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Functional microbial diversity in regenerating cutover peatlands responds to vegetation succession

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Summary

1. While establishment of vegetation is the most visual indicator of regeneration on cutover peatland, the reinstatement of belowground functions is less well understood. Vegetation succession results in differences in peat quality in terms of C availability. The respiratory response of the soil microbial community to ecologically relevant substrates (community-level physiological profile, CLPP) such as those found in rhizosphere exudates and litter hydrolysates, is thought to reflect the activity and functional diversity of the soil microbial community, especially those involved in turnover of soluble photosynthate-derived C.

2. The relationship between CLPP and typical regeneration stages was investigated at five European peatlands, each with up to five sites representing a gradient of natural regeneration stages. We aimed to determine whether unaided revegetation consistently affected soil microbial CLPP, which environmental factors explained variation in CLPP on the scale of individual peatlands, and if these factors were consistent across different peatlands.

3. Within each peatland, a decomposition index based on diagnostic bands in Fourier transform-infrared spectra indicated that regeneration had generally started from a common base and that the influence of vegetation on the decomposition index declined with depth. In parallel, differences in vegetation cover between regeneration stages resulted in significantly different CLPP, but this effect decreased rapidly with soil depth. The magnitudes of the effect of vegetation succession versus soil depth appeared to be linked with the age range of the regeneration gradients. Hence, the effect of vegetation on CLPP is effectively diluted due to the remaining organic matter. Specific plant species described significant proportions of CLPP variability but these species were not consistent across peatland types. The effects of soil depth appeared to be peatland-specific.

4. *Synthesis and applications*. Together, the results indicate significant responses of the microbial community to vegetation succession, with the strength of the effect probably dependent on quantities of labile C allocation to the soil microbial community. Therefore, particularly in the early stages of regeneration of cutover peatlands, CLPP could provide vital information about the relative importance of different plant functional types on potential rates of labile C turnover.

Key-words: restoration, peatlands, microbial diversity, succession, substrate induced respiration

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Introduction

Peatland ecosystems are globally threatened ecosystems despite harbouring approximately 25% of the terrestrial reserves of carbon (Gruber *et al*. 2004). Extensive drainage, afforestation and peat extraction have caused widespread destruction of peatlands in Europe (Chapman *et al*. 2003). In pristine peatlands, net carbon sequestration, defined as uptake of CO_2 and transformation into a long-lived pool of carbon, exceeds losses of C through net respiration (Belyea & Malmer 2004). In cutover peatlands, however, where large pools of carbon have been removed by extraction, lack of vegetation further increases net losses of carbon dioxide as soil respiration continues in the absence of photosynthetic fixation (Waddington, Warner & Kennedy 2002). Various restoration programmes to actively revegetate extensively cutover peatlands have been tested in North America and Europe in the last two decades (Gorham & Rochefort 2003; Rochefort & Price 2003). While establishment of vegetation is essential and perhaps the clearest indicator of regeneration on cutover peatland, whether the belowground functions unique to peatlands are reinstated upon recovery of the vegetation structure is less well understood. For example, some studies have shown that revegetation lowers the net efflux of carbon (Tuittila *et al*. 1999; Waddington & Warner 2001) and thereby shifts net ecosystem exchange closer to an actively carbon fixing state. Net ecosystem exchange depends upon the balance between production and decay of organic carbon and the role of regenerating peatlands in global C cycling can only be determined if we have an understanding of the factors that drive these processes.

Decay rates are determined by many factors, including the composition and/or activity of the microbial population, which may be altered in response to physico-chemical conditions and/or availability of labile carbon sources (Thormann 2006). In cutover peatlands, the majority of potentially available C is the remaining soil organic matter (SOM). Respiration rates from cutover surfaces are often very low after an initial flush of activity immediately post-harvest (e.g. Tuittila *et al*. 1999), indicating the low availability of SOM to the residual soil microbiota. Vegetation succession during regeneration can result in differences in SOM in terms of C availability, both through inputs of leachates of organic substrates from plant roots (often termed 'rhizodeposition') or from the stems of bryophytes, and eventually through plant litter. Trinder, Artz & Johnson (2008) demonstrated at one of the sites used in this study that a large proportion of newly fixed C is allocated below-ground to dissolved organic C and microbial biomass pools. Microbial biomass and net SOM mineralization rates are altered in harvested peatlands when active management has taken place to restore the vegetation (Fisk *et al*. 2003; Andersen, Francez & Rochefort 2006). Most microbial biomass estimates, however, provide no information about the relative importance or community structure of the microbial communities involved in cycling of labile vs. recalcitrant carbon. For example, Paterson *et al*. (2008) showed that, in a peaty podzol, labile C is utilized by a distinctly separate microbial community from that utilizing more recalcitrant plant litter (and presumably SOM) fractions. It follows that understanding the activity, community dynamics and drivers of microbiota involved in cycling of labile C in the early stages of regeneration of cutover peatlands could provide vital information about the likely fate of plant-derived C and the relative importance of different plant functional types in net C sequestration. Community-level physiological profile (CLPP), using relatively simple carbon compounds such as those found in rhizosphere exudates and litter hydrolysates, are thought to reflect the activity and functional diversity of the soil microbial community, especially those involved in turnover of soluble plant photosynthate C (Campbell *et al*. 2003) and can be used to compare between plant functional types and/or regeneration stages.

Artz, Chapman & Campbell (2006) showed that a large proportion of variation in CLPPs in peat may be attributed to the degree of peat decomposition. Yan, Artz & Johnson (2008) subsequently observed significant differences in CLPPs of rhizosphere microbial communities of common colonizing plant species on a single cutover peatland. In other ecosystems, however, changes in plant species composition have not universally been observed to influence soil microbial community composition or function (e.g. Kennedy *et al*. 2004). In this present study, we therefore analysed CLPPs of microbial communities from locations across Europe at varying stages of revegetation. The aims of this study were first, to evaluate whether vegetation succession *consistently* affects functioning of the microbial community in terms of catabolism of ecologically relevant carbon substrates. Secondly, we aimed to identify drivers of CLPP variability, in order to test whether substrate quality indicators, such as vegetation types and degree of peat decomposition explain significant proportions of variance in CLPP, and whether these drivers were *similar in type and magnitude* between peatlands of different geographical location, with inherent differences in botanical origin and historical peat formation. Finally, we discuss our findings in the light of whether CLPP could be used as an indicator of progress in peatland restoration.

Methods

EXPERIMENTAL SETUP

Nineteen sites were selected in five European cutover peatlands (Table 1) with relatively similar dominant vegetation. At the Finnish sites, ditch-blocking was carried out, and since cessation of extraction, the Baupte sites received summer additions of local drainage water (Table 1). Other than this, peatlands were unmanaged post-abandonment. Vegetation regeneration occurred unaided from local seed banks. Sites within peatlands were representative of the succession gradient present and took into account the history of peat production, cessation of extraction and the approximate minimum age of colonizing vegetation (Table 1) so that site factors represented a regeneration gradient after abandonment.

