Long-lasting modification of soil fungal diversity associated with the introduction of rabbits to a remote sub-Antarctic archipelago

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During the late nineteenth century, Europeans introduced rabbits to many of the sub-Antarctic islands, environments that prior to this had been devoid of mammalian herbivores. The impacts of rabbits on indigenous ecosystems are well studied; notably, they cause dramatic changes in plant communities and promote soil erosion. However, the responses of fungal communities to such biotic disturbances remain unexplored. We used metabarcoding of soil extracellular DNA to assess the diversity of plant and fungal communities at sites on the sub-Antarctic Kerguelen Islands with contrasting histories of disturbance by rabbits. Our results suggest that on these islands, the simplification of plant communities and increased erosion resulting from the introduction of rabbits have driven compositional changes, including diversity reductions, in indigenous soil fungal communities. Moreover, there is no indication of recovery at sites from which rabbits were removed 20 years ago. These results imply that introduced herbivores have long-lasting and multifaceted effects on fungal biodiversity as well as highlight the low resiliency of sub-Antarctic ecosystems.

1. Introduction

The distribution of organismal diversity in soils is linked to abiotic conditions and the nature of the aboveground plant community [1,2]. Mammalian herbivores can therefore affect soil communities via direct physical disturbance or indirectly via effects on plant cover and soil properties that induce changes in habitat and resource availability [2]. The direction and magnitude of such effects may differ greatly depending on ecosystem type and disturbance intensity [2,3].

The sub-Antarctic islands are extremely isolated. Their ecosystems are typically species-poor and characterized by an absence of indigenous mammalian herbivores [4]. Following their discovery in 1772, the Kerguelen Islands were subject to numerous alien plant and animal introductions that have resulted in substantial changes to ecosystem structure and functioning [4]. The most notable of these introductions is that of rabbits (Oryctolagus cuniculus) in 1874. They now occur at high densities both on the main island, Grande Terre, and several of the smaller ones [5]. Where rabbits occur, their grazing and burrowing activities have significantly affected the native vegetation, resulting in the decreased abundance of certain native species (e.g. Azorella selago, Pringlea antiscorbutica) and the over-dominance of others (e.g. Acaena magellanica). Moreover, rabbit-induced increases in erosion and changes to soil characteristics have tended to favour introduced plant species [5].
While the effects of alien herbivores on Kerguelen’s native vegetation are well described [5], their impacts on soil communities—in particular soil fungi—are poorly known. Fungal communities play a central role in terrestrial ecosystem functioning (e.g. in nutrient cycling) [6]. It has been assumed that the sub-Antarctic islands host limited fungal diversity and that the relative simplicity of these communities may make them particularly sensitive to disturbance [7]. However, the response of sub-Antarctic fungal communities to biotic disturbance-induced changes to plant communities has not been explored.

We hypothesized that (i) rabbit-induced changes to the structure of aboveground plant communities and to soil characteristics has altered the diversity and composition of soil fungal communities and (ii) such changes are long lasting. To test these hypotheses, we studied communities from sites on the Kerguelen Islands with different rabbit disturbance histories. We used soil metabarcoding to evaluate the composition of both fungal and plant communities [8]. These data were used to assess the relative importance of biotic and abiotic factors in shaping soil fungal assemblages.

2. Material and methods

Study sites on the Kerguelen Islands were located on the Isthme Bas (Grande Terre) where rabbits occur at high density, Ile Guillou where a successful eradication programme was undertaken in 1994 [5] and Ile Australia that has remained rabbit free (figure 1). In each area, we established five or six 10 m plots within which we collected soil samples—two for DNA metabarcoding and a third for soil physico-chemical analyses (e.g. organic matter content, acidity; electronic supplementary material, table S1)—and conducted vegetation surveys (e.g. plant assemblage and cover; figure 1c).

Using a previously described method, one or two extracellular DNA extractions were performed per soil sample [8]. Standard methods were used to PCR amplify metabarcodes for vascular plants (P6-loop of the chloroplast trn intron [9]) and fungi (nuclear ribosomal ITS1 [10]) from each extraction. In each case, a pair of PCR replicates along with extraction and PCR controls were conducted. Plant and fungal amplicons were paired-end sequenced on Illumina HiSeq (2 × 100 bp) and MiSeq (2 × 250 bp) platforms, respectively. Sequences were then filtered and clustered using an established workflow. Taxonomic assignments were made by comparison to reference sets derived by in silico PCR from public databases (EMBL, UNITE) as well as an exhaustive database for Kerguelen Island vascular plants.

