correlate with C cycling is necessary for improved modelling and predictions of carbon efflux in a changing environment[2].

One way to interrogate microbial interactions with soil C cycling is through the use of simplified microbial communities in sealed microcosms [3]. Soil microbial communities are complex but connection between all bacteria in a given soil is limited due to pore space and water connectivity available in a given soil [4]. Simplified communities allow for tractable communities that mimic microbial communities in a given pore space. These communities, while simplified, are still capable of complex lignocellulose degradation and high variance of C fates.

Further, climate change models predict changes in precipitation, with some areas receiving more water inputs and others receiving less [5]. By comparing soils that are maintained at constant moisture levels with samples that go through cycles of wetting and drying we can evaluate the effects of precipitation events on soil C efflux [6]. Changing levels of moisture content will influence microbial connectivity with substrates while prolonged dehydration may result in microbial dormancy, thus decreasing the amount of C cycling.

In our current "Terrestrial Microbial Carbon Cycling" SFA experiment we collected soils from 95 sites across the southwestern United States. Microcosms were created by making a 1:20 dilution of collected soils and using it to inoculate microcosms with sand and Ponderosa pine needle litter. Collected soils were used to inoculate 2 microcosms: one treated with constant moisture conditions and one treated with three wetting cycles followed by long periods of desiccation. We incubated the microcosms for 3 months and collected CO_2 emissions throughout the duration of the experiment. At the end of the experiment, we measured final dissolved organic carbon (DOC). From these measurements we found that soil microbial communities subjected to wetting and drying cycles had lower levels of CO_2 efflux and DOC (Figure 1A,B). We also identified microbial communities at the extremes of carbon use efficiency (CUE) to interrogate further with multi-omic analyses (Figure 1C,D).

The conversion of available C substrates into stable compounds is an important step in soil C cycling [7]. However, the production of stable compounds that remain in the soil by microbiota takes energy that is supplied through CO_2 emission. In a broad sense the amount of carbon stored to the amount of CO_2 emitted is the CUE [9]. CUE is therefore an important measurement for modelling soil C cycling and informing CO_2 efflux predictions [8]. By comparing high and low CUE communities we can identify microbial species or metabolic pathways that can be used as "traits" in C cycle models.

In this proposal, we aim to identify microbial taxa and metabolic pathways that correlate with CUE in constant and changing moisture conditions. Here, we propose shotgun metagenomic sequencing to identify microbial taxa and microbial pathways present in the microcosm communities. We also aim to create metagenome-assembled genomes (MAGs) that will provide important understanding of uncharacterized and under-characterized environmental taxa [9]. This analysis will allow for assessment of species-specific and community wide functional capabilities in ecologically coherent microbial communities.

Further, microbial taxa and metabolic pathways identified through metagenomic sequencing has been included in C cycle modelling to aid in prediction of C efflux [10]. In this proposal we aim to employ metagenomic and metatranscriptomic analyses to predict C fate. Then, we will harness metabolomic and proteomic analyses to validate the initial predictions and further refine predictive models. Finally, with the combined analyses we can determine if high CUE communities store C as more recalcitrant compounds than low CUE communities.

