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SOIL MICROBIAL DIVERSITY AND SPATIALITY ACROSS PEATLAND VEGETATION MOSAICS UNDERGOING RESTORATION IN THE SOUTHERN PENNINES, UK

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SUMMARY

Impacts of degraded blanket bog restoration on culturable and non-culturable soil bacterial and fungal biodiversity and spatial distribution were assessed. A total of 18 peat cores sampled from unvegetated areas and from 3 restored vegetation classes, remnant vegetation and grass dominated gullies were subjected to culturable microbial enumeration, molecular community profiling and pyrosequencing. Culturable bacterial and fungal counts in peat were two orders of magnitude lower than in peats supporting other vegetation classes. Key bacterial and fungal phyla involved in N and C cycling were identified and respective community structures and geospatial relationships identified were closely linked to above-ground restoration efforts. The study has delivered much needed information on how peatland restoration may impact soil microbial communities, the key drivers of ecosystem services.

KEY WORDS: Blanket bog, Bacteria, Fungi, Pyrosequencing, Restoration

INTRODUCTION

UK peatlands, which cover about 8% of the land area but store 40–50% of the nation's terrestrial carbon, are increasingly coming under threat as a result of historical land management, long-term anthropogenic N and S deposition and climate change (Holden *et al*., 2007). Evidence from data gathered on peat physico-chemistry coupled to primary productivity, hydrology and atmospheric chemistry suggest that what are currently regarded as net sinks risk becoming major sources of carbon (Worrall *et al*., 2009). In this respect, our limited understanding of the identity, distribution and functioning of soil microbial drivers of inter-linked C and N cycling in degrading UK moorland systems (Artz, 2009; Littlewood *et al*., 2010) gives particular cause for concern and additionally remains a fundamental barrier to their restoration and sustainable management.

From a microbiological perspective, plant community, soil temperature, pH and hydrological status are of crucial importance in determining microbial community structure and thus C and N cycling activity in northern peatland ecosystems (Artz, 2009). Decomposition and mineralisation of peat by heterotrophic soil bacteria and fungi is mainly limited to the predominantly oxic surface layers of peatlands, the acrotelm, and periodically the underlying mesotelm (Clymo and Bryant, 2008) during intermittent water table drawdown. The permanently saturated and anoxic catotelm, supports limited microbial decomposer communities but in cases of severe drought will be progressively exposed to increased oxidative decomposition and respiratory flux. Fungi appear to dominate the surface acrotelm

either as litter decomposing saprotrophs or root symbiotic mycorrhizal fungi (Thormann and Rice, 2006; Smith and Read, 2008). Within the mesotelm, fungi, including yeasts, facultative anaerobic bacteria and methanogenic archaea have previously been detected that were able to mobilise recalcitrant carbon sources through oxidative mineralisation or fermentative processes under water saturated conditions. Bacterial communities also show depth- and pH-related stratification but, unlike fungal counterparts, are regarded as being less influenced by plant community structure (Artz, 2009 and references therein). Artz (2009) had highlighted limited but highly promising studies of functional microbial community ecology that have been achieved through rapid developments in molecular DNA/RNA based barcoding techniques.

The Southern Pennines in Northern England hosts one of the most south-westerly extensions of European upland blanket bog, over 70% of which has been classified as degraded. In extreme cases areas are devoid of vegetation cover, exposing unconsolidated bare peat that is often gullied to the gritstone bedrock through severe water erosion (Tallis, 1997; Evans and Lindsay, 2010). Severe ecosystem degradation has, in part, been attributed to long-term historical anthropogenic loading of N, S ozone and metals and heightened susceptibility to climate change drivers (Caporn and Emmett, 2009).

Large-scale moorland restoration efforts in the area have involved lime and fertiliser application allowing lowland nurse grass (inc. *Festuca* and *Agrostis* spp.) cultivation and subsequent application of seed and heather (*Calluna vulgaris* L.) brash or planting to establish dwarf shrub cover and *Sphagnum* re-introduction (Moors for the Future Partnership, 2008). Key restoration goals are stabilisation and re-vegetation of bare peat to increase biodiversity and recover hydrological function and lost carbon sequestration potential.

The main aim in the first phase of this study at Holme Moss was to identify acrotelm and mesotelm bacterial and fungal communities in a restoration derived vegetation mosaic based on culture-dependent and -independent molecular DNA-based methodology. The two specific objectives were 1) vegetation-dependent enumeration of culturable bacteria and DNA-based identification of soil bacterial and fungal community and 2) GIS-based spatial analysis of plant-soil-microbial community interactions in restoration mosaics.

MATERIALS AND METHODS

The Holme moss study site, originally established in 2006 (Caporn *et al*., 2007), is located close to the the northern boundary of the Peak District National Park (53° 31’ 59” N; 01° 51’ 29” W; elevation 524 m a.s.l) in the southern Pennines. Restoration of extensive bare peat areas was initiated in 2008 with sucessful establishment of nurse grass swards and young (2 year-old) heather seedlings on formerly bare peat. Sampling close to a transmitter mast allowed inclusion of peat supporting a 25 year-old heather dominated stand which was established following mast erection in 1985 (P. Anderson, pers. comm.). In June 2010, three parallel line transects (approx. 300 m) were established in a due northerly direction to core (12mm dia. x 150mm) sample surface (acrotelm/mesotelm) bare peat and peat under vegetation mosaics encompassing 3 different stages of restoration, namely, nurse grass, young heather and 25-year heather. Peat supporting remnant dwarf shrub communities and naturally vegetated gullies were also sampled.

