Project Title	Electrical monitoring and control of environmental microbes
University (where student will register)	University of Warwick
Which institution will the student be based at?	As above
Theme (Max. 2 selections)	Climate & Environmental Sustainability ☐ Organisms & Ecosystems ☒ Dynamic Earth ☐
Key words	Environmental Microbiology, Engineering Biology, Bioelectricity
Please explain how the project fits within the NERC remit	The project fits within the NERC remit of "All aspects of environmental influence on, and effects of, microbial systems; bioremediation; microbial diversity." We will develop a novel approach for monitoring and enhancing microbial diversity and their biological activities.
Supervisory team (including institution & email address	**PI:** **Dr Munehiro Asally (Warwick)** m.asally@warwick.ac.uk **Co-I:** **Prof Gary Bending (Warwick)** Gary.Bending@warwick.ac.uk **Dr Magdalena Karlikowska (Cytecom Ltd)** m.karlikowska@cytecom.co.uk

Project Highlights:

- The project will use a novel electrophysiological cell-vitality detection technique to monitor biological activities of soil microbes
- Using the electrophysiological technique, the project will monitor the biological activity of soil from different locations
- The project will characterise the growth polarisation effect of electric field polarises on the growth of mycelial fungi

Overview (including 1 high quality image or figure):

Microbes in soil are a crucial part of our environment. For example, the mycelial networks formed by soil fungi in forests have been shown to promote plant diversity.

Intriguingly, recent studies show that mycelial networks can facilitate electrical communication between plants. Studies have also shown that applied electric fields can polarise the growth of mycelial fungi. These findings suggest that electricity may be an important part of soil ecology and a new route for environmental control. Bioelectrical paradigm could be complimentary to DNA-based understanding and technologies as it provides a framework for measuring and controlling the physiological activities of microbes.

The PI's group recently developed a novel electrophysiological approach for measuring cell vitality and detecting physiologically active microbial cells (Figure 1). We found that electric stimulation can induce cell-physiology-dependent response (hyperpolarisation in proliferative cells, and depolarisation in inhibited cells), and this resulted in the creation of the spinout company Cytecom. This technology could be used for monitoring physiological activities of microbes.

This project will use the electrophysiological technique for monitoring vitality of soil microbes. The project will have three objectives. Objectives 1 and 2 are aimed at establishing a bioelectrical measurement of microbial physiology and objective 3 is aimed towards establishing a bioelectrical approach to control the growth of microbes.

The first objective is to establish the electrophysiological assay with isolated bacterial and mycelia strains. Membrane potential and their dynamics upon electric stimulation will be recorded using fluorescence microscopy. The second objective is to perform electrophysiology assays with complex environmental soil samples to estimate the biological activities. The results will be compared to other established methods for measuring cell viability and vitality, namely colony forming assay, ATP assay and flow cytometry assay. The third objective is to use electrical stimulation for controlling the polarity of the growth of microbes. Cells will be grown in the presence and absence of externally applied electric fields and the polarity of the growth with respect to the angle of the electric field will be measured.

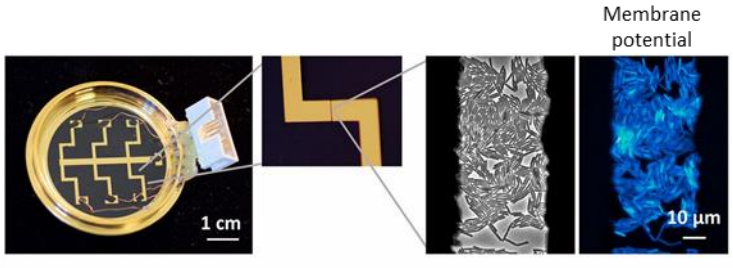

Figure 1. A) electrode dish for microbial electrophysiology developed in the PI's group. B) Membrane potential of cells are measured by fluorescence microscopy.