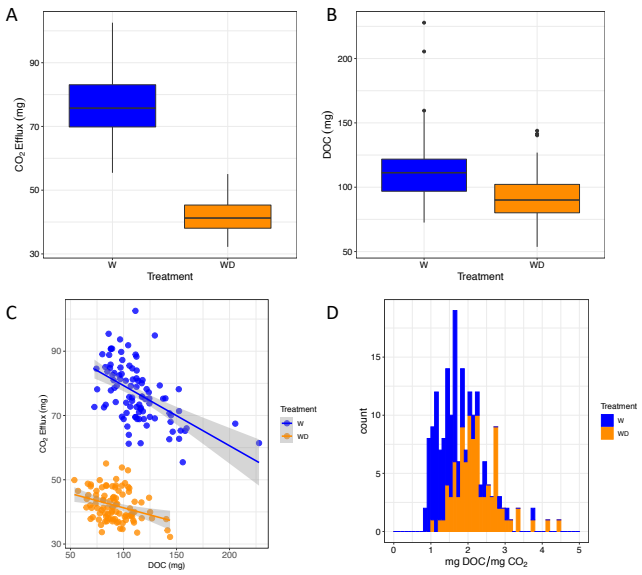

Figure 1. C efflux and DOC quantities of soil microcosms. A. CO_2 efflux by treatment (P<0.01). **B.** DOC by treatment (P<0.01). **C.** Scatter plot of CO_2 efflux compared to DOC. Regression lines shows best fit lines with 95% confidence intervals (W R^2 = 0.28; WD R^2=0.10). **D.** Histogram of mg DOC per mg CO_2. High CUE samples have a higher DOC to CO_2 ratio. For all graphs: W=wet treatment; WD=wet/dry treatment.

Approach:

A central approach for our Terrestrial Microbial Carbon Cycling SFA is the use of simplified soil communities that we can use to measure C cycling in microcosms. This proposal builds on a recent experiment where diluted extracts from 95 different soils collected throughout the southwestern United States were inoculated into sand microcosms. We provided Ponderosa pine needles for microbial degradation and measured the CO_2 efflux over a three-month incubation period with 2 moisture treatments: constant moisture and 3 cycles of wetting and drying. At the end of the incubation, we measured dissolved organic carbon (DOC) and collected samples for DNA extractions and freezer storage. From these measurements we were able to identify high (e.g. low CO_2 efflux and high DOC production) and low (e.g. high CO_2 efflux and low DOC production) CUE communities. Our SFA supports 16S gene sequencing of all our samples. This proposal is for deep metagenomic and metatranscriptomic sequencing of a subset of our samples that we identify as having the highest and lowest CUE (Task 1), metabolite and protein analysis of that same subset, and computer modelling to determine the relationships between sequencing data and the resulting metabolite pools (Task 2). Our samples will be prepared and shipped according to the standard protocols for each institution. We anticipate sample shipment would be possible by September 2020, or even sooner, as the initial experiment is complete. Samples are currently stored at -80C while awaiting DNA extraction.

Task 1. Determine the metabolic pathways that are enriched in high CUE communities versus low CUE communities. In task 1 we aim to identify metabolic pathways in high and low CUE communities by leveraging microcosm communities from a recent incubation experiment. In this recent experiment we calculated CUE (mg DOC/mg C emitted as CO_2) for 95 samples across 2 moisture treatments (Figure 1). From this experiment, we identified samples at the extremes of CUE. In this proposal we aim to further investigate the 6 highest and the 6 lowest CUE samples from each treatment. The total number of samples for this proposal is:
(6 high CUE samples + 6 low CUE samples) x 2 treatments = 24 total samples

1. **Metagenomics of microcosm samples.** We request access to the **Illumina HiSeq** sequencing platforms at **JGI** for characterization of microbial metagenomes from the 24 samples. We will aim for a sequencing depth of ~26 Gb per sample for a total of **624 Gb** for this analysis. We will extract DNA from these samples and send them to JGI for library

preparation and sequencing. Shotgun metagenome data will be compared with our 16S analysis to identify links between taxonomy and function. We will do this by creating metagenome-assembled genomes (MAGs) to characterize the function of microbes and determining how many of our 16S taxa are represented by MAGs. We also aim to combine metagenomic data to identify biochemical pathways that we can combine with the other omics data (metatranscriptomics, metabolomics, and proteomics) to identify links between soil microbes and CUE.

2. **Metatranscriptomics of microcosm samples.** At **JGI** we request access to the **Illumina HiSeq** sequencing platforms for sequencing the metatranscriptomes of the 24 samples. Extracted RNA will be sent to JGI for sequencing with a target depth of ~15Gb per sample for a total of **360 Gb** for this analysis. Metatranscriptomics will allow us to identify active taxa and functions that will indicate centrally important transcripts that are indicative of high and low CUE communities across moisture treatments.

Task 2. Determine the fate of soil C by characterizing the metabolites and proteins produced in high and low CUE communities. In this task we will determine the fate of soil C and in conjunction with task 1 determine if metagenomic pathways can help predict the metabolites that are produced.

