Applications must be submitted to the OVPR by 5:00 p.m. on the published application deadline. Refer to the SFSA guidelines, available on the OVPR website, for information regarding eligibility, allowable expenses, and other submission details. Applicants must complete each item on this application form. Completed applications are limited to a maximum of 1500 words on 3 pages or less, including any figures, tables, and the budget information. Applications greater than 3 pages will be returned without review. All applications must be signed by the faculty member and the department chair.

Name __Kathryn M. Docherty_________________________ Date ___9/30/15_________________________

Department _____Biological Sciences___________________ College__CAS__________________________

Email _kathryn.docherty@wmich.edu___________________ Phone # ___7-5654_____________________

Faculty rank__Assistant Professor_____________________

Title of proposed work __Long-Term Response of Microbial Communities to Multiple Climate Change Factors____

Amount requested____$2000

Date and title of any previous SFSA or FRACAA project

SFSA: “Belowground effects of fire as a restoration tool in Michigan tallgrass prairies” September 2013

1. Provide an abstract/succinct summary of the proposal (50 words or less):

The effects of climate change on microbial communities, which mediate much of the carbon cycle, are rarely studied with more than one treatment. This project will use soils collected from the only long-term climate change experiment that concurrently manipulates four variables to examine the multi-decadal effects on microbial community structure.
2. Proposed Work

**BACKGROUND:** Climate change is one of the most pressing global issues we face today. Recently brought into the media spotlight by President Obama's tour of Alaska and his efforts to limit the effects of future fossil fuel emissions on our planet, the social and political action that we take now will decide what type of home we leave for our children and grandchildren. Yet, our ability to respond to changes in Earth’s atmosphere and climate rely upon an accurate understanding of how terrestrial ecosystems can accentuate and mitigate the effects of human-induced climate variation. The most recent model used in the Intergovernmental Panel on Climate Change assumes that soil microbial decomposition, a critical feedback to global change, is a first-order decay process, proportional to the size of the carbon pool. However, this relationship is likely far more complex, and depends on how climate change factors influence soil microbial community composition and microbial physiology. Currently, we lack a robust understanding of how climate change will influence soil microbial communities and their long-term ability to sequester (or release) carbon from terrestrial ecosystems. While some studies have examined the effects of single factors (e.g. elevated CO₂, increased drought, increased nitrogen deposition), no studies to date have investigated the long-term effects of multiple concurrent climate change factors on soil microbial community composition and function. These types of experiments are crucial for providing realistic parameters for more accurate predictive models.

**EXPERIMENTAL DESIGN:** The Jasper Ridge Global Change Experiment (JRGCE), located in an annual grassland near Palo Alto, CA, is one of the few long-term climate change experiments that manipulates multiple combinations of climate change variables (i.e. a multi-factorial design). Originally established in 2001, the JRGCE has continuously manipulated four climate change variables in a multi-factorial design, yielding a total of 16 treatments. Treatments include all combinations of ambient and elevated levels of nitrogen deposition (N), atmospheric CO₂ concentrations (C), precipitation levels (P) and canopy temperature (T). The experiment's strength lies in the long-term measurements, but also the high number of replicates (8 per treatment) to maximize measurement of ecologically-relevant effects. The JRGCE was originally designed to examine the effects of multi-factorial climate change on grassland plant communities. However, the system also provides an excellent framework for examining the multi-factorial effects of climate change on the composition of soil microbial communities. Several long-term datasets that consider soil abiotic factors have been generated previously (e.g. pH, soil moisture, nitrogen concentrations). The annual grassland where the JRGCE is located is a nitrogen-limited system. Thus, most previous studies that have examined soil processes show that nitrogen deposition has important implications for soil microbial function. However, no studies to date have used molecular approaches to examine multi-year effects of the climate change treatments on soil microbial community composition. The proposed work relies on an archive of soils that have been collected annually from JRGCE from April 2001- April 2015, and are currently stored in an ultra-low temperature freezer in my laboratory at WMU. In the proposed work, I will examine microbial community structure from frozen soils collected from single and 2-way interactive treatments and controls from 2001, 2004, 2007, 2011 and 2015.
HYPOTHESES:

**H1: The effects of single climate change factors will vary over time.**
Early in the time series (2001, 2004), soil microbial community composition will be most influenced by N, but this effect will plateau over time. Elevated N will select for faster-growing heterotrophic microbial communities. C and T will induce slow changes in soil microbial community composition that will only be seen in a decadal time-scale. The effects of P will not be obvious, but will co-vary with inter-annual variation in precipitation patterns.

**H2. Combinations of climate change factors will either mitigate or enhance the effect of N.**
N will overwhelm any effects of other treatments in 2001. Later in the time series (2011 or 2015), C will reduce the effects of N, while T will increase the effect of N. This is because elevated C will select for a greater abundance of autotrophic organisms, while elevated T will select for a greater abundance of heterotrophic organisms, already under selection by N. P will influence N only as a function of inter-annual variation in precipitation. Combinations of other treatments, such as CxT, may mitigate the effects of each single variable.

