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Short communication

Substrate utilisation profiles of microbial communities in peat are depth dependent and correlate with whole soil FTIR profiles

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Abstract

A multiple substrate induced respiration (SIR) assay, using ^{14}C-labelled carbon sources, was used to evaluate community level physiological profiles (CLPP) of the microbial community in peat horizons of differing degrees of humification. The separation and grouping of the peat horizons by CLPP was similar to the pattern produced by analysis of the organic carbon chemistry of the peat horizons by Fourier Transform Infrared (FTIR) spectroscopy and therefore reflected the level of decomposition. Partial redundancy analysis showed that a large proportion (68.7%) of the variability in the CLPP data could be attributed to the ratio of polysaccharide to 'carboxylate' FTIR bands alone. The multiple substrate SIR technique may, therefore, be a powerful technique to further elucidate the influence of the microbial constituent of peat on the potential activity and patterns of cycling of labile carbon in peatlands.

Keywords: Functional microbial diversity; Substrate induced respiration; Community level physiological profiling; Peat; Whole soil FTIR

Peat is of global importance as one of the major terrestrial carbon sinks, with the estimated amount of carbon held in Northern peatlands amounting to ca. 30% of global C stocks (Gorham, 1991). With the exception of the pathways of methane formation and release (Segers, 1998), the microbially mediated routes and rates of carbon cycling through peatlands have received relatively little attention despite a growing number of reports of increasing losses of CO_2 leading to annual carbon balances showing a net loss of carbon (Vasander et al., 2003). Similarly, increasing losses of dissolved organic carbon (DOC) have been observed from many Northern peatlands over the past decade (Freeman et al., 2001). Many of these increases have been correlated with anthropogenic changes, either directly, in a change of use of peatlands leading to degradation of the hydrological status or through actual peat cutting (Tuittila et al., 1999), or indirectly, by climate change effects such as increased frequencies of drought phases (Freeman et al., 2004). Heterotrophic soil micro-organisms play a fundamental role in the release of CO_2 and DOC and differences in peat quality in terms of C availability are likely to influence microbial community structure and function. Dissolved organic matter (DOM) from degraded peatlands has been shown to be more humified than DOM from intact peatlands and the amounts to be inversely correlated with the total rates of CO_2 efflux from these peatlands, suggesting preferential respiration of labile carbon compounds (Glatzel et al., 2003). Other studies suggest that between 50% and70% of net soil respiration in peatlands is driven by the turnover of recent photosynthates (Komulainen et al., 1999). A multiple substrate SIR assay performed on whole soil, which has been effectively used to 'fingerprint' the respiratory response of soil micro-organisms to substrate additions and therefore the community level physiological profile (CLPP) of soil microbial communities, was first developed by Degens and Harris (1997) and recently miniaturised as MicroResp™ by Campbell et al. (2003). Multiple substrate SIR techniques, which typically use additions of chemically simple carbon compounds, are likely to target primarily the more copiotrophic, *r*-strategist, microorganisms within the soil community. If respiration in peatlands is primarily driven by labile carbon, it should therefore be feasible to distinguish CLPP in different peat horizons that

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reflect the decomposition status of the peat organic matter. Fourier Transform Infrared Spectroscopy (FTIR) distinguishes the composition of the principal chemical classes that soil organic matter and soil minerals are composed of, and has been successfully used on whole soils to describe the status of decomposition in different horizons (Chapman et al., 2001; Haberhauer and Gerzabek, 1999; Haberhauer et al., 1998), for example, through following the reduction of the carbohydrate markers with depth in organic soils. FTIR data can be used as a quantitative indicator of the composition of the soil organic matter using multivariate statistics to distinguish soil horizons (Haberhauer et al., 2000).