1. **Metabolomics and proteomics of microcosm samples.** We request access to the **EMSL** metabolomics and proteomics pipelines to identify metabolites and proteins produced in the microcosm samples. We aim to determine metabolite contents using **GC-MS** and **NMR** and to determine the protein contents with **LC-MS**. The resulting data will be analyzed as described below to determine if high CUE communities are associated with more recalcitrant metabolites and less degradation proteins than low CUE communities. We will also be able to identify differences in metabolites and proteins across the two water treatments.

2. **Modeling of multi-omics data.** With the data generated in this proposal we will be able to assemble MAGs and identify key characteristics of high and low CUE communities. With the metagenomic and metatranscriptomic data we aim to compare metagenomic data with metatranscriptomic data to identify expressed and non-expressed genes allowing us to identify active genes involved with microbial C usage. To integrate sequencing data with metabolomics and proteomics, we will map the identified enzymes and metabolites onto a master metabolic network generated using databases such as ModelSEED and KEGG. From this analysis we will be able to link transcript and enzyme abundance with metabolite pools and differentiate metabolic maps of high and low CUE communities. At **EMSL** we request access to computing resources as described below.

Regulatory Compliance: The laboratories at PNNL have the necessary permits from APHIS for working with soil samples and isolation of bacteria from soil.

EMSL Computing Approach

1. **Metagenome and metatranscriptome assembly and annotation.** We will require access to **Tahoma** for metagenome assembly and annotation using the ATLAS pipeline [11]. In the ATLAS pipeline, reads will be quality filtered, error corrected, and normalized before being assembled with HipMer [12]. We will then review the coverage and quality of the assembled contigs before using Prokka [13] to translate and functionally annotate proteins. Annotated metagenomes will serve as databases for the metaproteome searches in task 2.

2. **Metagenome assembly into metagenome-assembled genomes (MAGs).** The metagenome reads from the ATLAS pipeline after the normalization step and will use MetaBat (https://bitbucket.org/berkeleylab/metabat/src/master/) [14] to assemble MAGs. Completeness and contamination of each MAG will be estimated using CheckM (https://github.com/Ecogenomics/CheckM/wiki) [15] and its lineage-specific marker genes. Taxonomy will be identified either by the16S rRNA genes using BLASTN [16] if captured in the MAG or by amino-acid identity (AAI) using CompareM (https://github.com/dparks1134/CompareM) [17]. Assigned taxonomies will be compared to 16S sequencing analysis to estimate the level of taxonomic concordance across sequencing techniques.

☒ **By checking this box, I am confirming that participants on this proposal will NOT process, store, or transmit sensitive data (e.g. Personally Identifiable Information, Official Use Only, etc.) on Tahoma, Cascade or Aurora.**

Total CPU Hours Request for first year of proposal: 250,000 CPU hours					
Total GPGPU Hours Request for first year of proposal: 0 GPGPU hours					
Total Data Archive Request for first year of proposal: 10 TB					
Software Details	**Node Request (CPUs or GPGPUs)**	**Estimated # of jobs**	**Estimated Node Hours**	**Expertise of your investigators for these requests**	**EMSL Support Requested** *Specific Needs (e.g., compiling code, libraries needed, help running jobs, etc.)*
ATLAS	CPU	~100	100,000	Proficient	Initial help setting up jobs in Tahoma
MetaBat	CPU	~100	50,000	Proficient	Initial help setting up jobs in Tahoma
CheckM	CPU	~100	50,000	Proficient	Initial help setting up jobs in Tahoma
CompareM	CPU	~100	50,000	Proficient	Initial help setting up jobs in Tahoma

