Microbial methane cycling in Scottish peatlands (Ref IAP2-18-106)

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In partnership with University of Stirling, Faculty of Natural Sciences, Division of Biology and Environmental Sciences

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Overview

Peatlands are one of Scotland's most important natural assets, cover over 20% of Scotland's land area, and they are estimated to store around 1,600 Mt of carbon. Healthy peatlands function as sinks for greenhouse gases and carbon, and therefore play a crucial role in climate change mitigation. However, little is known about the microbiota in these environments and how this will affect the cycling of greenhouse gases (such as methane).

The central aim of this PhD project is to study the diversity and activity of methane cycling microbes (methanotrophs and methanogens) in Scottish peatlands, combining methods such as nucleic acid stable isotope probing (SIP) and next generation DNA and RNA sequencing to link microbial physiology to ecosystem functioning and response to environmental stress. In particular, we want to determine the impact of environmental changes on the microbial methane cycling capacities and carbon balance in Scottish peat.

The recent advances of molecular high-throughput approaches enable us for the first time to connect the microbial diversity and community characteristics of ecosystems with their ecological function.

This project is thus timely and further serves an important capacity-building function by training up scientists who are capable to link key carbon cycle processes as they occur in the field, with the microorganisms responsible for driving them.

Methodology

The key objectives of this project are to:

1. Determine the structural and functional characteristics of the microbial community in Scottish peatlands.

2. Use a functional metagenomic/transcriptomic approach to study the active microbiota in those peatlands using stable isotope probing (SIP) combined with high throughput sequencing. This will identify the active microorganisms responsible for cycling of methane.

3. Investigate impacts of changing peat conditions as a result of environmental or land use changes on the community structure and activity of microbes involved in the production and consumption of methane in peatlands.

The key areas the student will be working on are:

1. Determining trace gas fluxes and environmental parameters

We will use microcosms (soil/sediment in 120 ml serum bottles) and *in situ* gas sampling, and gas

chromatography methods as test systems to measure gas fluxes (CH_4, CO_2) in Scottish peatlands. Samples will be used for DNA/RNA analysis and enrichment/isolation of key methane cycling microbes.

2. Recording and analyzing patterns in microbial diversity

DNA and RNA will be extracted from fresh peat and from microcosms incubated under contrasting, although environmentally-relevant conditions. Incubation approaches will include, e.g. variations in oxygen, temperature, water regime, CH_4, CO_2, organic C, nitrogen compounds, trace elements, etc. Extracted DNA/RNA will be screened by PCR/qPCR and amplicon sequencing to target and quantify bacterial and archaeal 16S rRNA and fungal 18S genes/transcripts, as well as functional markers for essential C cycling processes in the peatlands (e.g. *pmoA*, *mmoX*: methane oxidation; *mcrA*: methanogenesis). This will provide molecular data on the distribution and diversity of the microbiota in these peatlands and how they respond to environmental changes.

3. Stable isotope probing (SIP) to identify the active key players in the sites

A functional, focussed metagenomic approach will be applied to study the active microbiota in peatlands using SIP (3, 4) combined with high throughput sequencing. SIP will be used to determine, in samples from key sites, which are the most active groups of microbes with respect to active methanogens and methanotrophs involved in methane cycling. ^{13}C-labelled substrates for analysis of the methane cycle by SIP will include CO_2 and/or methane. These experiments will determine which are the key active methanogens and methanotrophs in these peat environments.

4. Isolation of microbial key players

The information obtained from sequencing data will be used to isolate uncultivated key players in the methane cycle from these peatland environments. The gene sequence information will enable targeted isolations of methanotrophs and methanogens. Isolation approaches will include variations in oxygen, temperature, CH_4, CO_2, organic C, nitrogen compounds and trace elements. These approaches have proved successful in the past and complement ongoing projects in Pratscher's lab to isolate so far uncultivated methanotrophs from terrestrial environments (1, 2).

Timeline

See methodology (above) for more details on each key activity.

Year 1:

1. Determining trace gas fluxes and environmental parameters and their spatio-temporal variability [will extend into yr 2].
2. Recording and analyzing patterns in microbial diversity.

Year 2:

3. Stable isotope probing (SIP) to identify the active key players in the sites.

A. Sequencing data analysis of 2. and 3.

Year 3/3.5.

4. Isolation of microbial key players.

A. Sequencing data analysis of 3.

B. Thesis and paper writing.

Training & Skills

The student will receive and have access to the full variety of the extensive IAPETUS2-cohort training, including workshops and cohort meetings. This will enable the PhD student to develop broader transferable skills and knowledge.

In addition to the IAPETUS2-cohort training, the student will be trained in a range of scientific methods: Molecular microbial ecology (next generation sequencing, metagenomics, bioinformatics), biogeochemistry, microbial physiology (e.g. cultivation, SIP), microcosm design, data analysis and analytical GC assays. All methods proposed are established in the applicants' laboratories.

The student will join a vibrant laboratory working on a variety of aspects of environmental microbiology. They will use a range of innovative methodology and cutting edge techniques in biogeochemistry, microbiology and molecular ecology. The project will therefore provide excellent multidisciplinary training and an exciting research opportunity to interact between the fields of molecular microbial ecology, biogeochemistry and microbial physiology.

We anticipate that the PhD student will present their results in at least two meetings of the UK Microbial Molecular Ecology Group (MMEG) (e.g. 2020 and 2021) and at least one international symposium (e.g. ISME or Gordon Research Conference (GRC) on either "Applied and Environmental Microbiology (AEM)" or "Molecular Basis of Microbial One-Carbon Metabolism (C1)" in 2021 or 2022). The student will also have opportunities to network with project partners at Heriot-Watt and Stirling and to become a

member of the broader scientific community working on methane cycling in terrestrial environments.

References & Further Reading

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