Methodology:

Membrane potential dynamics will be measured by fluorescence time-lapse microscopy. The fluorescence intensities will be quantified for each cell using a deep-learning image segmentation tool. The change in membrane potential will be estimated in millivolt using the established equation based approaches (Ehrenberg *et al.*, 1988). Microbial cells will be treated with chemical stresses using inhibitors, and environmental stresses such as change in temperature and water availability, and cell vitality and viability will be measured using the electrophysiological assay (Cytecom), Flow cytometry, ATP assay and colony forming unit assay. For objective 3, the growth of mycelial cells in externally applied electric fields will be imaged by microscopy using the gold-electrode dish (Figure 1). Growth of mycelia in soil and on agar will be determined following application of electric fields and growth responses imaged using a time-lapse assay.

Training and skills:

- Electrical stimulation assay (microbial electrophysiology assay)
- Time-lapse fluorescence microscopy
- Macroscopic imaging
- Quantitative image analysis
- Flow Cytometry
- ATP enzymatic assays
- Plate assays

Partners and collaboration (including CASE):

Name of L1/L2 Partner (where applicable)	
Name of CASE partner (where applicable – project proposal **must** be accompanied by a letter of support from the CASE partner)	Cytecom Ltd.

Further information on partners and collaboration (including CASE):

Please see the supporting letter. In summary, Cytecom will make the following contributions.

- £3,500 (£1,000 per year) supplement to the Research and Training Support Grant for the duration of the studentship
- £1,000 to cover expenses associated with the 3-month industrial placement
- In-kind contributions:
  - The CyteCount machine (RRP £25,000) will be provided free of charge for the duration of the project.
  - 10% discount on the CyteCount consumables for the duration of the project
  - 10% discount on the CytePulse consumables for the duration of the project
  - Product development and data analysis support from Cytecom's Team with a total value of £30,000

Possible timeline:

Year 1: Electrical stimulation assays with isolated strains. Detailed characterisation of membrane potential dynamics of isolated and environmental microbes.

Year2: Detailed comparison of cell vitality and viability assays (electrophysiology, flow cytometry, ATP, plate assay)

Year3: Characterisation of the effects of external electric field on growth polarisation

Further reading:

(Gruler and Gow, 1990; Gow, 2009; Chang and Minc, 2014; Stratford *et al.*, 2019; Benarroch and Asally, 2020; Adee, 2023)

Journal articles:

Benarroch, J. M. J. M. and Asally, M. (2020) 'The Microbiologist's Guide to Membrane Potential Dynamics', *Trends in Microbiology*, 28(4), pp. 304–314. doi: 10.1016/j.tim.2019.12.008.

Chang, F. and Minc, N. (2014) 'Electrochemical Control of Cell and Tissue Polarity', *Annual Review of Cell and Developmental Biology*, 30(1), pp. 317–336. doi: 10.1146/annurev-cellbio-100913-013357.

Ehrenberg, B. *et al.* (1988) 'Membrane potential can be determined in individual cells from the nernstian distribution of cationic dyes', *Biophysical Journal*, 53(5), pp. 785–794. doi: 10.1016/S0006-3495(88)83158-8.

Gow, N. A. R. (2009) 'Transhyphal Electrical Currents in Fungi', *Microbiology*. doi: 10.1099/00221287-

130-12-3313.

Gruler, H. and Gow, N. A. R. (1990) 'Directed Growth of Fungal Hyphae in an Electric Field', *Zeitschrift für Naturforschung C*, 45(3–4), pp. 306–314. doi: 10.1515/znc-1990-3-427.

Stratford, J. P. *et al.* (2019) 'Electrically induced bacterial membrane-potential dynamics correspond to cellular proliferation capacity.', *PNAS*, 116(19), pp. 9552–9557. doi: 10.1073/pnas.1901788116.

Book:

Adee, S. (2023) *We are electric: The new science of our body's electrome*. Canongate Books.

Further details:

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