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|---|---|
| **Project Title** | Identifying microbial sources of nitrous oxide ($N_2O$) production |
| **University (where student will register)** | University of Warwick |
| **Theme (Max. 2 selections)** | Climate & Environmental Sustainability ☒<br>Organisms & Ecosystems ☒<br>Dynamic Earth ☐ |
| **Supervisory team (including institution & email address** | **PI:**<br>Ryan Mushinski<br>University of Warwick<br>Ryan.Mushinski@warwick.ac.uk<br><br>**Co-I:**<br>Gary Bending<br>University of Warwick<br>Gary.Bending@warwick.ac.uk |

| **Flagship Criteria Intake 2023** | **Score** | |
|---|---|---|
| CENTA2 L1/2 end-user co-development and supervision<br>2 for a L1/2 partner that have no prior CENTA collaborations:<br><br>Jacobs, Marine Biological Association, AstraZeneca | **(2+2)** | **0** |
| CASE project, confirmed by a specific letter of support from the CASE partner, projects without such a letter clearly stating commitment will not be awarded a point. | **1** | **0** |
| Diversity of the supervisory team (diversity towards e.g., gender, ethnicity, disability, and cultural background) | **1** | **0** |
| Career development of the supervisory team: specifically targeted at projects led by an academic who is seeking their first PhD student as lead supervisor. | **1** | **0** |
| Collaboration project with one of our Research Centre Partners (BGS, CEH, NCEO, NCAS) led by an academic from an HEI partner | **2** | **0** |
| Applicant co-development of the project | **1** | **0** |
| Sum | **10** | **0** |

**Project Highlights:**

- New research to unravel the sources of $N_2O$ from terrestrial systems.
- A blend of laboratory and field experience, accessing skills ranging from novel analytical chemistry to molecular techniques.
- Access to state-of-the-art instrumentation and opportunities for wider collaboration in UKRI-funded research projects.

**Overview**

Atmospheric nitrous oxide ($N_2O$) concentrations have increased by an alarming 20% since pre-industrial times (Myhre et al. 2013) and future increases are anticipated. With nearly 300 times the global warming potential of $CO_2$ over an atmospheric lifetime of approximately a century, $N_2O$ represents an important greenhouse gas and certainly the most important greenhouse gas in agricultural systems. Microorganisms are the primary source of $N_2O$, which release the gas as a by-product of nitrogen metabolism in soils, sediments, and water bodies. Global estimates indicate that fertilized agriculture as well as lands that are not currently cultivated contributes 24-56% of total annual $N_2O$ emissions. Moreover, $N_2O$ is the dominant ozone-depleting substance in the stratosphere that is not controlled by the Montreal Protocol (Ravishankara et al. 2009). It is important, therefore, to understand the processes that generate $N_2O$ if viable management strategies are to be developed that reduce fluxes to the atmosphere. While its importance to atmospheric chemistry is well understood, the spatial and temporal variation in $N_2O$ fluxes at regional scales remain poorly constrained. This has led to significant uncertainties in the climate models used to estimate emissions, assess mitigation strategies, and predict feedbacks of global change (Butterbach-Bahl et al. 2013). As a result, policymakers lack accurate information regarding how to control $N_2O$ emissions effectively.

To help better constrain sources of $N_2O$, this studentship will employ isotope-based methodology that allows the separation of specific soil microbial processes (nitrification vs. denitrification) responsible for $N_2O$ production in natural and managed ecosystems. The unique approach relies on the fact that $N_2O$ produced by nitrification has a significantly different intramolecular arrangement of N and O isotopes compared to $N_2O$ derived from denitrification (Hyodo et al. 2018). Thus, $N_2O$ derived from each source has a unique isotopic "fingerprint". This approach has been validated in a small number of laboratories worldwide and has yielded novel insights regarding the processes responsible for $N_2O$ production. The PhD student will continue the development of this method using a newly acquired isotope-ratio mass spectrometry system capable of measuring $N_2O$ isotopomers. This project will measure $N_2O$ isotopomers in agronomic and natural systems and couple these measurements to molecular characterisation of $N_2O$-producing microbes.

**Methodology:**

Aim 1. Develop methodology for quantifying $N_2O$ isotopomers on an Elementar isoprime precisION isotope ratio mass spectrometer coupled to an Elementar iso flow GHG peripheral. Here we will develop and validate working standards and subsequent methods.

Aim 2. Determine $N_2O$ bulk and isotopomer composition from natural and agronomic soils at a farm in central England. In conjunction with a BBSRC-funded project, we will collect in-situ headspace gas samples from soil and analyse isotopic $N_2O$ composition. A manuscript will be prepared to report the findings from this aim.

Aim 3. Couple microbial composition and activity to $N_2O$ isotopomers. Here we will culture $N_2O$-producing microbes from soil taken in Aim 2 and analyse their individual $N_2O$ isotopomer signal. A manuscript will be prepared to report the findings from this aim.

**Training and skills:**

Students will be awarded CENTA2 Training Credits (CTCs) for participation in CENTA2-provided and 'free choice' external training. One CTC equates to 1⁄2 day session and students must accrue 100 CTCs across the three years of their PhD.

Training during this fellowship includes a wide range of molecular techniques and analyses (traditional culturing, DNA extraction from soil, PCR, sequencing, and bioinformatics) as well as analytical chemistry (isotope ratio mass spectrometry, building sampling mesocosms). Field-based sampling and measurements from natural ecosystems will also be emphasized with additional training opportunities through possible collaboration with industry partners.

**Partners and collaboration (including CASE):**

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| Name of L1/L2 Partner (where applicable) | Not applicable |
| Name of CASE partner (where applicable – project proposal **must** be accompanied by a letter of support from the CASE partner) | Not applicable |

**Respiratory and Contact Infection Resilience of the Project:**

The School of Life Science at the University of Warwick has SOP's in place to allow research to continue in light of respiratory infection outbreak. This includes reducing the capacity of people in laboratory spaces, placing protective barriers between workstations, and working from home when possible. The laboratory portion of this work will proceed as normal, within the scope of the SOP's. All meetings associated with this project will be in line with current guidelines. The field component will proceed within the confines of a subsequent SOP - to be developed between the PI in accordance with all University- and government-mandated requirements.

**Possible timeline:**

Year 1: Develop methodology for quantifying $N_2O$ isotopomers.

Year 2. Determine $N_2O$ bulk and isotopomer composition from natural and agronomic soils

Year 3. Couple microbial composition and activity to $N_2O$ isotopomers.

**Further reading:**

Butterbach-Bahl K, Baggs EM, Dannenmann M, Kiese R, Zechmeister-Boltenstern S. (2013). Nitrous oxide emissions from soils: how well do we understand the processes and their controls? *Phil. Trans. R. Soc.* B3682013012220130122.

Hyodo A, Malghani S, Zhou Y, Mushinski RM, Toyoda S, Yoshida N, Boutton TW, West JB. (2019). Biochar amendment suppresses $N_2O$ emissions but has no impact on $^{15}N$ site preference in an anaerobic soil. *Rapid Comm. Mass Spec*. 33: 165-175.

Myhre G, et al. (2013). Radiative forcing of the direct aerosol effect from AeroCom Phase II simulations. *Atmos. Chem. Phys.* 13: 1853-1877.

Ravishankara AR, Daniel JS, Portmann RW. (2009). Nitrous oxide ($N_2O$): the dominant ozone-depleting substance emitted in the 21st century. *Science* 326: 123-125.

**Further details:**

Please add project/institutional contact details including a link to the application website if applicable

Environmental Processes Lab Website: www.ryanmushinski.com