Replicate peat cores ($n = 3$, 30 cm depth) were taken in late autumn at an experimental plot at Middlemuir Moss in the North East of Scotland, UK. This site represents a cut-over raised peatland where peat extraction (both manual and block-cutting) was abandoned in several stages between ca. 1950 (the plot used in this study) and 1995. Extensive plant recolonisation at this plot resulted in ca. 10 cm of newly formed peat on top of a residual peat reservoir of >2.5 m depth. The vegetation is typical of degraded raised mires, dominated by *Eriophorum vaginatum*, *Calluna vulgaris, E. angustifolium*, *Sphagnum papillosum* and *S. capillifolium*. The mean annual water table is ca. −10 cm. Cores were cut into 4 different horizons based on their apparent level of humification on the Von Post (1922) scale. The horizons were designated as the moss (M) layer (humification index of H1, 0–3 cm), the decaying moss (D) layer (H3; 3–6 cm), newly formed (N) peat (H6; 6–9 cm) and old catotelm (O) peat below the cut horizon (H8; 25–28 cm). Differences in the level of decomposition were confirmed by FTIR analysis using a Nicolet Magna-IR 550 FTIR spectrometer (Nicolet Instruments Limited, Warwick, UK) over the wavenumber range 4000–350 cm^{-1} of zirconium ball-milled freeze-dried samples. FTIR data were normalised by subtraction of the minimum value and subsequent division by the average over the spectral range prior to statistical analyses. For MicroResp™ analyses, peat samples were cut to approximately 5 mm^3 and homogenised by manual mixing. The MicroResp™ assembly was prepared as described by Campbell et al. (2003) except that samples were weighed to 0.30 ± 0.01 g $well^{-1}$. The assay was performed with 15 ^{14}C-labelled carbon sources at 200 Bq $well^{-1}$ in a carrier solution of unlabelled parent compound (Table 1) and no addition control. Final concentrations of substrates were at the theoretical maximum amount that can be completely oxidised considering the total oxygen per well available (Table 1). Detection of evolved $^{14}CO_2$ was by liquid scintillation counting as per Campbell et al. (2003) except that the detection plate contained rolled filter papers moistened with 40 μl of 2 M NaOH. Several incubation times (4, 20, 48, 120 h) were tested to establish the optimum incubation length for the statistical separation of CLPP for the peat horizons. The patterns of $^{14}CO_2$ generation for all time points were analysed by principal components analysis (PCA) and subsequent canonical variate analysis (CVA) of the PC scores. The PCA step was used to reduce the number of variables for CVA so that the sample number exceeded the number of variates for CVA and the minimum number of PC scores needed to explain more than 95% of the total data variability were used for CVA. Significance of CVA groupings was established by permutation analysis (1000 repetitions of Monte Carlo simulations, Genstat 7th edition).

Table 1
Carbon sources used in the CLPP test and probabilities that utilisation of a particular carbon source is unaffected by moisture content manipulations of the peat horizon samples between 80% and 95% GMC

Carbon source	Non-radioactive addition in mg-C g^{-1} soil (wet weight)	Peat horizon[a]			
		Moss	Decaying moss	New peat	Old peat
U-^{14}C-Glucose	0.125	0.265	0.428	0.008*	0.819
1-^{14}C-Galactose	0.125	0.091	0.538	< 0.001**	0.915
U-^{14}C-Arabinose	0.125	0.095	0.355	0.007*	0.122
U-^{14}C-Xylose	0.125	0.092	0.627	0.738	0.707
U-^{14}C-Sucrose	0.125	< 0.001**	0.950	0.002**	0.970
1-^{14}C-Mannitol	0.115	0.393	0.242	0.691	0.992
U-^{14}C-Glucosamine	0.125	0.035*	0.449	0.633	0.795
N-acetyl-D-1-^{14}C-glucosamine	0.125	0.336	0.421	0.034*	0.989
U-^{14}C-Benzoic acid	0.116	0.583	0.925	0.021*	0.923
Phenylethyl-1-^{14}C-amine	0.075	0.031*	0.926	0.071	0.949
U-^{14}C-Glycine	0.167	0.719	0.677	0.128	0.884
U-^{14}C-Lysine	0.107	0.133	0.644	0.909	0.830
U-^{14}C-Arginine	0.136	0.169	0.409	0.646	0.937
U-^{14}C-Aspartic acid	0.167	0.003*	0.195	0.529	0.327
U-^{14}C-Glutamic acid	0.139	< 0.001**	<0.001**	0.122	0.475

*/**Statistical significance of GMC effect as tested by ANOVA. *Significant at $p \leqslant 0.05$. **Significant at $p \leqslant 0.0033$, adjusted using the Bonferroni correction (Miller, 1991) on a horizon basis, to ensure $\alpha \leqslant 0.05$ (type I error).

[a]See text for description of the properties of the peat horizons.