Appendix 1: References

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2. Wieder WR, Grandy AS, Kallenbach CM, Bonan GB (2014). Integrating microbial physiology and physio-chemical principles in soils with the MIcrobial-MIneral Carbon Stabilization (MIMICS) model. Biogeosciences. 11:3899-3917. https://doi.org/10.5194/bg-11-3899-2014
3. Zegeye EK, Brislawn CJ, Farris Y, Fansler SJ, Hofmockel KS, Jansson JK, Wright AT, Graham EB, Naylor D, McClure RS, Bernstein HC (2019). Selection, succession, and stabilization of soil microbial consortia. mSystems. 4(4). https://msystems.asm.org/content/4/4/e00055-19
4. Smith AP, Bond-Lamberty B, Benscoter BW, Tfaily MM, Hinkle CR, Liu C, Bailey VL (2017). Shifts in pore connectivity from precipitation versus groundwater rewetting increases soil carbon loss after drought. Nature Communications. 8:1335. https://doi.org/10.1038/s41467-017-01320-x
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8. Parks DH, Rinke C, Chuvochina M, Chaumeil PA, Woodcroft BJ, Evans PN, Hugenholtz P, Tyson GW (2017). Recovery of nearly 8,000 metagenome-assembled genomes substantially expands the tree of life. Nature Microbiology. 2:1533-1542. https://doi.org/10.1038/s41564-017-0012-7
9. Wieder WR, Bonan GB, Allison SD (2013). Global soil carbon projections are improved by modelling microbial processes. Nature Climate Change. 3:909-912. https://doi.org/10.1038/nclimate1951
10. Lal R (2004). Soil carbon sequestration impacts on global climate change and food security. Science. 304(5677):1623-1627. https://doi.org/10.1126/science.1097396
11. White III RA, Brown J, Colby S, Overall CC, Lee J, Zucker J, Glaesemann KR, Jansson C, Jansson JK (2017). ATLAS (Automatic Tool for Local Assembly Structures) – a comprehensive infrastructure for assembly, annotation, and genomic binning of metagenomic and metatranscriptomic data. PeerJ Preprints. 5:e2843v1. https://doi.org/10.7287/peerj.preprints.2843v1
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17. Parks DH, Rinke C, Chuvochina M, Chaumeil PA, Woodcroft BJ, Evans PN, Hugenholtz P, Tyson GW (2017). Recovery of nearly 8,000 metagenome-assembled genomes substantially expands the tree of life. Nature Micro. 2:1533-1542. https://doi.org/10.1038/s41564-017-0012-7

Appendix 2: CVs

VANESSA L. BAILEY. **Pacific Northwest National Laboratory**

Scientist (V), Team lead
Phone: 509-371-6965; Fax: 509-371-6946; Email: vanessa.bailey@pnnl.gov

EDUCATION

1999 Ph.D., Soil Science, University of Alberta, Edmonton, Alberta, Canada
1994 B.S.A., Soil Science, University of Manitoba, Winnipeg, Manitoba, Canada

PROFESSIONAL EXPERIENCE

- 2017- present: **Team Lead** for Ecosystem Sciences/Biological Sciences Division, PNNL
- 2014- 2017: **Associate Division Director** for the Microbiology Group, PNNL
- 2002-present: **Scientist** Microbiology/Biological Sciences Division, PNNL
- 2000-2002: **Postdoctoral Researcher**, Microbiology/Biological Sciences Division, PNNL
- 2000: **Soil Scientist,** Alberta Agriculture, Food, and Rural Development, Edmonton, Alberta, Canada.

PROFESSIONAL AFFILIATIONS

- American Geophysical Union, Soil Science Society of America, American Society for Microbiology, International Society for Microbial Ecology

AWARDS, HONORS, APPOINTMENTS

- The Environmental and Molecular Sciences Laboratory (EMSL), Wiley Fellow
- Courtesy Faculty, Dept of Crop and Soil Sciences, Oregon State University
- Adjunct Professor, Dept of Crop and Soil Sciences, Washington State University

FUNDING

- Initiative Lead, Predicting Ecosystem Resilience through Multiscale Integrative Science: Laboratory Directed Research and Development:
    - Initiative is comprised of 11 PI-led projects
    - Total investment is $2.9 million/y for 4 years
- Co-Investigator for the US Department of Energy-Biological and Environmental Research **Genome Sciences Program** led by Los Alamos National Laboratory, funded in October 2018 for 3y ($225K/y to PNNL).
- Principal Investigator for the US Department of Energy-Biological and Environmental Research **Terrestrial Ecosystem Science** research program at PNNL which was funded in October 2017 ($800K/y).
- Principal Investigator for the US Department of Energy-Biological and Environmental Research **Terrestrial Ecosystem Science** research program at PNNL which received preliminary funding in 2013, and began as a funded project in October 2014 ($700K/y).
- Investigator for the DOE-BER program, **Carbon Sequestration in Terrestrial Ecosystems**. This multi-lab program (Oak Ridge, Argonne, and Pacific Northwest National Laboratories) ran from 1999-2011 with a total funding of ~$3M/y.
    - In 2008, I was the task lead (~$400K/y) for Microbial Ecology on that project.