Replicate cores ($n = 3$) were extracted from each site (October–December 2003), and sectioned within 3 days into four horizons

Table 1. Sampling locations, site histories and vegetation composition

Peatland (abbreviation)	Location and altitude, metres above sea level	10-year average air temperature (°C)	Site history/depth of remaining peat	Site code	Regeneration stage*	Dominant vegetation, listed in order of abundance	Minimum age of plant community (y)
Middlemuir Moss, Scotland, UK (SC)	57°36′N, 2°9′W, 110 m	8·0	Block harvested (manual) for generations, combination of block harvesting/milling since 1960s until 1993. Abandoned without further management, 0·7–3·1 m remaining peat	A	B	Mostly bare, isolated *E. vaginatum*, *Campylopus introflexus*	< 5
				B	E1	*S. cuspidatum*, *S. auriculatum*, *E. vaginatum*	5–10
				C	E2	*E. angustifolium*, *S. auriculatum*, *S. cuspidatum*	5–10
				D	A	*Sphagnum* spp., *C. vulgaris*, *Deschampsia flexuosa*	> 50
Chaux d'Abel, Jura Mountains, Switzerland (CH)	47°10′N, 6°57′E, 1040 m	6·4	Block harvested (manual/small commercial scale) until 1963. No further management, > 1·2 m peat remaining	A	E	*S. fallax*, *P. strictum*, *P. commune*, *Eriophorum* spp.	> 29
				B	A1	Same species, intermediate between A and C	> 42
				C	A2	*S. fallax*, *P. strictum*, *E. vaginatum*, *Vaccinium* spp.	> 51
				D	I	*S. magellanicum*, *S. fuscum*, *S. rubellum*, *Vaccinium* spp. (no survey data)	Intact
Le Russey, Jura Mountains, France (FR)	47°18′N, 6°7′E, 867 m	7·7	Block harvested (manual/small commercial scale) from 1968–1984. No further management, 1–2 m peat remaining	A	B	Bare peat	> 4
				B	E1	*Sphagnum fallax*, *E. angustifolium*, *E. vaginatum* (rare)	> 22
				C	E2	*S. fallax*, *E. angustifolium*, *E. vaginatum*, *Calluna vulgaris*	> 21 (< 40)
				D	I	ND† (no survey data)	Intact
Aitoneva, Finland (FI)	62°12′N, 23°18′E, 156 m	4·2	Block harvested 1942–1948; Milled 1951–1975, ditches blocked in 1994 (Tuittila *et al.* 1999), 1·25 m peat remaining	A	B	Bare peat, no vegetation‡	10
				B	E1	*Eriophorum vaginatum*‡	10
				C	E2	*Carex rostrata*‡	10
				D	E3	*C. rostrata*, *S. fallax*‡	10
				E	E4	*Eriophorum vaginatum*‡	10
Baupte, France (FB)	49°17′N, 1°21′W, 4 m	11·4	Milled until ca. 2000 (ongoing harvesting in other parts). Remaining peat ca. 1 m	A	B	Bare peat, no vegetation	5–10
				B	E	*E. angustifolium*, *Hypnaceae*	5–10

*Regeneration stages as predefined on the basis of visual site surveys: B, bare; E, early; A, advanced; I, Intact. †ND, not determined. ‡Survey data only available as averages.

reflecting presumed different stages of decomposition starting from a common base. Due to lack of information prior to this experiment on the location of the horizon from which peat regeneration started, it was deemed more appropriate to use a common sampling strategy based on measured depth (0–5 cm, 5–10 cm, 22·5–27·5 cm and 42·5–47·5 cm) and subsequent analysis of the degree of decomposition (as described below). In cases where surface horizons contained only a thin layer of mosses of < 5 cm on top of remaining cutover peat, only the vegetative layer was sampled. Although predominant cover for such sites was *Sphagnum*, some samples contained small quantities of sedge roots. Each sample was cut into 1 cm^3 sub-samples and mixed manually. At least five sub-samples were pooled to maximize sample homogeneity.

VEGETATION SURVEYS

Site vegetation was surveyed during 2003, using either point-quadrat (at CH; Goodall 1952) or per cent cover techniques (at FI, SC, FB and FR; Buttler 1992) of three replicate plots (varying between 0·33–2·25 m^2). Plant cover was determined with a grid of 10×15 cells fixed at 20 cm height. Percentage cover was calculated from point quadrat data by 100× number of cells with a contact/total number of grid cells. Data were normalized to sum of cover within each peatland to account for variability introduced by the survey techniques. We tested for differences between prescribed regeneration stages within each peatland in plant composition, using permutated canonical variate analysis following dimension-reducing Principal Components Analysis (PCACVA) of the cover data under two null hypotheses: (i) there are no differences between plant communities of different regeneration stages within each peatland, and (ii) there are no differences between communities amongst the peatlands (described in detail below).

CLPP ANALYSES

CLPPs were determined using the MicroResp™ assay, which differs from the commonly used Biolog technique in that it is less dependent on growth of soil microorganisms, instead quantifying the mineralization of C substrate additions to the soil community (Campbell *et al*. 2003). Samples (further cut to approximately 5 mm^3 and manually mixed) were weighed to $0{\cdot}30 \pm 0{\cdot}01$ g $well^{-1}$ into 2 mL-deep-well microtitre plates. Fifteen radiolabelled carbon sources (see Artz *et al*. 2006 for details) were added at 200 Bq $well^{-1}$. Plates were sealed using MicroResp gas-permeable plateseals (MEL Ltd, Aberdeen, UK). Evolved $^{14}CO_2$ was captured on rolled filter papers with 40 µL of 2 M NaOH in the detection plate. Assemblies were clamped and incubated at 25 °C for 48 h. Detection plates received 200 µL of Optiphase 'Supermix' scintillation fluid (Perkin Elmer, UK) after incubation and counts (1 min $well^{-1}$) were recalculated as percentage utilization of added ^{14}C label.

