The effects of restoration on bacterial community structure in a montane blanket bog

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Summary

This study focuses on bacterial community structure in restored and intact montane blanket bogs. Molecular community analysis approaches were employed to profile microbial community structures. Preliminary results showed that season predominantly influenced bacterial community structure. Multi-dimensional scaling (MDS) plots revealed that bacterial communities from an intact site appeared to have greater homology with each other, as these communities clustered more tightly together, when compared with those from the restored site. This may suggest less homology between bacterial communities in the restored site, possibly due to previous anthropogenic activities such as draining and cutting.

Key index words: restored, intact, T-RFLP, Ribotype

Introduction

Peatlands are located globally at all latitudes and altitudes, with large areas found in northern Europe. In Ireland both high rainfall (between 800 and 2,800 mm yr^{-1}) and poor drainage in its central lowlands have contributed to its prevalence of peatland (Avery, 1990; IPCC, 2008). Nevertheless, the introduction of large scale, mechanised turf extraction schemes and afforestation programmes in the 1940s and 1950s has led to a 92% loss of raised bogs and an 82% loss of blanket bogs in Ireland (IPCC, 2008.). Peatlands are responsible for approximately 40-60% of global methane emissions of which ~74% are derived from microbial activity (Upton *et al.,* 2000; Galand, 2004; Archer, 2005). Methane is considered to be 20 times more effective than CO_2 as a greenhouse gas, and as global atmospheric concentrations are increasing by approximately 1% yr^{-1}, great attention has focused upon peatlands (Hales *et al.,* 1996; Mc Donnald *et al.,* 1999; Upton *et al.,* 2000).

In recent times, knowledge of microbial communities in peatlands has greatly increased with particular focus on microbes associated with methane formation and consumption (methanogenic archaea and methanotrophic bacteria respectively). While it is particularly important to understand the role these groups have in peatlands, it is equally important to examine the general microbial populations present in these niche-specific ecosystems. Numerous studies have identified many heterotrophic bacteria as abundant in peat, including members of the genera *Bacillus*, *Pseudomonas*, *Achromobactor* and *Arthrobacter* (Gilbert and Mitchell, 2006).

This research is part of the 'Bogland project', funded by the Environmental Protection Agency (EPA), with a view to developing a sustainable protocol for peatland management in Ireland. 'Bogland' comprises a number of sub-projects which examine different aspects of four peatland types in Ireland, including their social, economic and environmental importance. This study focuses on how microbial community structures vary across different peatland types, in conjunction with their response to anthropogenic disturbance. This paper discusses preliminary data relating to the general bacterial community structure found in a montane blanket bog, comparing restored and intact sites. Culture-independent molecular community analysis approaches have been used to profile microbial community structures.

Methodology

Samples were collected from a montane blanket bog in the Slieve Bloom mountain range located between Co. Laois and Co. Offaly, Ireland. This blanket bog contains two sites of interest, a restored site (Glenlahan) and a relatively intact site (The Cut), 2km apart. Both sites are situated > 200m above sea level and are part of a larger blanket bog complex covering a total area of 2230ha. pH of both sites averaged approximately pH 4. Glenlahan was chosen as one of Bogland's core sites as it is a focus of the EU Life programme, set up for the conservation of natural habitats in the 1990s.

Sites were sampled over two seasons; spring and summer 2006. Samples were taken in triplicate, using a 1m auger to examine two depths (0-20cm and 30-50cm). As the bed rock was quite shallow (~50cm), the 30-50 cm sample was not always obtainable.

Samples taken back to the laboratory were sectioned into two depths (0-20cm and where possible 30-50cm). Total DNA extraction was carried out using 10g of peat from each section, after homogenisation, using the Cambio total DNA extraction™ kit. Extracted DNA underwent PCR amplification, targeting the general bacterial region of the 16S rRNA gene via universal primer set F27 and

R1492, prior to terminal restriction fragment length polymorphism (T-RFLP) analysis. PCR amplicon mixtures produced underwent digestion with the restriction endonuclease *Msp*I prior to analysis on a Beckman Coulter CEQ 8000 automated sequencer.

Amplicon profiles generated were initially processed via the statistical package 'RiboSort' (Scallan *et. al.*, 2008). Data sets of ribotypes were treated initially using the Bray-Curtis index as a measure of similarity between ribotypes, using Primer software which generated a number of nonmetric multidimensional scaling (MDS) plots. MDS ordinations are typically interpreted based on the distance between points; where ribotypes appear close together these can be regarded as having similar bacterial community compositions, whereas the communities with the lowest similarity are represented on the plot by points located furthest apart. The MDS plots described here were used to visualise the relationships between bacterial communities (as determined by their TRFLP profiles) across season and site.

Results

The mean ribotype number (Table 1) from each site was determined to examine any differences between bacterial ribotype number over season (spring 2006 and summer 2006) and depth (0-20cm and 30-50cm). Overall, ribotype number in both sites decreased markedly between spring 2006 and summer 2006, at all depths. Nevertheless fluctuations in ribotype number did occur in the intact site between depths 0-20cm and 30-50cm, as mean ribotypes number for Spring 2006 increased from 43 in the 0-20cm depth to 65 in the 30-50cm depth. The opposite occurred in Summer 2006 where mean ribotype number fell from 13 at depth 0-20cm to 5 at depth 30-50cm.