We used non-metric multidimensional scaling (NMDS) to ordinate fungal and plant communities. Vector fitting and multivariate analysis of variance (PERMANOVA [11]) were used to examine the effects of soil conditions and, in the case of fungal communities, biotic parameters (i.e. plant assemblages characteristics) on community composition. Ordinations for different datasets were compared using Procrustes analyses. All statistical analyses were conducted in R using the vegan package [12,13].

Full methodological details are given in the electronic supplementary material.

3. Results

Sequencing of plant metabarcodes generated 1 328 674 reads, which after filtering resulted in 25 unique sequence types. Ordinations of plant communities inferred from metabarcoding and botanical surveys were highly similar (table 1; electronic supplementary material, figures S5 and S6). Moreover, there was a strong linear correlation between the relative abundances of plant species in both datasets (p < 0.001; electronic supplementary material, figure S4). This suggests that on the Kerguelen Islands, current plant cover is well represented by soil DNA. Our results imply two distinct situations. Undisturbed plots on Ile Australia that are characterized by species-rich, native-dominated plant...
communities and disturbed—either naturally on Ile Australia or as a result of rabbits on Isthme Bas and Ile Guillou—plots with species-poor communities dominated by *Acaena* or *introduced* *Taraxacum* spp. (figure 1c; electronic supplementary material, figures S2 and S5). Vector fitting indicates a correlation between plant community composition and organic matter content ($p, 0.05$; electronic supplementary material, figure S6).

For fungi, 8 602 068 reads were obtained; a total of 547 molecular operational taxonomic units (MOTUs) were identified with per plot diversity varying markedly between sites (electronic supplementary material, table S3). Fungal communities also showed striking compositional differences with axes of discrimination related to (i) the density of plant cover, soil organic matter content and carbon–nitrogen ratio and (ii) plant community diversity, that distinguishes rich and undisturbed native communities (AU05-AU07, AU09) from the others (vector fitting, $p < 0.01$; figure 2a). Analyses using PERMANOVA confirm that each of the parameters identified by vector fitting explains a significant proportion of the variance ($p < 0.05$; electronic supplementary material, Results). Ordinations of fungal and plant communities were more similar to one another than would be expected by chance (table 1; electronic supplementary material, figure S6). This result suggests that the composition and richness of fungal and plant communities are co-structured across plots.

Full details of results are provided in the electronic supplementary material.

### Table 1. Results of Procrustes analyses for pairwise comparisons of ordinations of plant and fungal communities inferred from surveys and metabarcoding. Significant correlations indicate that ordinations were more similar to one another than would be expected by chance. See the electronic supplementary material for details.

<table>
<thead>
<tr>
<th></th>
<th>Procrustes sum of square</th>
<th>correlation in a symmetric Procrustes rotation</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>botanical survey-based plant communities/fungal communities</td>
<td>0.62</td>
<td>0.62</td>
<td>0.004</td>
</tr>
<tr>
<td>DNA-based plant communities/fungal communities</td>
<td>0.49</td>
<td>0.71</td>
<td>0.001</td>
</tr>
<tr>
<td>botanical survey-based plant communities/DNA-based plant communities</td>
<td>0.23</td>
<td>0.98</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Figure 2. Environmental parameters determining the structure of soil fungal communities. (a) Ordination of soil fungal communities with vector fitting of biotic and abiotic variables (NMDS stress = 0.134). Grey and black arrows represent non-significant and significant (at $p < 0.01$) variables, respectively. *Acaena magellanica*, per plot frequency of *A. magellanica*; other natives, per plot frequency of other native plants; introduced species, per plot frequency of introduced plants; OMC, soil organic matter content; C/N, carbon/nitrogen ratio; pH, soil acidity; Shannon index, plant community diversity; plant cover, aboveground plant cover. (b) Per plot fungal MOTU richness relative to soil pH and plant community diversity (Shannon index). Circle size proportional to the number of fungal MOTUs. See the electronic supplementary material for details.
are or were rabbit-affected, respectively (figure 2; electronic supplementary material, figure S7). We found no consistent differences in soil parameters nor have any reason to assume that limited dispersal explains the differences (electronic supplementary material, Results, table S2 and figure S3). Instead, the co-structuring of soil fungal and plant communities (table 1; electronic supplementary material, figure S6) suggests that differences between fungal communities are the result of contrasting rabbit disturbance histories. Similar trends in soil community composition have been observed in other ecosystems when pressure from mammalian grazers was high [2,3]. We hypothesize that simplification of plant communities as a result of rabbit activity reduces the number of soil microhabitats, and thereby soil fungal diversity (figure 2b). The exclusion of the cushion plant A. selago from areas affected by rabbits may be particularly significant in terms of explaining differences between soil fungal communities. Specifically, the cushion growth form has been shown to modify local abiotic conditions and promote soil microhabitat diversity [14]. This hypothesis is also consistent with our results for individual plots on the Isthme Bas where more diverse fungal communities were found under mixed species cover than mono-specific Acaena cover (figure 2b; electronic supplementary material, figures S2 and S7).