PHYSICOCHEMICAL AND ORGANIC MATTER CHARACTERIZATION

Carbon and nitrogen were determined by combustion at 1100 °C (CNS-2000 LECO). Due to lack of carbonates, total carbon was taken as total organic carbon (TOC). Total soluble carbon was determined in 0·5 M K_2SO_4 extracts (1010 Bioritech Analyzer), followed by 5% orthophosphoric acid acidification for measurement of soluble organic carbon (SOC). Total soluble nitrogen was measured colorimetrically as NO_3^-, after oxidation with persulfate. Differences in level of decomposition were characterized by diamond attenuated total reflectance FTIR spectroscopy (Nicolet Magna-IR 550 FTIR spectrometer, Warwick, UK) over the wave number range 4000–350 cm^{-1} of zirconium ball-milled freeze-dried samples. FTIR data were normalized and a simple index of decomposition (PS-COO, previously used by Artz *et al*. 2006) was calculated from the ratio of the polysaccharide diagnostic band with maximum at 1030 cm^{-1} (indicative of C-O stretching and O-H deformation) vs. the aromatic/aliphatic carboxylate band at 1600 cm^{-1} (indicative of lignin or other aromatic C = C stretching and/or asymmetric C-O stretch in COO-) (Parker 1971; Cocozza *et al*. 2003; Artz *et al*. 2008). Microbial biomass C and N were estimated by fumigation extraction using a peat-modified protocol (Williams & Silcock 1997). Water table data were collected during at least monthly observations. Ecosystem respiration data were collected from replicated plots on representative vegetation, using dark chamber-based flux measurements with portable infrared gas analysers (EGM-PP Systems, Hitchin, UK) and were expressed as average ecosystem respiration rates (mg CO_2 m^{-2} h^{-1}) as described by Tuittila *et al*. (1999). Instantaneous net ecosystem CO_2 exchange rates were determined using the same system with transparent closed chambers. Data were collected during the growing season at Aitoneva, La Chaux d'Abel and Le Russey in 2003 and 2004 (June–September), at Middlemuir (June–September 2004) and Baupte (May–September 2004–2005).

STATISTICAL ANALYSIS

Differences in PS-COO ratio were tested using general linear models that incorporated the nested sampling design. Differences in vegetation composition between regeneration stages in each peatland were assessed using permutated PCACVA of arcsin-transformed per cent cover data. CLPP data were arcsin-transformed and preliminary Principal Components Analysis was used to visualize differences on the basis of peatland type, site and horizon. This was performed on the covariance matrix in Genstat for Windows (9th edition, VSN International), using Monte Carlo permutation (999 repetitions). Arcsin-transformed CLPP data were further analysed using redundancy analyses (RDA; Canoco for Windows 4·5, Biometris, The Netherlands). The hierarchical structure of the data set required that effects at each level were tested separately, by exclusion of the relevant higher spatial structure as covariables (Lepš & Šmilauer 2003). The hierarchical structure was coded using 'dummy' factors ('regeneration stage' × 2–5, 'core' × 3, 'sampling horizon' × 4). As permuted RDA requires balanced designs, missing values within each data set (0–5% of values) were replaced with the average of remaining replicates for affected carbon substrates. This ensures that 'there is no effect of missing replicates on estimated average or variance of that combination of factors for the permutation test' (Legendre & Anderson 1999). We tested hypotheses (i) and (ii) within the data set for each peatland by performing split-plot type restricted permutations (999 repetitions) of all canonical axes in blocks defined by the respective covariables. The effect of various alternative characteristics of each peatland (e.g. vegetation cover; Tables 1 and 2) was tested at the appropriate level within the hierarchy using forward selection of variables after permutation testing (999 repetitions). Prior to RDA, the CLPP data set was analysed using detrended correspondence analysis to confirm that gradient lengths indicated the suitability of a linear model (RDA) for further analyses.

Table 2. Significance of vegetation differences between regeneration stages; and available hydrological and carbon exchange summaries

Peatland abbreviation	Regeneration stage	Plant cover (%)	Significance of differences in plant cover†	Average annual water table (cm) and range (min/max)	Average growing season ecosystem respiration in g CO_2 m^{-2} h^{-1}; (min/max)	Average growing season instantaneous daytime net ecosystem exchange in g CO_2 m^{-2} h^{-1}; (min/max)	Modelled C balance in g CO_2 m^{-2}; (Reference)
SC	B	5*	A	27 (54/–7)§	0·06 (0·01/0·22)	–0·02 (–0·07/0·00)¶	ND
	E1	100	B	–1 (6/–11)	0·13 (0·02/0·28)	0·25 (–0·12/0·51)	ND
	E2	100	B	–1 (6/–11)	0·22 (0·07/0·42)	0·39 (0·02/0·79)	ND
	A	100	C	11 (33/1)	0·37 (0·17/0·57)	0·23 (–0·15/0·53)	ND
CH	E	100	A	16 (10–28)	0·67 (0·20/1·82)	0·58 (0·02/1·58)	ND
	A1	100	B	14 (5–28)	0·79 (0·23/2·00)	1·00 (0·28/2·18)	ND
	A2	100	C	16 (10–26)	0·88 (0·37/1·45)	0·62 (–0·03/1·36)	ND
	I	100	D‡	ND	ND§	ND	ND
FR	B	0	A	5 (16/–1)	0·06 (0·00/0·23)	–0·06 (0·00/–0·23)	–22 (1)¶
	E1	100	B	14 (23/2)	0·28 (0·02/0·54)	0·46 (0·04/0·85)	67–118 (1)
	E2	100	C	14 (22/7)	0·42 (0·03/0·40)	0·65 (0·01/1·19)	93–175 (1)
	I	100	D‡	ND	ND	ND	ND
FI	B	0	A	1 (14/–6)	0·06 (0·00/0·20)	0	ND
	E1	24	B	10 (32/–2)	0·24 (0·03/0·81)	0·45 (0·05/1·35)	~30–100 (2)
	E2	42	C	–30 (–20/–36)	0·22 (0·03/0·76)	0·66 (0·19/1·40)	~35–95 (2)
	E3	100	D	–18 (–1/–36)	0·07 (0·00/0·25)	0·19 (0·00/0·79)	~70/140 (2)
	E4	80	E	–10 (3/–22)	0·39 (0·07/0·85)	0·90 (0·17/1·85)	ND
FB	B	0	A	61 (97/19)	0·60 (0·07/3·51)	ND	ND
	E	79	A	55 (94/11)	0·33 (0·03/1·44)	–0·76 (–0·06/–1·12)	ND

*Values scaled to 100% within each peatland (cf. Methods). †Determined by permutated (999 Monte Carlo repetitions) PCACVA. Values sharing a letter within each peatland are not significantly different ($P > 0·05$). ‡assumption based on earlier assessments at CH (Laggoun-Défarge, 2008) and FR (A. Siegenthaler *et al.* personal communication) due to lacking survey data. §Negative values indicate site with periodic or consistent (FI-C, D) flooding. ¶Negative values indicate net loss of CO_2. References given for modelled carbon balances are in (1) Bortoluzzi *et al.* (2006; includes minor inputs from CH_4 fluxes), and (2) Kivimäki, Yli-Petäys & Tuittila (2008).

Results

ORGANIC MATTER COMPOSITION

As it was unknown a priori whether regeneration established from a common base within each peatland, the PS-COO indices (in Supporting Information Fig. S1) were used to test this. Except in FR, the degree of decomposition of the lowest horizon was not significantly different between sites. The relative abundance of polysaccharides (i.e. labile C substrates) over more recalcitrant C, increased with regeneration, not only in the top horizons but also with depth as litter and root exudate inputs increase (Supporting Information Fig. S1).