METHODS: To address the hypotheses described above, soil microbial community composition from 55 archived samples will be analyzed. These include 11 samples from 2001, 2004, 2007, 2011 and 2015, corresponding to the ambient control, treatments with elevated N, T, C, P alone, and elevated NxT, NxC, NxP, TxC, TxP, and CxP. DNA will be extracted from 0.5 g of archived soil using a PowerSoil DNA Isolation Kit (MoBio Labs). DNA quality will be checked visually using gel electrophoresis and DNA concentrations will be measured using a Qubit 2.0 fluorometer quantitation system (Life Technologies). Extracted DNA will then be sent to Michigan State University’s Genomics Core Facility for 16S rRNA library preparation and amplicon sequencing using a Mi-Seq instrument (Illumina). Briefly, this procedure involves amplifying the 16S rRNA marker gene that is present in all Bacteria. The sequence of all amplicons is then determined using a massively-parallel sequencing approach that yields millions of sequence reads per sample. Sequence data will be processed and curated using the mothur 1.3.6 pipeline. Bioinformatics processing using this pipeline removes any sequences that are not the appropriate length, represent chimeras, or do not correspond to the 16S rRNA gene. Once sequences are associated with the appropriate taxonomic identifiers, relative abundances of community members will be determined. A variety of multivariate statistical approaches will be used to visualize and analyze the data. Community similarity will first be visualized using a non-metric multidimensional scaling (NMDS) ordination approach to determine whether communities under each treatment differ a) from the controls and b) from each other over time. Other environmental datasets are also available from JRGCE at these time points (pH, soil moisture, nitrogen concentrations). The influence of each variable on community composition will be determined using redundancy analysis (RDA).

3. **Contribution of Proposed Work.**
The proposed work will provide the first assessment of the effects of multi-factor climate change on soil microbial communities using a 14-year time scale. There are no other climate change studies that have run as long as the JRGCE with continuous treatments. This will
provide an important first step toward building accurate microbial decomposition parameters into predictive climate models. The accuracy of these models is crucial for predicting the future effects of climate change, as well as for assessing whether proposed mitigation effects will have the desired outcomes.

Publication: The proposed work will result in at least one peer-reviewed scientific publication. External Funding: The proposed work will provide important preliminary data for two planned proposals to expand analyses to all combinations of treatments and the entire time series. The first proposal will be submitted to NSF Division of Environmental Biology in January 2016. The second will be submitted to the Department of Energy in 2016. No requests for proposals have been made yet, but the DOE usually has a call related to climate change and metagenomics which will be an excellent fit for an expanded version of this project. Undergraduate Education: This project will provide a research experience for at least one undergraduate researcher to prepare his/her Lee Honors College thesis.

5. Reputation of Researcher and WMU.
In addition to providing a publication and preliminary data for proposals for external funding, the proposed work will utilize archived soil from a high profile experiment. The JRGCE was founded by Dr. Christopher Field, who is affiliated with the Carnegie Institution for Science and Stanford University and is in the National Academy of Sciences. Dr. Field is a co-chair of the IPCC working group that contributed to the IPCC 5th Assessment Report on Climate Change, and has testified before Congress about the importance of taking action to prevent further climate change. Publications and proposals involving this work will include Dr. Field as a co-author, and he will ensure that significant results of this work will be included in the next IPCC report. Collaboration with this prestigious project and researcher will increase my reputation as an early-career investigator. Additionally, producing important results that will influence predictive climate models will increase the reputation of WMU as a research institution.


<table>
<thead>
<tr>
<th>Item</th>
<th>Cost</th>
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<tbody>
<tr>
<td>MoBio Labs PowerSoil DNA Isolation Kit</td>
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<tr>
<td>Cubit HS-DNA quantitation kit</td>
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<tr>
<td>16S library prep and Mi-Seq sequencing (at MSU)</td>
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<tr>
<td>Consumables</td>
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<td><strong>TOTAL</strong></td>
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6.2. Budget Justification.
The total amount requested is $2000. $500 will be used to purchase a kit for extracting DNA from 55 samples from MoBio Labs. $90 will be used to purchase a High Sensitivity DNA quantitation kit for determining concentrations of extracted DNA. $1,200 will support 16S rRNA library construction and Mi-Seq sequencing using an Illumina platform at Michigan State University Genomics Core Facility. $210 will be used to purchase supplies, including latex gloves, tubes, pipette tips, agarose, etc. required to complete this project.
Faculty member signature: Kathryn M. Doctery

Acknowledgement of Department Chair:
☐ The department chair acknowledges submission of the SFSA application.
☐ The department chair acknowledges the proposed expenses are reasonable and necessary.

Chair’s signature: [Signature] Date: 5/29/15

For OVPR use only:
Faculty member completed previous reporting requirements: Yes ☑ No ☐
Funding decision: Funded ☐ Not funded: ☐

Date received: SEP 29 2015

Research and Sponsored Programs