# Functional microbial diversity in cutover peatlands responds to restoration and is directed by labile carbon

***Running title:*** **Peat CLPP**

**Rebekka R.E Artz [1*], Stephen J. Chapman[1], Andy Siegenthaler[2], Estelle Bortoluzzi[3], Mika Yli-Petays[4] and André-Jean Francez[5]**

[1]*Macaulay Land Use Research Institute, Craigiebuckler, Aberdeen, AB15 8QH UK*

[2]*Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Station de Lausanne, Antenne romande, CH-1015 Lausanne, Switzerland*

[3]*Université de Franche-Comte, Laboratoire de Chronoécologie, La Bouloie, 25030 Besancon CEDEX, France*

[4]*Peatland Ecology Group, Department of Forest Ecology, University of Helsinki, P.O. Box 27, FI-00014, Helsinki, Finland & Finnish Forest Research Institute, Parkano Research Unit, Kaironiementie 54, FI-39700 Parkano, Finland*

[5]*Université de Rennes 1, UMR-CNRS ECOBIO n° 6553, Campus de Beaulieu, 35042 Rennes CEDEX, France*

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*Corresponding author:* Rebekka R.E. Artz, The Macaulay Institute, *fax:* (+44) (0)1224 498207; *e-mail:* r.artz@macaulay.ac.uk

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**Summary**

1. While the establishment of vegetation is the essential and clearest indicator of regeneration on cutover peatland, the reinstatement of the belowground functions unique to peatlands are less well understood. Carbon turnover rates, which are partly determined by the composition and activity of the soil microbial population, may be altered in response to the physico-chemical conditions and/or the availability of labile carbon sources.
2. The relationship between microbial functioning, as assessed by community level physiological profiles (CLPP), and typical peatland regeneration phases was investigated at five peatlands in Europe, each with up to five sites representing a gradient of natural peatland regeneration. We aimed to determine whether spontaneous revegetation had a significant effect on the CLPP of the soil microbial community, which environmental factors explained the variation in CLPP on the scale of individual peatlands, and if these factors were consistent across a larger spatial scale.
3. Peatland location (26 %) and horizon depth (11.7 %) of the samples had the strongest influence on the CLPP patterns at the larger spatial scale. Within each peatland, 'site' and sampling depth were the primary determinants. Tested at the spatial scale of individual peatlands, various vascular plant species were the primary alternative site factors, explaining between 12.3 and 25.7 % of CLPP variance at each 'site', where significant site separation occurred. Substrate quality indicators, and in some case the size of the microbial biomass, were the primary alternative factors which explained CLPP variance at the sampling depth level. Within peatlands undergoing restoration, similar trends in CLPP responses to

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recovery stages were observed and these trends were correlated with measures of C substrate quality and especially labile C pools.

4. *Synthesis and applications.* This correlation between microbial responses, climatic variability, and substrate quality is potentially useful for predicting long term belowground responses of regenerating peatlands and determining the key vegetation species that drive such belowground responses.

## Introduction

Peatlands are a threatened environment in many parts of the world despite harbouring approximately 30% of the global reserves of soil carbon. Extensive drainage, afforestation and extraction for fuel and horticultural peat have caused extensive destruction of peatlands (Moore 2002; Chapman *et al.* 2003). In pristine peatlands, net carbon sequestration, defined as the uptake of $CO_2$ and transformation into a long-lived pool of carbon, exceeds the losses through net respiration (Belyea & Malmer 2004). In cutover peatlands, where a large pool of carbon has already been removed by extraction, the lack of vegetation further increases net losses of carbon dioxide as soil respiration continues in the absence of photosynthetic fixation (Waddington *et al.* 2002; Vasander *et al.* 2003).