VEGETATION COVER

Within each peatland, the prescribed regeneration stages had significantly differing plant cover (Table 2, Fig. 1), with exception of the two early regeneration stages in SC and in FB. Despite initial visual similarities, there were no vegetated regeneration stages that had identical cover across the peatlands ($P < 0·05$). Figure 1 illustrates this point as the trajectory of regeneration is different in each of the peatlands with a gradient of regeneration stages.

C SINK FUNCTION

In common with many abandoned cutover peatlands where recolonization is unaided, C sink strength was still low (Table 2). It was outside our remit to model C balances from empirically obtained fluxes; thus, only the range and average values are reported here. For comparison, however, we report published figures from modelling studies of these sites where available, that indicate that sink strength does increase in the regeneration gradients. Table 2 also indicates the water table ranges, which reflect the lack or difficulty (FB and FI) of hydrological management.

RELATIONSHIP BETWEEN VEGETATION COVER AND CLPP

In all peatlands, CLPPs displayed patterns that were specific for regeneration stages (Fig. 2). CLPPs were differentiated in upper horizons, but this effect diminished in lower horizons. In peatlands with a relatively short history of regeneration (SC, FI and FB), there was no separation of CLPPs at the lowest horizon while in peatlands with wider chronological gradients, distinct differences between CLPPs of different sites could be observed (Fig. 2). The regeneration stage factor

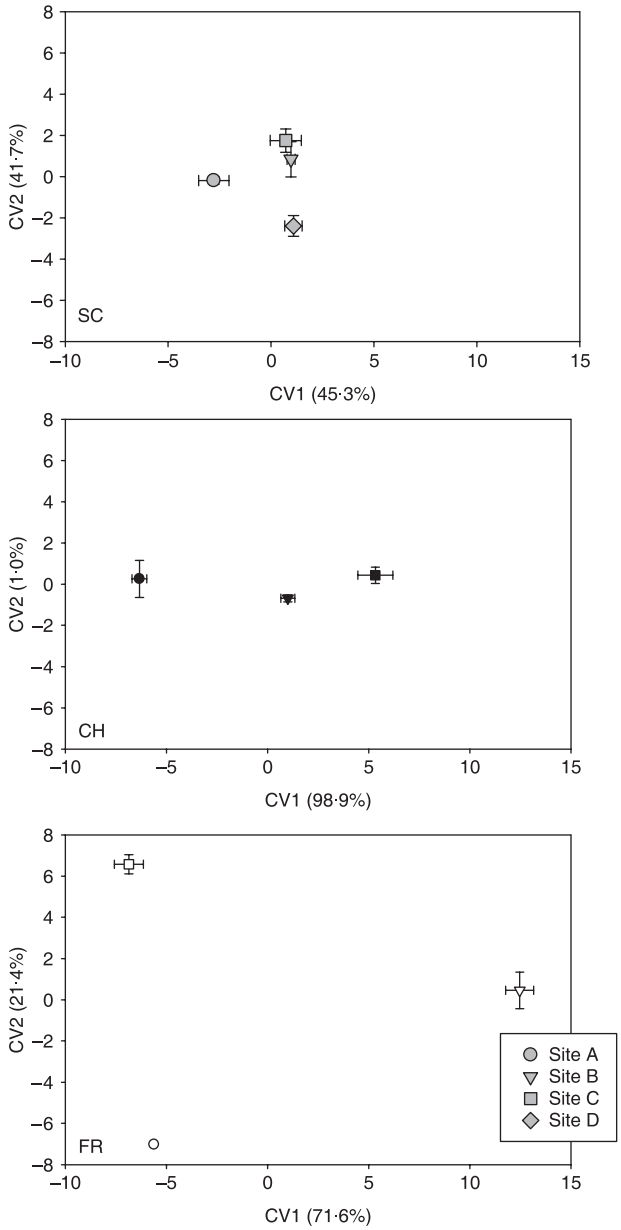

Fig. 1. Ordination plot of PCACVA of vegetation cover for the regeneration gradient sites at the Scottish (SC), French (le Russey, FR) and Swiss (CH) peatlands. The trajectories show significant separation of different regeneration stages. Cover data were not available for intact control sites at FR and CH. Data shown are means ± SEM (n = 3).

had a highly significant effect on CLPPs in all peatlands except FB (Table 3). The core parameter was not significant within any peatland, indicating there was no within-site heterogeneity in microbial community structure. The horizon factor explained the largest proportion of variance (Table 3). CLPPs of different regeneration stages were not significantly different in the horizon forming the underlying base of regeneration, except for FR (in Supporting Information Table S1). This is in agreement with the decomposition index, which, in the lowest horizon, was only significantly different in the FR regeneration gradient (Supporting Information Fig. S1). These 'dummy' factors may, of course, be describing effects of other differences between samples. We therefore performed further RDA to test the influence of various alternative factors, for example, physicochemical and (micro)biological differences (Tables 1 and 2).

EFFECTS OF REGENERATION STAGE AND HORIZON ON CLPP

Within each peatland, cover of certain vascular and/or bryophyte species, as well as age of the plant community and water table, were the best alternative descriptors at the level of regeneration stages (Table 4). In agreement with a lack of statistical differences in vegetation composition, there was no significant effect of site specific alternative variables in FB. Figure 3 shows the directional effect of significant variables in RDA at the site level. The effects of some plant species appear to be overlapping (e.g. *S. fallax* and *S. angustifolium* in FI, *E. vaginatum* and *V. oxycoccus* in FR). The loadings of CLPP substrates did not show any obvious correlation with any environmental variable tested (Fig. 3), with the exception of glycine and lysine, which were always positively correlated with *E. vaginatum* cover where this species occurred.

The alternative factors explaining significant components of CLPP variance at the horizon level were different in each peatland (Table 4). The bulk soil C:N ratio only explained 5·1% of CLPP variance in FR. Levels of soluble organic C and N explained highly significant proportions of CLPP variance in FR and FI. The size of microbial biomass C played a minor role in explaining variance in SC, yet explained over 50% in FB. Microbial biomass N content was significant in explaining CLPP variance in CH and FR. The PS-COO index was only a significant factor within SC, where it explained over a third of CLPP variance.

