### PROGRESS REPORT ON PROJECT SUPPORTED BY A PADDY PALLIN FOUNDATION SCIENCE GRANT

#### **Report Type**

12 Month

Date submitted 25/7/2017

#### **1. PROJECT IDENTIFICATION**

#### 1.1 Project title

"The untold story of underground communities: fungi and soil seed-banks in Mountain Ash forests"

#### **1.2 Administering Organization**

The Australian National University

#### 1.3 Project Leader

Name: Elle Bowd Email: <u>elle.bowd@anu.edu.au</u> Work Phone: (02) 61251097 Mobile Phone: 0422802195

#### 2. PROJECT DESCRIPTION AND OBJECTIVES

#### 2.1. 100 word Project Summary

Fungi and soil seed-banks have key functions in ecosystems, particularly following disturbance. Despite this, we know little to nothing about them. Mountain Ash forests in Victoria offer a unique opportunity to examine these communities and the influence of disturbance. My research takes advantage of next generation DNA sequencing to generate baseline data on the disturbance thresholds and successional trajectories of fungi and soil seed-banks. Contributing to 34 years of long-term monitoring in these forests, this research will provide important new insights into forest management, particularly with respect to the conservation of fungi and soil seed-banks.

#### 2.2 Summary of original objective of the project

The objective of this project is to fill knowledge gaps about fungi and soil seed-banks by addressing two important questions:

**1)** What is the effect of time since fire on soil seed-banks and fungi along a chronosequence spanning 150 years in Mountain Ash forests?

**2)** How does the composition of fungal communities and soil seed-banks compare post-fire to areas subject to clear-fell logging in similarly aged Mountain Ash forests?

Through this research we aspire to produce detailed management recommendations for the conservation of fungal and floristic communities. This will highlight species or groups at high risk of local extinction. Furthermore, we aim to set a precedent for the inclusion of fungi and soil seed-banks in long term monitoring sites across Australia, and indeed worldwide.

#### **3. PROJECT OVER DURATION OF FOUNDATION GRANT**

#### **3.1** Have there been changes to the project?

This could include changes to the research Project resulting from funding being at a lower level than requested. By indicating changes to the budget, aims and research plan in the Report, you are requesting approval from the Research Grants Committee for a revision of the Project. A 'satisfactory' assessment of the Report and the Project by the Research Committee means that the revision has been approved.

The only change that has been made to the project is the reduction in study sites from 90 to 81. This was only because some disturbance types were difficult to find or access. For example, during fieldwork some 'undisturbed' and 'three fire' site types were not accessible due to road closures and treacherous conditions, and therefore I could only sample four and six replicates respectively.

#### 3.2 What were your research plans and objectives for the period covered by this report?

During the year I have been supported by the Paddy Pallin foundation (September 2016 - 2017) I had planned to complete my first field season (fungi) across nine disturbance types in the Victorian Central Highlands. For each site, I planned to sample nine soil cores and record the projective cover of each vascular plant species. Following fieldwork I aimed to have soil moisture content analysis, DNA/RNA laboratory work and DNA sequencing completed by September 2017.

## **3.3** Did the research project proceed as planned? What have you achieved over this period? Outline the research findings to date.

In November 2016 – February 2017 I underwent my 'fungi' field season in the Victorian Central Highlands (See Fig 1). My field sites spanned across Toolangi State Forest, Yarra State Forest and the Yarra Ranges National park. In total, I measured 81, 25 x 25 m sites and took 729 soil cores (nine from each site). Soil was stratified based on depth (0-10cm and 20-30cm) and pooled respectively. For each depth, three 50ml falcon tubes full of soil were taken for fungal DNA and soil moisture content analysis. An additional sample was taken from the top 10cm of the soil and mixed in LifeGuard soil preservation solution for fungal RNA analysis. All samples were kept on ice and transported to a -20°C freezer at the end of each day. This allowed the DNA and RNA to remain stable and not degrade. Remaining soil of each depth was weighed, air-dried and bagged for later chemical analyses. In each site I also recorded the projective cover of 121 plant species.

On my return to the Australian National University, I sent off my fungal DNA samples for extraction and completed soil moisture content analysis of all 162 samples. Following this I started my RNA extractions, and hope to have all lab work completed by September 2017.

I requested the funding from the Paddy Pallin foundation to assist me with purchasing laboratory equipment and DNA sequencing. Already I have used to funds to purchase RNA extraction kits and the Lifeguard preservation solution (for RNA preservation). These funds have been fundamental in allowing me complete my first field season and commence laboratory work. Remaining funds will be used to pay for DNA sequencing.

As I am yet to complete my laboratory work I am yet to have any research finding to report. However, I hope to begin writing my first paper at the end of this year (2017).



Fig 1. 'Fungi' fieldwork season.

## **3.4 Have you experienced any difficulties that have affected the progress of the research project?** (yes/no)

No.

**3.5 What are your research plans and objectives, including publication plans, for the coming year?** In the next year I aim to complete my second field season (soil seed-banks) and germination experiments. Soil seed-banks will be collected from the same 81 sites sampled for fungi in 2016. Soil samples will be pooled, homogenised and mixed thoroughly before two large subsamples are taken and stored at between 15-20°C in zip lock bags during transport to the greenhouse. In the greenhouse, soil will be air dried, and the two subsamples per site will be evenly spread on a tray of 2cm of sterilised sand and randomly allocated to be treated by heat (80°C in an oven) for 1 hour 15 minutes, smoke water, or no treatment (control) to stimulate germination in greenhouse conditions [32]. Trays will be examined weekly until germinants are able to be identified and recorded.

Furthermore in 2017, I will be working on disseminating my results about fungal communities in high ranking, international journals. I aim to have at least four papers published at the end of my PhD in 2019.

#### 4. ACADEMIC OUTPUTS

**4.1 Publications and other academic outputs directly related to this project. (Please list all publications and those manuscripts accepted for publication, for the period covered by this report)** None as yet.

4.2 Evidence of scholarly impact and contribution. Is there evidence that this research project is having/has had and impact in the research field or the broader public domain?

If yes, give details (For instance, standard citation data on articles published in ISI journals, citations to books, republication, other invitations, newspaper/media/expert commentary).

None as yet.

# 4.3 End-user interaction and other project outcomes If there are examples of the impact of this research project not covered in item 4.2 above please provide details. For example, introduction or modification of standards/protocols, preparation of proposals for funding from other agencies as a result of outcomes from this project.

No additional information. Please see 4.2

#### 5. ATTACHMENTS & OTHER MATERIAL

Please provide as separate files, any figures, graphs, images and other material that cannot be included in this form. Please also provide updated material (text and images) that can be used to revise your project summary on the Foundation's web site. Please provide text in Microsoft Word format and images in JPEG format with a minimum size of 600 x 400 pixels. If this is the final project report, the web page summary must be updated to reflect the outcomes of the project. Is any material being forwarded as additional attachments? No additional material has been attached, as this is a progress report and the project summary has not changed from the original application. No additional material has been attached as there are no results to report as yet and the project summary or plan has not changed from the original application.