- Investigator, the USDA research team, the **Consortium for Agricultural Soils Mitigation of Greenhouse Gases** (2002-2004, ~$300K).
- Principal Investigator: Laboratory Directed Research and Development (These are panel-reviewed, highly competitive projects that create new areas of expertise). Noteworthy projects include:
    - *Wetland Microbiomes in Transition* (~$280K/year, 3 years)
    - *Signatures of Environmental Perturbation – Microbial Community and Organic Matter Resilience* (~$500K/year, 2 years)
    - *Community Diversity and Functional Redundancy of Cellulytic Microbial Communities in Soil* (~$250K/year, 3 years)

SELECTED PUBLICATIONS (*h*-index of 26 from >60 publications, >3,776 total citations)

Vera-Gargallo B., T. Roy Chowdhury, J.M. Brown, S.J. Fansler, A. Duran-Viseras, C. Sanchez-Porro, and **V.L. Bailey**, et al. 2019. "Spatial Distribution of Prokaryotic Communities in Hypersaline Soils." Scientific Reports 9.

Bailey V.L., C. Hicks Pries, and K. Lajtha. 2019. "What do we know about soil carbon destabilization?." Environmental Research Letters 14, no. 8:Article Number 083004. PNNL-SA-143015. doi:10.1088/1748-9326/ab2c11

Yan, Z., T. Wang, L. Wang, X. Yang, P. Smith, M. Hilpert, S. Li, J. Shang, **V. Bailey**, and C. Liu. 2018. “Microscale water distribution and its effects on organic carbon decomposition in unsaturated soils.” Science of the Total Environment, 644: 1036-1043. https://doi.org/10.1016/j.scitotenv.2018.06.365

Bailey VL, B Bond-Lamberty, K DeAngelis, AS Grandy, CV Hawkes, K Heckman, K Lajtha, R Phillips, BN Sulman, KEO Todd-Brown, and MD Wallenstein. 2017. "Soil carbon cycling proxies: understanding their critical role in predicting climate change feedbacks." Global Change Biology 24(3):895-905. doi: 10.1111/gcb.13926

Smith AP, B Bond-Lamberty, BW Benscoter, MM Tfaily, R Hinkle, C Liu, and **VL Bailey**. 2017. "Shifts in pore connectivity from precipitation versus groundwater rewetting increases soil carbon loss after drought." Nature Communications. 10.1038/s41467-017-01320-x

Elshahed MS, NH Youseff , AM Spain, C Sheik, FZ Najar , LO Sukharnikov, BA Roe, JP Davis , PD Schloss, **VL Bailey,** and LR Krumholz. 2008. "Novelty and uniqueness patterns of rare members of the soil biosphere." Applied and Environmental Microbiology 74(17):5422-5428.

Jastrow, J.D., J.E. Amonette, and **V.L. Bailey**. 2007. Chemical, biological, and physical mechanisms controlling soil carbon turnover and their potential application for enhancing carbon sequestration. Climatic Change 80: 5-23. Invited.

Bailey, V.L., J.L. Smith, and H. Bolton, Jr. 2002. Fungal to bacterial ratios in soils investigated for enhanced C sequestration. Soil Biology and Biochemistry 34 (7): 997-1007.

TAYTE P. CAMPBELL **Pacific Northwest National Laboratory**

Post-Doctoral Researcher
Phone: (509) 371-7889; Email: tayte.campbell@pnnl.gov

RELEVANT EXPERIENCE

Microbial and molecular bench work. Metagenomic, genomic, and transcriptomic analyses. Multivariate statistics.