Significant ($p<0.005$) separation of peat horizons in terms of CLPP patterns was already achieved after 4 h of incubation (Fig. 1). The value for the mean Mahalanobis distance (the distance between two points in multidimensional space) was used as an indicator of inter-horizon separation. The maximum value for this parameter was reached after 48 h of incubation (Fig. 1). The 48 h incubation period was therefore used to standardise subsequent experiments and for comparison with FTIR data. A potential drawback of soil-based systems for CLPP could be the induction of anaerobic conditions during incubation. We were not able to follow the redox potential or dissolved oxygen content of individual wells due to system constraints, but had determined the concentrations of added carbon based on available oxygen in the well to minimise the risk of oxygen limitation. Conditioning at a preset moisture content, usually 40% gravimetric moisture content (GMC), has been routinely applied prior to soil SIR assays in order to alleviate the effects of soil water content (West and Sparling, 1986). However, peat is usually waterlogged and also becomes irreversibly hydrophobic on drying below 65% GMC. We therefore tested the effect of moisture content influences on CLPP patterns within the range of naturally occurring GMC in peat, specifically 80%, 85%, 90% and 95% GMC, using identical peat horizons to the main experiment taken from a plot at Middlemuir Moss similar in regeneration age, but with a slightly differing surface vegetation (e.g. *Deschampsia flexuosa*, indicative of drier and more minerotrophic conditions). The FTIR signatures of these samples were not statistically different from those from the first

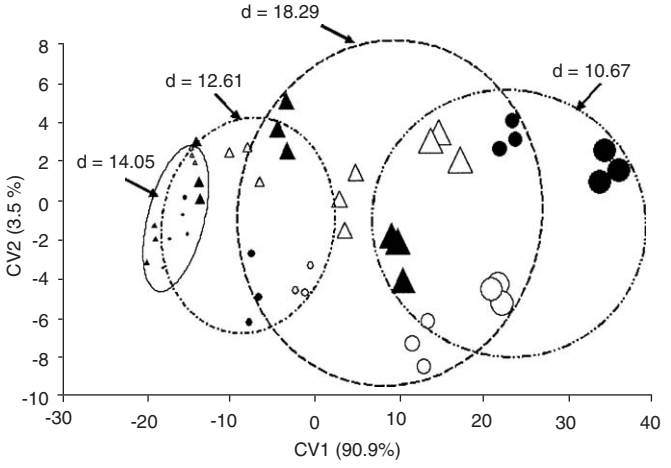

Fig. 1. Graphical representation of CVA scores for CLPP patterns of moss horizons (filled circles), decaying moss (open circles), new peat (open triangles) and old peat horizons (filled triangles), showing clearly that incubation time is the prime determinant of horizon separation. Size of the symbols indicates incubation length from shortest (4 h) to longest (120 h). Circles drawn around data clusters represent the different incubation lengths (4 h, unbroken line; 20 h, dash–dotted line, 48 h, dashed line; and 120 h, dash–dot–dotted line) and are of no statistical significance. To estimate the Mahalanobis distances (the distance between points in multidimensional space), repeat CVA was carried out on each data set per time point and reported as the values for *d*.

experiment (data not shown). Artificially created variations in the soil moisture content did not have a significant effect on carbon substrate mineralisation in the most humified horizon (Table 1), where anaerobic conditions would be more likely to develop due to the lower porosity of humified material compared to fresh or decaying moss horizons. Where significant differences in the extent of mineralisation occurred, there was no clear trend as to the type of carbon substrate affected (Table 1). It is possible that under the wetter conditions, the rate of substrate utilisation was influenced by diffusion gradients of the carbon source. In general, however, the data suggest that there was no systematic oxygen limitation in the experimental setup.

FTIR analysis on the peat horizon samples used for the first experiment showed a decline of the polysaccharide markers (absorption band indicative of C–O stretching at 1030 cm^{-1}) and relative increase of lignin-like (1513 cm^{-1}) and aliphatic structures (2850 and 2920 cm^{-1}) with depth, as expected (data not shown). Spectral bands indicative of 'carboxylate' (R-COO—), alkene (C–C) and/or aromatic C═C structures also increased in relative terms with depth (1600 cm^{-1}; Bourdon et al., 2000). Multivariate analysis by CVA confirmed that the horizons as defined using the von Post scale differed significantly ($p<0.05$) in their FTIR spectra. The first two canonical variate axes explained 97.15% and 2.69% of the variability after CVA on the first 6 PC scores (explaining >99.5% of the variability). We then compared these two multivariate datasets by Procrustes rotation. Orthogonal Procrustes rotation rotates a configuration to maximum similarity with another configuration but requires that the datasets used for the test contain a minimum number of variables that describe the original data with sufficient accuracy (Carlosena et al., 1995). CVA utilising the PC scores of the CLPP dataset was repeated with the maximum number of PC scores so that the CVA contained data representing >99.2% of the variability. Comparison of the two datasets by Procrustes rotation was therefore valid; the maximum permissible amount of the variability (in both cases more than 99%) was explained by the first 2 axes used in both datasets. Group separation in the fitted FTIR data followed a similar pattern as in the CLPP data both within and between the peat horizons (Fig. 2). In a study of biodegradation of DOM from different types of soils, Kalbitz et al. (2003) found that carbohydrates are degraded preferentially where high carbohydrate content of the soil DOM permits this. In samples of low initial carbohydrate content there was little change in the carbohydrate content during degradation, but relative increases in aromatic compounds, lipids, sterols and free fatty acids were observed. FTIR reports on constituents that are present in concentrations greater than 1% of the bulk matter and will primarily reflect the macromolecular compounds such as carbohydrates, lignin and waxes. However, the disappearance of the polysaccharide markers with depth reflects the decreasing potential to supply mono or