Discussion

Within all peatlands except FB, the regeneration stages were a significant factor in explaining CLPP variance. There was a strong trend (Fig. 2) in the surface horizons of FB that separated the revegetated from the bare site. We therefore conclude that, within peatlands, vegetation succession consistently altered CLPP of the soil microbial community [hypothesis (i)]. The proportion of variance explained by regeneration stages also increased where the range of stages was larger (in the order: FB – FI – SC – CH – FR, Table 3) with the highest proportion explained in peatlands CH and FR, where the gradient ranged between bare or early regeneration to intact reference sites (Table 1). It is therefore feasible that CLPPs were not significantly different in FB simply because the plant community is still at the earliest stage of succession (Table 1), and hence, the *quantity* of overall allocation of plant-derived labile C may not be sufficient to elicit a significant response. Such a relationship has recently been shown by Dijkstra, Cheng & Johnson (2006) who reported that priming (increased SOM turnover resulting

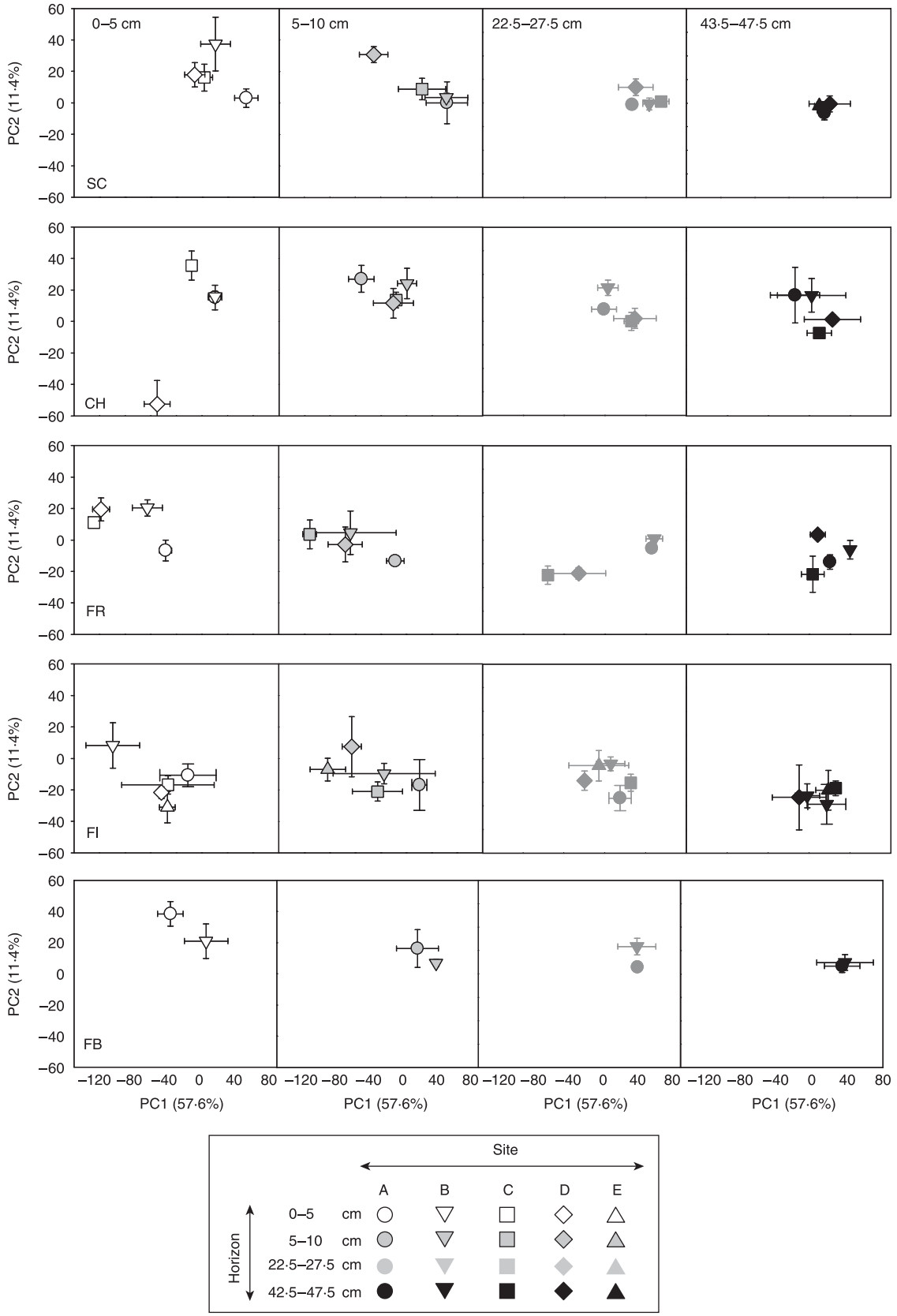

Fig. 2. Ordination plot of PCA of the CLPP data set. Data were separated into different figures for each peatland (SC, CH, FR, FI and FB, as indicated in Table 1). Sample distribution shows a clear effect according to peatland location, but within each peatland, site and horizon effects are also visually apparent. Data shown are means ± SD (n = 3), codes as indicated in Table 1.

Table 3. Variance decomposition of the effects of 'regeneration stage', 'core' and 'horizon depth' on peat microbial CLPP

Peatland	Factor tested	Covariable	DF	Permuted blocks	Variance explained (%)†
SC	Regeneration stage (S)	N/A	3	C	13·9***
	Core (C)	S	8	H	1·5^{NS}
	Horizon depth (H)	C	36	None (unrestricted)	23·4***
		Total			37·3
CH	Regeneration stage (S)	N/A	3	C	19·5***
	Core (C)	S	8	H	1·8^{NS}
	Horizon depth (H)	C	36	None (unrestricted)	20·4***
		Total			39·9
FR	Regeneration stage (S)	N/A	8	C	23·7***
	Core (C)	S	36	H	1·8^{NS}
	Horizon depth (H)	C		None (unrestricted)	36·4***
		Total			60·1
FI	Regeneration stage (S)	N/A	4	C	12·7***
	Core (C)	S	10	H	1·3^{NS}
	Horizon depth (H)	C	45	None (unrestricted)	26·0***
		Total			38·7
FB	Regeneration stage (S)	N/A	1	C	6·0^{NS}
	Core (C)	S	4	H	8·9^{NS}
	Horizon depth (H)	C	18	None (unrestricted)	43·2***
		Total			43·2

†Estimated using Monte Carlo permutation testing (999 permutations) in RDA within blocks defined by the co-variables: ***$P < 0{\cdot}001$; **$P < 0{\cdot}01$; *$P < 0{\cdot}05$; ^{NS}, not significant.