EDUCATION

2019 Ph.D., Biology and Biomedical Sciences, Washington University in St. Louis, MO
Multi-omic understanding of the evolution of xenobiotic tolerance in bacterial isolates and communities

2015 M.S. Environmental Science, Brigham Young University, Provo, UT
Tree Islands of Fertility Structure Bacterial Community Assembly and Functional Genes Contributing to Ecosystem Processes

2013 B.S. Environmental Science, Brigham Young University, Provo, UT
Minor: Chemistry

PROFESSIONAL EXPERIENCE

- 2019-present: Postdoctoral RA, Ecosystem Sciences, PNNL
- 2019-present: Adjunct Professor, Washington State University – Tri-cities, Richland, WA
- 2015: Research Intern, Max Planck Institute for Chemical Ecology

FUNDING

- 2013: **ORCA Undergraduate Research Grant**

PUBLICATIONS (*h*-index of 3 from 6 publications, >24 total citations)

Campbell TP, Sun X, Patel VH, Sanz C, Morgan D, Dantas G, (2020). The microbiome and resistome of chimpanzees, gorillas, and humans across host lifestyle and geography. *ISME Journal*.

Aanderud ZT, Bahr J, Robinson DM, Belnap J, **Campbell TP**, Gill RA, McMillan B, St. Clair S, (2019). The burning of biocrusts facilitates the emergence of a bare soil community of poorly-connected chemoheterotrophic bacteria with depressed ecosystem services. *Frontiers in Ecology and Evolution*. 7:467.

Anthony WE, Carr RR, DeLorenzo DM, **Campbell TP**, Shang Z, Foston M, Moon TS, Dantas G, (2019). Development of *Rhodococcus opacus* as a chassis for lignin valorization and bioproduction of high-value compounds. *Biotechnology for Biofuels*. 12:192.

Roell GW, Carr RR, **Campbell TP**, Shang Z, Henson WR, Czajka JJ, Martin HG, Zhang F, Foston M, Dantas G, Moon TS, Tang YJ, (2019). A concerted systems biology analysis

of phenol metabolism in *Rhodococcus opacus* PD630. *Metabolic Engineering*. 55:120-130.

Henson* W, **Campbell* TP,** DeLorenzo* D, Gao Y, Berla B, Kim SJ, Foston M, Moon TS, Dantas G, (2018). Multi-omic elucidation of aromatic catabolism in adaptively evolved *Rhodococcus opacus*. *Metabolic Engineering*. 49:69-83. *Denotes equal contribution.

Aanderud ZT, Schoolmaster D, Rigby D, Bybee J, **Campbell TP,** Roundy B, (2017). Soil mediates the impact of fine woody debris on invasive and native grasses as whole trees are mechanically shredded into firebreaks in piñon-juniper woodlands. *Journal of Arid Environments.* 137:60-68.

PRESENTATIONS

Campbell TP, DeLorenzo DM, Henson WR, Gao Y, Berla B, Kim SJ, Foston M, Moon TS, Dantas G, (2019). Multi-omic analysis of adapted *Rhodococcus opacus* strains for the characterization of aromatic metabolism. **Poster Presentation**. 2019 Genomics Sciences Program Annual Principal Investigator (PI) Meeting, DOE, Washington D.C., 24-27 February.

Campbell TP, Sun S, Sanz C, Morgan D, Dantas G, (2018). The effect of captivity on chimpanzee and gorilla gut microbiota and their resistomes. **Poster Presentation**. Lake Arrowhead Microbial Genomics 2018, Lake Arrowhead, CA, 16-20 September.

APPENDIX 3 – Resources Requested

NSLS-II

If you are interested in using the XFM at NSLS-II, please complete the information below.

NSLS-II RESOURCE REQUEST					
Resource	**# of Samples**	**Estimated date of sample shipment to NSLS-II**	**Units of Requested (specify unit)**	**Project Team Expertise per Resource**	**Support Requested**

EMSL

For questions about the EMSL resource request form, please contact the appropriate Capability Lead: https://www.emsl.pnnl.gov/emslweb/scientific-capabilities