Various restoration programmes to actively revegetate extensively cutover peatlands have been tested in both North American and European countries in the last two decades (Gorham & Rochefort 2003; Rochefort & Price 2003). While the establishment of vegetation is the essential and clearest indicator of regeneration on cutover peatland, the reinstatement of those belowground functions unique to peatlands are 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 effects a shift in

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net ecosystem exchange closer to an actively carbon fixing state. Carbon sequestration depends upon the balance between production and decay. Decay rates are determined by the composition and/or activity of the microbial population, which may be altered in response to the physico-chemical conditions and/or the availability of labile carbon sources (Thormann, 2006). Differences in peat quality in terms of C availability following peatland restoration, through inputs of rhizoexudates and litter of pioneer vegetation, are likely to influence microbial community functioning and therefore, ultimately, rates of net soil respiration. We previously optimised a multiple substrate induced respiration (SIR) technique for use with peat which uses relatively simple carbon compounds such as those likely to be found in found in rhizosphere exudates and litter hydrolysates (Artz *et al*. 2006). Thus, a 'fingerprint' of the respiratory response of soil micro-organisms to substrate additions can be obtained, sometimes termed the "community level physiological profile" (CLPP), which reflects the activity and functional diversity of the soil microbial community (Lehman & Garland, 1997).

We showed previously that a large proportion of the variation in CLPP in peat can be attributed to a spectroscopic indicator of the level of decomposition of the peat (Artz *et al.* 2006). This conforms to the hypothesis that substrate quality is a major driver of microbial activity in peat (Waddington *et al*. 2001; Andersen *et al.* 2006). In addition, some studies suggest that 50% to 70% of net soil respiration in peatlands is driven by the turnover of recent photosynthates (Komulainen *et al.* 1998). Both Fisk *et al.* (2001) and Andersen *et al.* (2006) showed that the size and carbon mineralization activity (here, respiration of the soil organic matter) of the microbial community is altered in mechanically harvested peatlands when active management has taken place to restore the vegetational characteristics.

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In the present study, we therefore analysed the CLPP of microbial communities from field locations across Europe at varying stages of natural revegetation by *Sphagnum* spp. and other peatland indicator species. The aims of this study were i) to identify the drivers of CLPP variability on the large (between geographically separated peatlands) and small (i.e. within a peatland) scale, ii) to test the hypothesis that the vegetation structure of regenerating peatlands affects the CLPP of the soil microbial communities, and iii) to investigate whether the CLPP of peat samples could be used as an indicator of the restoration process.

## Methods

### SITES & EXPERIMENTAL SETUP

Various sites (total $n$ = 19), representative of the gradient of spontaneous regeneration present within each peatland, were selected in five previously cutover peatlands in Europe (Table 1). The sites were chosen within each peatland on the basis of the history of peat production, cessation of extraction and the approximate minimum age of the colonising vegetation (Table 1) as well as the composition of the surface vegetation recolonising these sites (Table 1). Based on these, all sites were assigned to a 'restoration stage' (Table 1) prior to sampling. More information about the selected peatlands and sites may also be found in Francez *et al.* (This issue). Replicate cores ($n$ = 3) were extracted from each site in the five peatlands during October-December 2003, and sectioned within 3 days into 4 different sampling horizons reflecting different stages of decomposition. These horizons were designated horizons 3 (surface layer 0-5 cm), 4 (5-10 cm), 6 (22.5-27.5 cm) and 8 (42.5 to 47.5 cm). In some cases, the horizon 3 layer contained only a thin layer of vegetation of < 5 cm on top of the remaining cutover peat. In these cases, only

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123 the vegetative layer was sampled. All samples (19 sites × 3 replicate cores × 4 sampling
124 horizons, giving a total $n$ = 228) were each cut into 1 $cm^3$ sub-samples and mixed
125 manually. At least 5 sub-samples were pooled for each sample to maximise sample
126 homogeneity.
127
128 CLPP ANALYSES
129 CLPP were determined using the MicroResp™ assay (Campbell et al., 2003) with
130 modifications (Artz et al., 2006). Briefly, the composite peat samples were cut to
131 approximately 5 $mm^3$ and homogenised further by manual mixing. Samples were weighed
132 to 0.30 ± 0.01 g $well^{-1}$ into a 2 ml deepwell microtitre plate. The assay was performed
133 with 15 radiolabelled carbon sources (U-$^{14}$C-Glucose, 1-$^{14}$C-Galactose, U-$^{14}$C-Arabinose,
134 U-$^{14}$C-Xylose, U-$^{14}$C-Sucrose, 1-$^{14}$C-Mannitol, U-$^{14}$C-Glucosamine, N-acetyl-D-1-$^{14}$C-
135 glucosamine, U-$^{14}$C-Benzoic acid, Phenylethyl-1-$^{14}$C-amine, U-$^{14}$C-Glycine, U-$^{14}$C-
136 Lysine, U-$^{14}$C-Arginine, U-$^{14}$C-Aspartic acid, U-$^{14}$C-Glutamic acid) and a no addition
137 control. The sources were added at 200 Bq $well^{-1}$ in a carrier solution of unlabelled parent
138 compound, which was at the maximum concentration that could be oxidised given the
139 oxygen available in each well (Campbell et al., 2003). Each well was sealed using a
140 MicroResp™ gas-permeable plate seal (MEL Ltd, Aberdeen, UK). Evolved $^{14}CO_2$ was
141 captured on rolled filter papers in the detection plate moistened with 40 µl of 2 M NaOH.
142 The entire assembly was clamped and incubated at 25°C for 48 h. This incubation period
143 was previously determined as the optimal time point for incubations for similar peat
144 samples (Artz et al., 2006). Cumulative mineralization was determined by addition of 200
145 µl of Optiphase 'Supermix' scintillation fluid (Perkin Elmer, UK) to the detection plate
146 wells and counts (1 min $well^{-1}$) were recalculated as percentage utilisation.
147