Table 1. Mean ribotype number of bacterial communities in both a restored and an intact mountain blanket bog site over season and depth. (ns = no sample).

Season	Spring 2006		Summer 2006	
Depth	0-20cm	30-50cm	0-20cm	30-50cm
Site				
Glenlahan-Restored	26	ns	11.5	ns
The Cut - Intact	43	65	13	5

MDS plots (Figures 1a and 1b) of the restored and intact sites revealed that season had the greatest affect on bacterial community structure. A distinct separation can be clearly observed between communities from each season. Depth, only measurable in the intact site, appeared to have relatively little impact on communities, yet mean bacterial ribotype number increased in spring 2006 between depths 0-20cm and 30-50cm, in comparison to summer 2006 where ribotype number decreased markedly between the same depths.

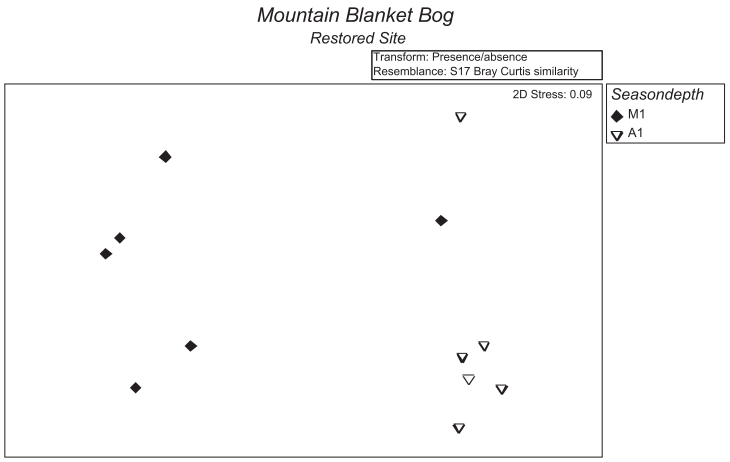

Figure1a. MDS plot of seasonal effects on bacterial community structure for samples taken from Glenlahan (restored site). (M= spring 2006, A=summer 2006, 1=depth 0-20cm).

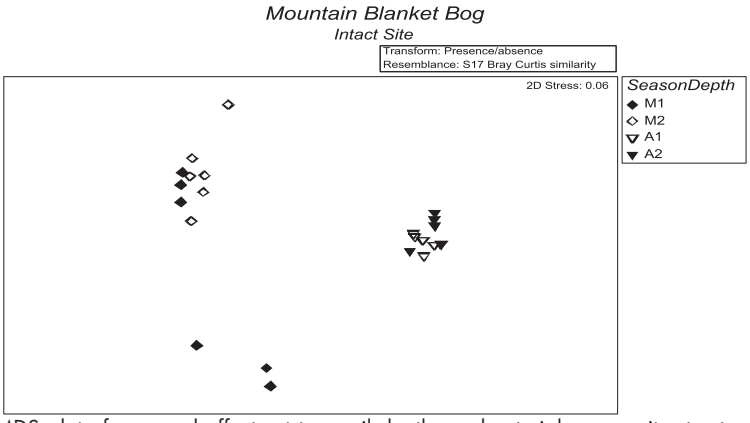

Figure1b. MDS plot of seasonal effects at two soil depths on bacterial community structure for samples taken from The Cut (intact site) (M= spring 2006, A=summer 2006, 1= depth 0-20cm, 2 = depth 30-50cm).

Discussion

Preliminary results show many similarities between bacterial community structure in both the restored and intact sites. Season appeared to be the more influential factor affecting bacterial community structure as seen in mean ribotype number analysis and in both MDS plots. Depth, although having relatively little influence on bacterial communities in the intact site, did affect mean ribotype number. This is possibly due to interactions within each depth combined with seasonal changes in the environment.

Season was the only factor examined in the restored site, as the 30-50cm depth sample was unobtainable during sampling. Again season appeared to have a large impact as ribotype number did decrease across season by over 50%. These changes were possibly due to temperature increases during the summer months, leading to an increase in drought in these predominantly wet environments. This in turn may lead to more favourable conditions for bacterial communities which are usually less dominant in wetter conditions.

As previously mentioned, both MDS plots showed a distinct divide between communities in spring 2006 and summer 2006. Yet, it was interesting to note bacterial communities from the intact site formed tight clusters, for each season, compared with the more scattered appearance of bacterial communities displayed in the MDS plot of the restored site. This possibly indicates greater homology between bacterial communities in the intact site and less homology between communities in the restored site. This variation in homology between the two sites may be the result of previous perturbations that occurred in the restored site due to drainage and harvesting of peat.

These results display preliminary findings on the effects of restoration on bacterial community structure in a montane blanket bog. Further multivariate analysis needs to be carried out to fully analyse effects of environmental factors and anthropogenic activities on montane blanket bog bacterial community structure.

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