EMSL RESOURCE REQUEST ***Add lines as needed for # of resources***					
Resource	**# of Samples**	**Estimated date of sample shipment to EMSL**	**Units of Requested (specify unit)**	**Project Team Expertise per Resource**	**EMSL Support Requested**
Orbitrap Mass Spectometer	24 microcosm soils	June/July 2020	400 hours	Beginner	Technician for instrument operation
Mass Spectrometer: GC-MS	24 microcosm soils	June/July 2020	75 hours	Beginner	Technician for instrument operation
FT-ICR Mass Spectrometer	48 microcosm extractions (water and methanol extractions)	June/July 2020	200 hours	Beginner	Technician for instrument operation
750 MHz NMR Bokan	24 microcosm soils	June/July 2020	400 hours	Beginner	Technician for instrument operation
C, H, N, S Analyzer	24 microcosm soils	June/July 2020	24 hours	Beginner	Technician for instrument operation
TAHOMA	N/A	N/A	250,000 hours	Proficient	Technical Support

JGI

For questions about the JGI resource request form, please contact jgi-jira+pmosupport@lbl.gov.

JGI RESOURCE REQUEST			
Resource	**# of Samples**	**Estimated date of sample shipment to JGI**	**Analysis Support Requested beyond JGI's standard pipelines**
Sequencing resources:			
Bacterial/archaeal *de novo* genomes (single organisms)			
Bacterial/archaeal single cells: *samples for FACS sorting*			
Bacterial/archaeal single cells: *cells to be sequenced*			
Bacterial/archaeal RNA-seq (single organism)			
Bacterial/archaeal resequencing			
Fungal *de novo* genomes			
Fungal RNA-seq (single organism)			
Fungal resequencing			
Algal *de novo* genomes			
Algal RNA-seq (single organism)			
Algal resequencing			
Plant *de novo* genomes			
Plant RNA-seq (single organism)			
Plant resequencing			
Metagenome (no iTags)	24 samples	May – August 2020	None
Stable Isotope Probing (SIP) metagenomics prep, *samples to be fractionated (*limit 36 samples)			
Metatranscriptome	24 samples	May – August 2020	None
Other sequencing request (methylation, FAIRE, ChIP-seq)			
Metabolomics resources:			
Polar metabolite analysis (LC/MS) – with/without stable isotope labeling, limit 200 samples			

Non-polar metabolite analysis (LC/MS) – with/without stable isotope labeling, limit 500 samples			

Organism detail (add rows if needed)				
Kingdom	***Genus***	***Species***	***Genome size (Mb) – for eukaryotes only***	

Environmental samples (add rows if needed)	
Number of samples	**General description of metagenome sample and source**
24	Soil microcosm communities originating from soil inoculum from New Mexico, USA.

JGI SYNTHESIS RESOURCE REQUEST	
Resource	**# of constructs/libraries**
Constructs <5 Kb	
Constructs 5-10 Kb	
Constructs >5 Kb	
Combinatorial library	
sgRNA library	
Other	
Total request in Kb (request must be between 50- 500 Kb):	

Biosafety Information (required for all proposals including a synthesis component):

Are any of the genes or fragments to be synthesized:

1. Related to the pathogenicity of an organism?* □ Yes □ No
2. Known to or has potential to encode any form of infectious agent or viral life-cycle component?* □ Yes □ No
3. Known to have any toxicity, or the likelihood that this project might increase toxicity?* □ Yes □ No

4. Intended for use in creating a vaccine?* □ Yes □ No

Comments (required if you answered Yes to any of the above):

Biosecurity, Biosafety, Biocontainment, and Environmental screening *

Describe the Biosecurity, Biosafety, Biocontainment, and Environmental aspects of your proposed research, including both the current aspects and the long term implications of the work (desirable or otherwise). Describe what you will do (and who you will collaborate with) to address any aspects of concern and how you will mitigate any undesirable outcomes. This information will be critically assessed during the JGI's DNA Synthesis Internal Review process, and your research will be delayed if the reviewers request modifications to your proposal due to insufficient consideration or description of these aspects.

Lay Description *

The JGI reviews all DNA synthesis proposals for Biosecurity, Biosafety, Biocontainment, and Environmental safety. In order to facilitate a better understanding of your proposal for our reviewers, please provide a lay description of your proposal (excluding scientific jargon), so that non-scientific/technical experts can better assess the broader aspects and implications of the work in the context of the research that is proposed.