Bacterial and fungal colony forming units (cfu g^{-1} soil) were enumerated from homogenised cores (n = 3 per vegetation class per transect) via dilution plating on Tryptone soy agar

medium or Potato dextrose agar medium with antibiotic selection. DNA was extracted from the middle sample of each triplicate set of core samples using a Powersoil DNA extraction kit. Extracted community DNA was used as a template for polymerase chain reaction (PCR) to enable denaturing gradient gel electrophoresis (DGGE) fingerprinting and sequencing of the bacterial and fungal microbial communities from the phylogenetically informative ribosomal RNA genes (16S-rRNA and ITS-rRNA genes, respectively). Amplified DNA was also cloned and randomly sequenced using the conventional Sanger method (Hirsch *et al*., 2010). Extracted DNA was further subjected to high-throughput pyrosequencing (Roche 454), targeting the same ribosomal genes to enable significantly larger numbers of microbes to be identified from each sample (Hirsch *et al.* 2010). DGGE community fingerprints were subjected to phylogenetics coupled to multivariate cluster analyses using appropriate molecular software packages. Microbial counts and 16S- and ITS- rRNA gene richness estimates were spatially interpolated for the study area using the Inverse Distance Weighting (IDW) technique in ArcGIS.

RESULTS AND DISCUSSION

The focus of this study has been on the upper horizons of peat, the acrotelm and mesotelm, supporting plant roots and root associated microbial communities that play a crucial role in primary peatland productivity. Mean counts for both bacteria and fungi were typically within the order of 10^6 cfu g^{-1} soil for all vegetated samples, whereas bare peat supported a mean of 5.6×10^4 and 2.7×10^4 cfu g^{-1} bacteria and fungi, respectively. Significant reduction in culturable bacterial and fungal counts in non-vegetated bare peat confirmed earlier preliminary findings (Caporn, 2007). The well known “rhizosphere” microbial enrichment response was apparent in the raised count data (Cheng and Gershon, 2007). However, rich and complex bacterial and fungal community DGGE banding patterns were observed in all samples. Band ‘richness’ estimations suggested the highest bacterial diversity in young and 25-year heather whereas similar fungal richness estimates were apparent across vegetation and bare peat. Spatial interpolation of bacterial and fungal counts and richness estimates allowed visualisation of site-specific variation. Bacterial richness appeared to be inversely related to fungal richness and lower bacterial and fungal counts were located in bare peat areas compared to other vegetation in the mosaic.

Sanger sequencing of bare peat and original vegetation generated preliminary information on the differential presence of bacterial phyla including *Acidobacteria, Actinobacteria, Verrucomicrobia and Cyanobacteria*. The former genera have been commonly identified in the periodically oxic mesothelm of Siberian peatlands (Artz, 2009). *Acidobacteria* have been identified in surface cores of heavy metal enriched moorlands in the Southern Pennines (Linton *et al*., 2007) that also develop in rhizospheres in mine trailings. *Planctomycytes* and related *Verrucomicrobiales* have been shown to be methanotrophs in the oxic acrotelm. Actinobacteria detected are likely to be involved in litter decomposition along with fungi in the oxic acrotelm. Fungal diversity encompassed three of the five subdivisions, *Zygomycota, Ascomycota and Basidiomycota*. Ascomycete species in bare peat showed close affinity to species in Scottish cutover peat and uncultured ectomycorrhizal *Pezizomycotina* (Artz *et al*., 2007). ITS sequences with high affinities to cellulose and lignin degrading basidiomycete yeasts and metal tolerant *Verticillium* species were identified.

Preliminary analysis of over 100,000 sequences generated via Roche 454 pyrosequencing from the 18 peat cores representing bare peat and the 5 vegetation classes has been extremely informative. Success in sequence assignment to phyla, genera and species levels was much

higher for bacteria than fungi. Of a total of 22 bacterial phyla, Proteobacteria (mean 55%) and Acidobacteria (23%) representation dominated in bare peat and all vegetation classes. The large Proteobacteria phylum contains soil bacteria involved in carbon, nitrogen and sulphur cycling (Kesters *et al*., 2006) whilst the latter are common in acidic soils including peatlands (Artz, 2009 and references therein). Diversity estimates highlighted a trend to increasing richness from bare eroded peat, through early restoration, to remnant dwarf shrub and gully classes. Identified fungal sequences highlighted the predominance of Ascomycota that accounted for between 57-87% of ITS sequences. This confirms and extends literature in peatlands (Artz, 2009), for example, the identification of the basal class *Archaeorhizomyces* that have been recently identified associated with the coniferous roots in boreal Eurasian and North American forests (Rosling *et al*., 2011). Basidiomycetes were the second most common fungal sub-division (8.5-27.6%) with a high predominance in peat supporting gully vegetation. *Neocallimastigomycota*, a novel phylum containing anaerobic cellulose and lignin degrading ruminant fungi (Griffith *et al*., 2010), were represented in all peat samples but predominated in peat supporting remnant dwarf shrubs. Limited numbers of sequences of symbiotic arbuscular mycorrhizal fungal phylum *Glomeromycota* were restricted to gully peat that hosted naturally regenerated acid grass species known form symbiotic arbuscular mycorrhizas (Smith and Read, 2008).

Multivariate analyses of randomly subsampled sequences assigned to homology-based operational taxonomic units (OTU) through genetic distance estimates, clearly highlighted vegetation class-specific selection on both bacterial and fungal communities. PCA ordination confirmed shifts in bacterial and fungal communities with a clear separation of bare peat and peat supporting recent re-establishing grass and heather cover from well establised 25-year restored heather, gully and remnant dwarf shrub vegetation. Further analyses of functional fungal and bacterial grouping will be presented at the congress.

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