Although there are broad differences in fungal communities between study sites, similarities between individual plots suggest additional processes are at work. At two naturally disturbed plots on Ile Australia (AU08 and AU10), soil fungal communities are more similar to those on Isthme Bas and Ile Guillou (figure 2; electronic supplementary material, figure S7). In this case, we suggest that disturbance-induced erosion has promoted a loss of soil organic matter, and thereby lowered soil fungal diversity. If so then rabbits may also be mediating differences in soil fungal diversity via a direct impact on resource availability [15]. Burrowing activities would promote increased soil erosion; consistent with this, the plot with the lowest soil fungal richness is also substantially eroded (IB16; electronic supplementary material, figure S7). We also identified relationships between soil parameters and soil fungal community diversity with no potential link to rabbit-induced disturbance history. Specifically, plots with waterlogged, highly acidic soils are associated with lower soil fungal diversity on both Ile Australia (AU07) and Ile Guillou (GU15) (figure 2b; electronic supplementary material, figure S7). In this case, our results imply that soil acidity limits fungal richness directly.

Rabbits were eradicated from Ile Guillou 20 years ago [5] but our analyses indicate that soil fungal communities on this island are yet to recover (figure 2r; electronic supplementary material, figure S7). Indeed, both plant and fungal communities on Ile Guillou are more similar to the rabbit-disturbed communities on Isthme Bas than they are to the undisturbed ones on Ile Australia (figures 1c and 2r; electronic supplementary material, figures S5–S7). We suggest that the current status of fungal communities on Ile Guillou reflects slow recovery of the native vegetation following rabbit eradication. That the vegetation on this island has not yet begun to show signs of recovery has been linked to (i) slow growth rates in dominant species (e.g. Azorella) and (ii) changes in abiotic conditions (e.g. soil, climate) that may have induced a stable state more favourable to introduced species (e.g. Taraxacum sp.) [4,5].

The introduction of rabbits to Kerguelen has led to changes in plant communities and soil characteristics that have modified the availability of both microhabitats and resources to soil fungi. Our study illustrates the multifaceted and long-lasting effects of introduced herbivores on fungal diversity as well as the overall sensitivity of sub-Antarctic ecosystems to such introductions. Rabbit-induced changes to soil fungal communities may have had flow-on effects in terms of lost functionality. The functional characterization of these communities will provide new insights into the resistance and resilience of threatened sub-Antarctic ecosystems.

Data accessibility. Filtered DNA sequences and soil physico-chemical data are provided in the electronic supplementary material. Unfiltered DNA sequences, as well as vegetation survey data, are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.h534.

Authors’ contributions. P.C. and P.T. designed the study; P.C., R.C.W. and F.H. performed sampling; J.P. and L.G. performed DNA and soil analyses. J.P. processed and analysed the data; J.P., R.C.W. and P.C. interpreted the results. J.P. drafted the manuscript with contributions from the other authors. All the authors approved the final version.

Competing interests. L.G. and P.T. hold patents related to use of the P6 loop of the chloroplast trn (UAA) intron for plant identification from degraded template DNA as well as the primers used to amplify this region. These patents restrict commercial applications and have no impact on the use of this locus by academic researchers.

Funding. We thank the Institut Polaire Paul Emile Victor (IPV) Programme 136 (led by M. Lebourvier) for financial support and fieldwork coordination.

Acknowledgements. We thank Marine Pouvreau, Marion Lombard, Julie Végrin and Françoise Cardou for field assistance; Nigel Gilles Yoccoz for providing the Kerguelen plant DNA database; Cindy Arnoldi and Jérôme Poulenard for help with soil analyses.

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