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148 PHYSICOCHEMICAL CHARACTERISATION

149 Carbon and nitrogen contents were determined by combustion at 1100°C with a
150 CNS-2000 LECO apparatus, on dried and milled peat samples. Due to the total lack of
151 carbonates, total carbon was taken to be total organic carbon (TOC). Total soluble organic
152 carbon was determined in 0.5 M $K_2SO_4$ extracts using a 1010 Bioritech Analyzer. Total
153 soluble carbon was analysed first, then the sample was acidified with a 5%
154 orthophosphoric acid solution to remove inorganic carbon and soluble organic carbon
155 (SOC) was measured. Total soluble nitrogen was measured colorimetrically as $NO_3^-$, after
156 oxidation with persulfate (Williams *et al.,* 1995). Differences in the level of humification
157 of the samples were characterised by diamond attenuated total reflectance FTIR
158 spectroscopy using a Nicolet Magna-IR 550 FTIR spectrometer (Nicolet Instruments
159 Limited, Warwick, U.K.) over the wavenumber range 4000-350 $cm^{-1}$ of zirconium ball-
160 milled freeze-dried samples. FTIR data were normalised by subtraction of the minimum
161 value and subsequent division by the average over the spectral range. The ratios of the
162 peak of the polysaccharide band (1030 $cm^{-1}$) to the 'carboxylate' (1600 $cm^{-1}$) FTIR
163 marker was used as an index of the level of humification (Artz et al., 2006). Microbial
164 biomass C and N were estimated by fumigation extraction using a protocol modified for
165 peat (Williams and Silcock, 1997). Annual water table data were collected during at least
166 monthly observations during the year of sampling. Annual soil respiration data were
167 collected using dark chamber-based gas flux measurements with portable infra red gas
168 analysers (EGM-PP Systems, Hitchin, UK) and were expressed as average respiration
169 rates (mg $CO_2$ $m^{-1}$ $h^{-1}$) (Alm et al. 1997; Tuittila et al. 1999, Kivimäki et al., this issue).
170 Data were collected at Aitoneva, La Chaux d'Abel and Le Russey during 2003, at
171 Middlemuir during 2003-2004, and in Baupte during 2004-2005.

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## VEGETATION SURVEYS

The vegetation at all sites was surveyed during 2003, using either point-quadrat (at Chaux d'Abel; Goodall, 1952) or percent cover techniques (at Aitoneva, Middlemuir, Baupte and Le Russey; Buttler, 1992) of 3 replicates of randomly chosen plots (varying between 0.33 - 2.25 $m^2$) within each site. Plant cover was determined with the help of a plexiglass rectangular 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 normalised to the sum of all cover within each peatland to account for variability introduced by the survey techniques.