Table 4. Effect of alternative environmental variables† on CLPP variance

Peatland	SC	CH	FR	FI	FB
Variable					
'Site' level					
Sphagnum spp.‡	N/A	NS	N/A	N/A	N/A
Sphagnum cuspidatum† (S-cus)	1·6*	N/D	N/A	NS	NS
Sphagnum angustifolium (S-ang)	N/A	N/D	N/A	2·1**	NS
Sphagnum fallax (S-fal)	N/A	N/D	NS	5·9***	NS
Carex nigra (C-nigr)	N/A	9·4***	NS	N/A	NS
Eriophorum vaginatum (E-vag)	NS	3·6***	10·9***	4·4**	NS
Eriophorum angustifolium (E-ang)	NS	N/A	1·6*	N/A	NS
Erica tetralix (E-tet)	10·6***	N/A	N/A	N/A	NS
Betula nana (B-nana)	N/A	6·2*	N/A	N/A	N/A
Molinia caerulea (M-caer)	NS	4·4***	NS	N/A	N/A
Vaccinium oxycoccus (V-oxy)	N/A	NS	2·4*	N/A	NS
Minimum age of plant community (age)	NS	NS	9·8*	N/A	NS
Water table (WT)	2·5*	2·1*	NS	NS	NS
Average total respiration	NS	NS	NS	NS	NS
Total explained (%)	14·7	25·7	24·7	12·3	NS
'Horizon' level					
Total carbon (%)	NS	NS	NS	NS	NS
Total nitrogen (%)	NS	NS	NS	NS	NS
C/N ratio	NS	5·1*	NS	NS	NS
Soluble organic carbon	NS	NS	NS	3·2*	NS
Soluble organic nitrogen	NS	14·2***	NS	21·5***	NS
C/N ratio (solubles)	NS	7·6***	NS	NS	NS
Level of decomposition (FTIR 1030:1600 cm^{-1})	NS	NS	36·2***	NS	NS
Microbial biomass C	NS	NS	5·0*	NS	46·7***
Microbial biomass N	7·8*	16·7***	NS	NS	NS
Microbial biomass C/N	NS	NS	NS	NS	NS
Average total respiration	NS	NS	NS	NS	NS
Total explained (%)	7·8	43·5	41·2	24·6	46·7

†Only significant variables determined using forward selection in Monte-Carlo permutation tests (999 repeats): ***$P < 0{\cdot}001$; **$P < 0{\cdot}01$; *$P < 0{\cdot}05$; NS, not significant. ‡Used where point-quadrat data did not identify to species level. N/A, not applicable, where species was not part of the plant community. N/D, not detected, where point-quadrat data did not identify to species level. Vegetation variables used % cover.

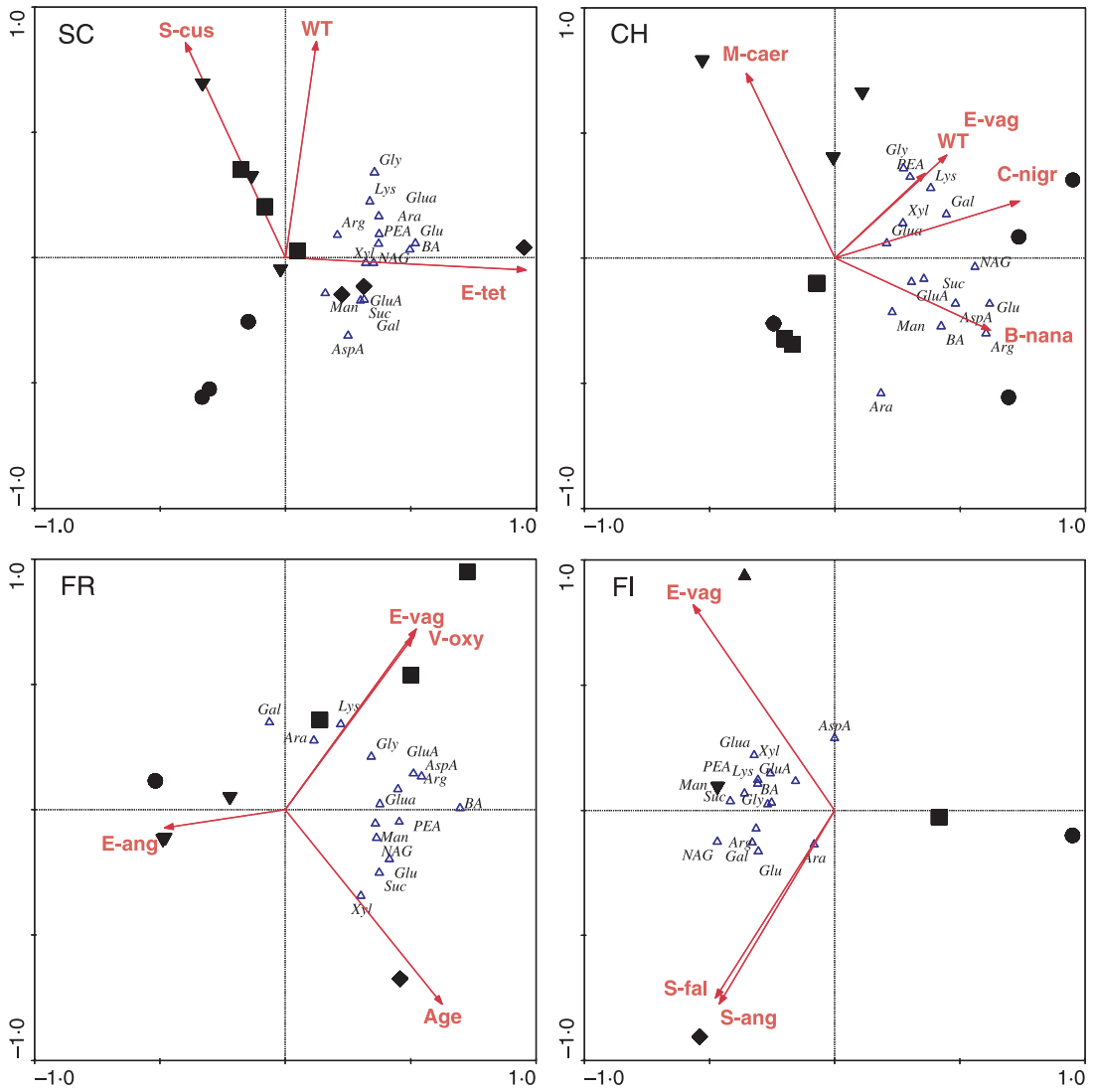

Fig. 3. Ordination plot of effects of alternative explanatory variables in RDA on CLPP from individual peatlands at the 'site' level. Effects of environmental variables which contribute significantly (see Table 4, also for abbreviations) to explaining CLPP variance are shown as projected arrows, with labels in bold. Sites shown are as described in Table 1 for each peatland, respectively, and are depicted as follows in the graphs: Site A (circles), Site B (downward triangles), Site C (squares), Site D (diamonds) and Site E (FI only, upward triangles). Loadings of carbon substrates are shown as crosses, with labels in italics. N.B. Missing values in the environmental data set lead to averaging of CLPP data for such data (e.g. vegetation at FI reported as averages only, see Table 1; hence, only one corresponding CLPP point).

from inputs of labile C, Kuzyakov, Friedel & Stahr 2000) in a grassland ecosystem was dependent on photosynthetically active plant biomass.

