

<b>Project Title</b>	Environmental microbes and gas fluxes: A systematic study of volatile reactive nitrogen oxides
<b>University (where student will register)</b>	University of Warwick
<b>Which institution will the student be based at?</b>	As above
<b>Theme (Max. 2 selections)</b>	Climate & Environmental Sustainability <input checked="" type="checkbox"/> Organisms & Ecosystems <input checked="" type="checkbox"/> Dynamic Earth <input type="checkbox"/>
<b>Key words</b>	Global change, Ecosystem ecology, Nitrogen gases, Microbial Ecology
<b>Supervisory team (including institution &amp; email address)</b>	<b>PI:</b> Dr. Ryan M. Mushinski University of Warwick Ryan.Mushinski@warwick.ac.uk  <b>Co-I:</b> Professor Gary Bending, University of Warwick gary.bending@warwick.ac.uk

### Project Highlights:

- You will assess soil emissions of an understudied group of nitrogen trace gases ( $\text{NO}_2$ ) along a gradient of soil carbon and nitrogen in natural and managed ecosystems.
- You will explore soil microbiomes to uncover correlations between community structure and  $\text{NO}_2$  emissions as well as investigate novel intersections between carbon- and nitrogen-cycle processes.

### Overview:

A range of pollutant gases, and especially nitrogen (N) compounds ( $\text{N}_2\text{O}$ ,  $\text{NO}$ ,  $\text{NO}_2$ , etc.) are emitted to the atmosphere from terrestrial sources, including agriculture and natural ecosystems.<sup>1,2,3</sup> These gases are extremely important for a myriad of reason including their contribution to climate change and urban air pollution. Common agricultural practices such as fertilization and irrigation will continue to increase, likely resulting in high emissions. In natural ecosystems, atmospheric deposition of N has become increasing prevalent, also stimulating emissions from soil. However, N-gas forecasts from terrestrial sources are hampered by (1) a lack of field-based measurements and (2) an incomplete understanding of the processes associated with production and consumption of these gases. This project aims to better quantify N-gas fluxes from terrestrial systems as well as map the various mechanisms associated with emission and consumption in soil.

The N-cycle is a significant source of atmospheric N-gases; however, the conditions leading to both emission and consumption are extremely complex. This is particularly true for the suite of  $\text{NO}_2$  gases ( $\text{HONO}$ ,  $\text{HNO}_3$ , organic nitrates, and particulate nitrates), which are much less studied than  $\text{N}_2\text{O}$ ,  $\text{NO}$ , and  $\text{NO}_2$ , but represent an important driver of climate via their contribution to the oxidizing capacity of the atmosphere. Additionally, there are potential intersections between N-cycling microbes and those involved in carbon-cycling, which may affect transformations of N in the soil. For example, N-cycle products (e.g.,  $\text{NH}_2\text{OH}$  and  $\text{NO}$ ) can react extracellularly with reactive oxygen species (ROS = OH,  $\text{O}_2^-$ ,  $\text{HO}_2$ ,  $\text{H}_2\text{O}_2$ ) generated by heterotrophic microbes to produce reactive N compounds such as nitrite

and NO<sub>2</sub>. These types of understudied reactions in soil represent a major gap in our understanding of the N-cycle which prevents us from scaling these processes to the regional and global scales. This study will explore the response of N-gases to soil N and carbon (C) amendments in various terrestrial ecosystems and systematically evaluate the processes responsible for emissions.

**Methodology:**

The approach will be to couple microbiological analysis to field-based measurements taken from the University of Warwick – Wellesbourne Campus. The student will target differing ecosystem types (agriculture, woodland, grassland), each of which will have a N fertilization gradient and carbon substrate amendment. Field-based fluxes of NO, NO<sub>2</sub>, N<sub>2</sub>O, and NO<sub>x</sub> will be measured, while soil ROS and N-cycle rates will be measured from sampled soil to allow for the establishment of predictive relationships between these variables and to provide evidence for the role of ROS in reactive N production. These samples will also be subjected to amplicon (16S and ITS) and metagenomic sequencing to identify changes in microbial community composition and functional potential, respectively. Additional microcosm studies will further allow the student to vary other edaphic conditions to evaluate reaction mechanisms (e.g., soil water content, oxic vs. O<sub>2</sub>-free, and sterilized vs. unsterilized soil).

**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 (microbial culturing, DNA extraction from soil, PCR, sequencing, and bioinformatics) as well as analytical chemistry (nitrogen oxide quantification, reactive oxygen extraction from soil and subsequent quantification, and building sampling microcosms). Field-based ecology will also be emphasized.

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

Both Dr. Mushinski and Professor Bending have substantial expertise in nitrogen cycle biogeochemistry as well as plant-soil-microbe interactions, evidenced by publications in the *Proceedings of the National Academy of Sciences*, *New Phytologist*, and *Soil Biology and Biochemistry*.

**COVID-19 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 COVID-19. 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 virtual. The field component will also proceed; however, a subsequent SOP will be developed between the PI and student to adhere to all University- and government-mandated requirements.

**Possible timeline:**

**Year 1:** Quantify production of N-gases (especially NO<sub>x</sub>) along gradients of soil N and carbon in terrestrial ecosystems.

**Year 2:** Use culture-dependent and -independent methods to identify soil microbiomes, specifically focusing on heterotrophic microbes that produce extracellular ROS as well as N-cycle taxa.

**Year 3:** Explore how N-cycle intermediates and products (e.g, NO and NH<sub>2</sub>OH) react extracellularly with ROS produced by heterotrophic bacteria and fungi to form various reactive N products.

**Further reading:**

- [1] Rees, R.M. et al. (2013). Nitrous oxide emissions from European agriculture – an analysis of variability and drivers of emissions from field experiments. *Biogeosciences* 10: 2671-2682.
- [2] Mushisnki, R.M. et al. (2019). Microbial mechanisms and ecosystem flux estimation for aerobic NO<sub>y</sub> emissions from deciduous forest soils. *Proceedings of the National Academy of Sciences* 116: 2138-2145.
- [3] Hudman, R.C. et al. (2012). Steps towards a mechanistic model of global nitric oxide emissions: implementation and space-based constraints. *Atmospheric Chemistry and Physics* 12: 7779-7795.