## STATISTICAL ANALYSIS

The CLPP data were transformed for statistical analyses using the arcsin function. Data were analysed using redundancy analyses (RDA) using Canoco for Windows 4.5 (Biometris, Wageningen, The Netherlands). The hierarchical structure of the dataset required that each level of the hierarchy was tested separately while keeping the next lower spatial level arrangement intact (Lepš and Šmilauer, 2003). The structure was coded using 'dummy' variables for each possible group of samples (i.e. peatland type × 5, site × 5, cores × 3, sampling horizon × 4). We tested all hypotheses at two spatial scales: first within all peatlands, and secondly, in each individual peatland. Missing values (i.e. mostly missing 'sites' to balance the design) were replaced with the average value for each carbon substrate over all samples of each peatland group. The effect of each spatial level (peatland type, followed by site and finally sampling horizon) was therefore analysed in a separate RDA by exclusion of the relevant higher spatial structure as covariables (see Tables 2 and 3). The effects were tested by performing split-plot type restricted

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197 permutations (999 repetitions) of all canonical axes in blocks defined by the respective
198 covariables.

199 The effect of the various alternative site or horizon-specific characteristics (Table
200 1, 4) was tested at the appropriate level within the hierarchy by excluding the statistical
201 effect of the higher spatial structure of the dataset and using forward selection of variables
202 after permutation testing (999 repetitions). Prior to RDA, the CLPP dataset was analysed
203 using detrended correspondence analysis to confirm that the gradient lengths indicated the
204 suitability of a linear model (RDA) for further analyses.

205

206 **Results**

207 LOCATION AND SAMPLING DEPTH ARE SIGNIFICANT DRIVERS OF CLPP
208 VARIABILITY AT BOTH SPATIAL SCALES

209 In all countries, we observed distinct differences in the CLPP of samples obtained from
210 the gradients of regeneration. In an example shown in Fig. 1, the CLPP data for the
211 Scottish peatland simultaneously illustrate the site-specific pattern of substrate utilisation
212 as well as a general decrease in the amount of carbon substrate utilised with depth. Initial
213 principal component analysis of all 228 CLPP patterns showed strong separation of data
214 and suggested a large effect of peatland location (Fig 2). Redundancy analysis of the
215 combined dataset from all five European peatlands showed significant separation of the
216 samples from separate locations (Table 2, 26 % of variance explained). The site factors
217 only explained a further 2.3 % to the total CLPP variance at the larger spatial scale (Table
218 2). The core factor was not significant. Finally, at the lowest level of the spatial structure,
219 the sampling depth explained 11.7 % of the CLPP variance at the larger spatial scale. The
220 three tested levels of the hierarchical structure of our dataset only explained a total of 40
221 % of the total CLPP variance.

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222 At the smaller spatial scale, i.e. within each peatland, the 'site' parameter had a
223 significant effect on the CLPP of peat from all countries except Baupte, France (Table 3).
224 The 'core' parameter was also not significant within any of the individual peatlands.
225 Within each peatland, the sampling horizon factor explained the largest proportion of the
226 variance in each case (Table 3).
227 These 'dummy' factors may, of course, be describing the effects of other
228 differences between samples at each of the hierarchical levels. We therefore performed
229 further RDA for the influence of the various available climatic, physicochemical and
230 (micro)biological data at each level. We used the same hierarchical higher level structures
231 (i.e. 'peatland', 'site', 'core', as appropriate), but selected those measured variables that
232 best explained the CLPP variance at that level by forward selection.
233
234 CLIMATIC VARIABILITY, SURFACE VEGETATION AND SUBSTRATE QUALITY
235 ARE ALTERNATIVE DESCRIPTORS OF LOCATION AND HORIZON EFFECTS
236 The average values and range of the characterised environmental variables are
237 summarised in Tables 1 and 4, for site and horizon specific variables, respectively. The
238 specific responses of some of these variables (e.g. microbial biomass C and N) to
239 spontaneous regeneration are discussed elsewhere in this issue by Francez *et al.*
240 At the larger spatial scale of all five peatlands, the only climatic characteristic that
241 significantly accounted for CLPP variance was the 10-year average air temperature. This
242 factor described a total of 4.2 % (Table 5), which is much lower than the effect of the
243 'peatland' dummy variables (Table 2). At the large spatial scale, the percent cover of
244 various vegetation types, minimum age of the plant community and average water table
245 explained significant, but very minor parts of the CLPP variance (Table 5). Combined,
246 these alternative factors only explained 6.4 % of variance. At the peat horizon level, the

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247 C:N ratio of soluble fractions and the level of decomposition (as defined by the FTIR ratio
248 of spectral bands indicative of polysaccharide versus carboxylate content) described most
249 of the total explained variance at the larger spatial scale (Table 5), while other factors such
250 as total C and N and microbial biomass N played a slightly lesser role. The PS-COO ratio
251 can be compromised in peat with high pH values, as the pH value affects the level of free
252 acid and hence the carboxylate band region. PS-COO still, however, explained a
253 significant, and similar, proportion of the variance when samples with high pH values (e.g.
254 FB) were excluded from RDA (not shown).