There is a wealth of literature demonstrating differences in functional responses in soil ecosystems in response to vegetation or land-use change (e.g. Schipper *et al.* 2001; Graham & Haynes, 2005) but the underlying cause of this change is not often identified. Here, the largest proportion of variance at the level of regeneration stage was explained by the proportional surface cover of vascular plant species (Table 4). Komulainen *et al.* (1998) presented data which suggested that 50 to 70% of net soil respiration in peatlands may be driven by the turnover of recent photosynthates. We observed a positive relationship between glycine/lysine utilization and cover of *E. vaginatum* in RDA. It is unknown whether glycine and lysine inputs from rhizoexudates of *E. vaginatum* differ from other peatland vegetation, although various reports suggest that rhizoexudate quality differs considerably between peatland plant species (Ström, Mastepanov & Christensen 2005; Ström & Christensen 2007). Yan *et al.* (2008) also identified lysine (but not glycine) as one of five carbon substrates that differentiated rhizosphere CLPPs of *E. vaginatum*, *E. angustifolium*, *Calluna vulgaris* and uncolonized peat. Fenner *et al.* (2004) demonstrated that *Sphagnum* significantly contributes to the DOC pool via leaching of recent photosynthates. Paradoxically, here, *Sphagnum* cover only explained significant amounts of CLPP variance in sites where bryophytes were a predominant part of the vegetation (i.e. FI, SC, Table 4). Despite observed inhibitory effects of *Sphagnum* leachates on decomposition rates of other functional litter types (Verhoeven

& Toth, 1995), we did not observe any inhibitory effects in CLPP attributable to their cover. Plant phenolics affect microbial activities in two ways. Low molecular weight phenolics are thought to be toxic to microorganisms or interfere with N dynamics due to utilization as C substrates thus exacerbating N limitation (e.g. Clein & Schimel, 1995). Larger phenolics bind with proteins, and hence, inhibit turnover of organic C and N-containing macromolecules (e.g. Hättenschwiler & Vitousek, 2000) rather than the turnover of substrates that can be directly taken up by microorganisms. Fierer *et al*. (2001) demonstrated that toxic effects may be limited in soils with pre-existing exposure to plant-specific phenolics. In the peatlands we studied, *Sphagnum* tissues or their diagnostic neutral sugars were found throughout profiles (Artz *et al*. 2008; Laggoun-Défarge, 2008); thus, a direct toxic effect is unlikely. Indirect effects of polyphenol:protein complex inhibition on turnover of complex C and N would not be observed in our test utilizing simple C sources that can be directly taken up by microbes.

Significant effects at the horizon level occurred in all peatlands. In most cases, substrate quality indicators, such as total C or N, were the major alternative explanatory factors of CLPP variability. Only in the Baupte peatland (FB) and to a lesser extent in the Scottish site (SC) was CLPP variance attributable to differences in microbial biomass C (Table 4). Dissolved organic matter (DOM) from degraded peatlands was 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, suggesting preferential respiration of labile carbon compounds (Glatzel *et al*. 2003). Indeed, the proportion of variance explained by the horizon generally increased in peatlands with a narrower range of regeneration stages (in the order: CH – SC – FI – FR – FB, Table 3). If FR is excluded on the basis that sites are not truly compatible because the lowest horizons were significantly different, it appears that the horizon effect is gradually 'diluted' as vegetation succession increases, presumably through changes in labile C inputs, that is, increased root penetration and rhizodeposition, and ultimately, increased depths of plant litter. Our data therefore predominantly indicate that the magnitude of the effect of vegetation succession appears to be moderation of the level of mineralization by changes in the *quantity* of labile C inputs rather than *differential inputs* of substrate types [hypothesis (ii)].

CLPP AS BIOLOGICAL INDICATOR OF RESTORATION SUCCESS?

The observed relationships between functional microbial community responses to regeneration stages and the occurrence of particular plant species indicate that it may be possible, with further research, to distinguish, for example, vegetation characteristics that are indicative of a return of the microbial communities involved in cycling of labile C to that of a reference state. The CLPP assay is, however, only a potential utilization assay and probably not a true reflection of *in situ* labile C turnover rates, as substrate utilization is assessed in isolation, and therefore, does not address potential resource competition for the multitude of substrates amongst the pool of photosynthate-derived C; neither will a 15 substrate-based test accurately describe the full microbial catabolic diversity involved in cycling of labile plant-assimilated C. Particularly in early stages of regeneration of cutover peatlands, however, CLPP could provide vital information about the relative importance of different plant functional types on potential rates of labile C turnover. The dominant horizon-specific differences, however, complicate such investigations as the effects of inputs of new photosynthetically fixed C are diluted against a large reservoir of residual organic C. Although definitive proof of restoration success will always be measured by how closely a restored site resembles an intact peatland in terms of vegetation, hydrological conditions and net carbon balance, this is costly in man-hours, equipment and analysis. The observed concurrence of CLPPs with regeneration stages, which run along a gradient of increasing C sink strength, indicate that it may be possible to identify features of labile C turnover that indicate a return to an actively C- fixing state. Our relatively limited range of regeneration stages within the examined peatlands, however, precluded this at this stage. In summary, determination of CLPP may help to focus restorative efforts in peatlands in a way that considers more than just the above-ground habitat.

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References

Andersen, R., Francez, A-J. & Rochefort, L. (2006) The physicochemical and microbiological status of a restored bog in Québec: identification of relevant criteria to monitor success. *Soil Biology & Biochemistry*, **38**, 1375–1387.

Artz, R.R.E., Chapman, S.J. & Campbell, C.D. (2006) Substrate utilisation profiles of microbial communities in peat are depth-dependent and correlate with whole soil FTIR profiles. *Soil Biology & Biochemistry*, **38**, 2958–2962.

Artz, R.R.E., Chapman, S.J., Robertson, A.H.J., Potts, J.M., Laggoun-Défarge, F., Gogo, S., Comont, L., Disnar, J-R. & Francez, A-J. (2008) FTIR spectroscopy can be used as a screening tool for organic matter quality in regenerating cutover peatlands. *Soil Biology & Biochemistry*, **40**, 515–527.

Belyea, L.R. & Malmer, N. (2004) Carbon sequestration in peatland: patterns and mechanisms of response to climate change. *Global Change Biology*, **10**, 1043–1052.

Bortoluzzi, E., Epron, D., Siegenthaler, A., Gilbert, D. & Buttler, A. (2006) Carbon balance of a European mountain bog at contrasting stages of regeneration. *New Phytologist*, **172**, 708–718.

Buttler, A. (1992) Permanent plot research in wet meadows and cutting experiment. *Vegetatio*, **103**, 113–124.

Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S. & Potts, J.M. (2003) A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments. *Applied and Environmental Microbiology*, **69**, 3593–3599.

Chapman, S.J., Buttler, A., Francez, A-J., Laggoun-Défarge, F., Vasander, H., Schloter, M., Combe, J., Grosvernier, P., Harms, H., Epron, D., Gilbert, D. & Mitchell, E. (2003) Exploitation of northern peatlands and biodiversity maintenance: a conflict between economy and ecology. *Frontiers in Ecology and the Environment*, **1**, 525–532.

Clein, J.S. & Schimel, J.P. (1995) Nitrogen turnover and availability during succession from alder to poplar in Alaskan taiga forests. *Soil Biology & Biochemistry*, **27**, 743–752.