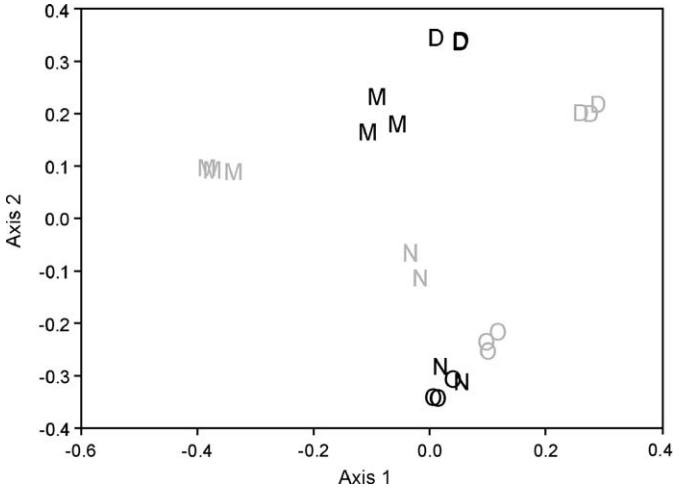

Fig. 2. Procrustes rotation of canonical variate axes 1 and 2 for the FTIR dataset after 48 h of incubation (black) after fitting to the CLPP dataset (grey). Both analyses clearly show similar group separation in multi-dimensional space of the peat horizons moss (M), decaying moss (D) new peat (N) and old peat (O) Axis 1 represents 65.9% and 97.1% of the dataset variability for the CLPP and FTIR data, respectively, and axis 2 represents 33.5% and 2.7%, respectively.

oligosaccharides and this may explain some of the observed correlation of the CLPP and FTIR datasets. We calculated the ratios of the peak of the polysaccharide band ($1030\,cm^{-1}$) to the 'carboxylate' ($1600\,cm^{-1}$) FTIR marker as an index of decomposition (Prasad et al., 2000). We used this ratio as an environmental variable in a partial redundancy analysis (RDA) of the CLPP data. The 1030/1600 ratio alone explained 68.7% of the total variability of the CLPP dataset and was highly correlated ($r = 0.892$) with axis 1, which differentiated primarily horizons M, D, and N/O (not shown). The differentiation between the most decomposed horizons N and O, however, occurred along axis 2 (21.1% of variance in partial RDA) and is not influenced by this FTIR ratio. We investigated other FTIR decomposition indices such as $1630/1510\,cm^{-1}$ ('carboxylates' to amide II and aromatic C=C) and $1630/2920\,cm^{-1}$ ('carboxylates' to aliphatics) (Haberhauer et al., 1998) after removing the statistical effect of the 1030/1600 ratio, but these did not correlate with axis 2 and did not significantly improve the amount of CLPP variability explained. Peat below the zone of root penetration is reported to have severely decreased concentrations of labile organic carbon in both the solid soil matrix and the DOM pool (Glatzel et al., 2003). In the same study, the authors observed that higher decomposition status of peat was significantly correlated with decreased CO_2 production potential. Glatzel et al. (2004) showed that the input of fresh (labile) carbon through re-establishing plant root exudates or their litter is the major driver of net loss of CO_2 to the atmosphere on highly decomposed peat undergoing restoration following surface harvesting. Hence, C sequestration in the initial phases of peatland restoration may be slow. The substrates chosen here were selected to represent a cross-section of substrates commonly found in root exudates (Campbell et al., 1997), phenolic compounds, and monosaccharide markers of peatland vegetation (Bourdon et al., 2000). As such, this assay may provide a valuable technique to study the effects of early vegetational succession in cutover peatlands in terms of their differential inputs of labile carbon on the microbially driven C turnover and sequestration. The concentration range of carbon additions used in this study is within that observed in root exudates (Wheatley et al., 2001; Griffiths et al., 1998), which have been shown to produce no significant short term changes to the soil microbial community composition as assessed using molecular techniques by Pennanen et al. (2004) and Griffiths et al. (1998). Indeed, if combined with molecular techniques, the ^{14}C-MicroResp^{TM} assay could elucidate some of the key relationships between microbial diversity and function in the cycling of labile C in peat.

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