Cocozza, C., D'Orazio, V., Miano, T.M. & Shotyk, W. (2003) Characterization of solid and aqueous phases of a peat bog profile using molecular fluorescence spectroscopy, ESR and FT-IR. *Organic Geochemistry*, **34**, 49–60.

Dijkstra, F.A., Cheng, W.X. & Johnson, D.W. (2006) Plant biomass influences rhizosphere priming effects on soil organic matter decomposition in two differently managed soils. *Soil Biology & Biochemistry*, **38**, 2519–2526.

Fenner, N., Ostle, N., Freeman, C., Sleep, D. & Reynolds, B. (2004) Peatland carbon afflux partitioning reveals that *Sphagnum* photosynthate contributes to the DOC pool. *Plant and Soil*, **259**, 345–354.

Fierer, N., Schimel, J.P., Cates, R.G. & Zou, J. (2001) Influence of balsam poplar tannin fractions on carbon and nitrogen dynamics in Alaskan taiga floodplain soils. *Soil Biology & Biochemistry*, **33**, 1827–1839.

Fisk, M.C., Ruether, K.F. & Yavitt, J.B. (2003) Microbial activity and functional composition among northern peatland ecosystems. *Soil Biology & Biochemistry*, **35**, 591–602.

Glatzel, S., Kalbitz, K., Dalva, M. & Moore, T. (2003) Dissolved organic matter properties and their relationship to carbon dioxide efflux from restored peat bogs. *Geoderma*, **113**, 397–411.

Goodall, D.W. (1952) Some considerations in the use of point quadrats. *Australian Journal of Scientific Research*, **5**, 1–41.

Gorham, E. & Rochefort, L. (2003) Peatland restoration: a brief assessment with special reference to *Sphagnum* bogs. *Wetlands Ecology and Management*, **11**, 109–119.

Graham, M.H. & Haynes, R.J. (2005) Catabolic diversity of soil microbial communities under sugarcane and other land uses estimated by Biolog and substrate-induced respiration methods. *Applied Soil Ecology*, **29**, 155–164.

Gruber, N. *et al*. (2004) The vulnerability of the carbon cycle in the 21st century: An assessment of carbon-climate-human interactions. *Toward CO_2 stabilization: Issues, Strategies and Consequences* (eds C.B. Field & M.R. Raupach), pp. 45–76. Island Press, Washington, D.C.

Hättenschwiler, S. & Vitousek, P.M. (2000) Role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends in Ecology & Evolution*, **15**, 238–243.

Kennedy, N., Brodie, E., Connolly, J. & Clipson, N. (2004) Impact of lime, nitrogen and plant species on bacterial community structure in grassland microcosms. *Environmental Microbiology*, **6**, 1070–1080.

Kivimäki, S., Yli-Petäys, M. & Tuittila, E-S. (2008) Carbon sink function of sedge and *Sphagnum* patches in a restored cut-away peatland. *Journal of Applied Ecology*, **45**, 921–929. .

Komulainen, V.M., Tuittila, E.S., Vasander, H. & Laine, J. (1998) Restoration of drained peatlands in southern Finland: initial effects on vegetation change and CO_2 balance. *Journal of Applied Ecology*, **36**, 634–648.

Kuzyakov, Y., Friedel, J.K. & Stahr, K. (2000) Review of mechanisms and quantification of priming effects. *Soil Biology & Biochemistry*, **32**, 1485–1498.

Laggoun-Défarge, F., Mitchell, E., Gilbert, D., Disnar, J-R., Comont, L., Warner, B. & Buttler, A. (2008) Cut-over peatland regeneration assessment using organic matter and microbial indicators (bacteria and testate amoeba). *Journal of Applied Ecology*, **45**, 716–727.

Legendre, P. & Anderson, M.J. (1999) Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. *Ecological Monographs*, **69**, 1–24.

Lepš, J. & Šmilauer, P. (2003) Hierarchical analysis of community variation. *Multivariate Analysis of Ecological Data Using Canoco*. Cambridge University Press, Cambridge, UK. pp. 141–144.

Parker, F.S. (1971) *Applications of Infrared Spectroscopy in Biochemistry, Biology and Medicine*. Adam Hilger, London.

Paterson, E., Osler, G., Dawson, L.A., Gebbing, T., Sim, A. & Ord, B (2008) Labile and recalcitrant plant fractions are utilised by distinct microbial communities in soil. *Soil Biology and Biochemistry*, **40**, 1103–1113.

Rochefort, L. & Price, J.S. (2003) Restoration of *Sphagnum* dominated peatlands. *Wetlands Ecology and Management*, **11**, 1–2.

Schipper, L.A., Degens, B.P., Sparling, G.P. & Duncan, L.C. (2001) Changes in microbial heterotrophic diversity along five plant successional sequences. *Soil Biology & Biochemistry*, **33**, 2093–2103.

Ström, L. & Christensen, T.R. (2007) Below-ground carbon turnover and greenhouse gas exchanges in a subarctic wetland. *Soil Biology & Biochemistry*, **39**, 1689–1698.

Ström, L., Mastepanov, M. & Christensen, T.R. (2005) Species-specific effects of vascular plants on carbon turnover and methane emissions from wetlands. *Biogeochemistry*, **75**, 65–82.

Thormann, M.N. (2006) Diversity and function of fungi in peatlands: a carbon cycling perspective. *Canadian Journal of Soil Science*, **86**, 281–293.

Trinder, C.J., Artz, R.R.E. & Johnson, D. (2008) Contribution of plant photosynthate to soil respiration and dissolved organic carbon in a naturally recolonising cutover peatland. *Soil Biology & Biochemistry*, **40**, 1622–1628.

Tuittila, E.S., Komulainen, V.M., Vasander, H. & Laine, J. (1999) Restored cut-away peatland as a sink for atmospheric CO_2. *Oecologia*, **120**, 563–574.

Verhoeven, J.T.A. & Toth, E. (1995) Decomposition of *Carex* and *Sphagnum* litter in fens – effects of litter quality and inhibition by living-tissue-homogenates. *Soil Biology & Biochemistry*, **27**, 271–275.

Waddington, J.M. & Warner, K.D. (2001) Atmospheric CO_2 sequestration in restored mined peatlands. *Ecoscience*, **8**, 359–369.

Waddington, J.M., Warner, K.D. & Kennedy, G.W. (2002) Cutover peatlands: a persistent source of atmospheric CO_2. *Global Biochemical Cycles*, **16**, 1002.

Williams, B.L. & Silcock, D.J. (1997) Nutrient and microbial changes in peat profile beneath *Sphagnum magellanicum* in response to additions of ammonium nitrate. *Journal of Applied Ecology*, **34**, 961–970.

Yan, W., Artz, R.R.E. & Johnson, D. (2008) Species-specific effects of plants colonising cutover peatlands on patterns of carbon source utilisation by soil microorganisms. *Soil Biology and Biochemistry*, **40**, 544–549.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Pairwise comparison of CLPP profile through horizons within each peatland

Fig. S1. Degree of decomposition.