255 At the spatial scale of the individual peatland, the percent cover of a few vascular
256 and/or bryophyte species, as well as, in some cases, the age of the plant community and
257 water table, appeared to be the best alternative descriptors at the 'site' level (Table 6).
258 There was no significant effect of any of the site specific alternative variables in FB. Fig.
259 3 shows the directional effect of each of the significant variables in RDA at the 'site' level
260 within each individual peatland. The effects of some plant species appear to be
261 overlapping (e.g. *S. fallax* and *S. angustifolium* in FI, *E. vaginatum* and *V. oxycoccus* in
262 FR). The loadings of the CLPP carbon substrates did not show any obvious correlation
263 with any particular environmental variables tested (Fig. 3), indicating that the main effect
264 was moderation of the level of mineralisation of all substrates by changes in the *quantity*
265 of labile C inputs rather than through differential inputs of substrate types. The alternative
266 factors explaining significant components of the CLPP variance at the 'horizon' level
267 appeared to be very different in each peatland (Table 6).

268

269 **Discussion**

270 We have shown in response to our first aim (i), that there are significant factors which
271 explain CLPP variance of the microbial communities of regenerating peatlands at both the

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large (between peatlands) and the small spatial scales (within each peatland). We have shown that long-term average air temperature data could be substituted for the 'peatland' location factor. Other, unreported, location-specific climatic factors (e.g. annual rainfall) may well be further explanatory variables at this level. It is nevertheless remarkable that such a relatively low amount of the variance (26 %) was explained at such a large geographical range. Other work in different ecosystems has previously demonstrated location specificity in the community structure of soil microbial communities at various scales. For example, Stevenson *et al.* (2004) successfully used a similar technique to differentiate samples on the basis of vegetation type on a small and landscape scale. They also reported remarkably little influence of the geographical location of their study sites.

In response to our second aim (ii), the vegetation structure included species with a small but significant alternative effect on CLPP variance at the 'site' level within each peatland except FB (Table 6) and at the larger spatial scale of all peatlands combined (Table 5). It is possible that the vegetational composition only explains minor amounts of CLPP variance at the larger spatial scale because the vegetation structure of the chosen peatlands does not overlap greatly. 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; Fisk *et al.* 2003; Graham & Haynes, 2005) but the underlying cause of this change is not often identified. In this study, the largest proportion of the variance was explained by the proportional surface cover of vascular plant species. This points to their influence on the microbial community through the composition and quantity of their rhizoexudate. Glatzel et al. (2004) showed that the input of 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. Crow and Wieder (2005) showed that vascular plants contributed 35-

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297 57% of total $CO_2$ efflux from peat surfaces. The carbon measured in their study was
298 primarily derived from rhizosphere processes, i.e. from root respiration as well as
299 microbial mineralisation of root exudates. Likewise, Fenner *et al.* (2004) demonstrated
300 that *Sphagnum* spp. contributes to the pool of dissolved organic carbon, via leaching of
301 recent photosynthates. In this study, a significant amount of CLPP variance was explained
302 by *Sphagnum* cover only where there bryophytes were a predominant part of the
303 vegetation in some of the studied sites (i.e. FI, SC).

304 Significant effects at the horizon level occurred in all peatlands at both spatial
305 scales. In most cases, substrate quality indicators, such as total or soluble C or N and their
306 ratio, or the level of humification, were the major alternative explanatory factors of CLPP
307 variability. In the Baupte peatland and in the two Jura mountain sites (CH and FR),
308 however, there were predominant effects of the C or N content of the microbial biomass.
309 Dissolved organic matter (DOM) from degraded peatlands has been shown to be more
310 humified than DOM from intact peatlands and the amounts to be inversely correlated with
311 the total rates of $CO_2$ efflux from these peatlands, suggesting preferential respiration of
312 labile carbon compounds (Glatzel et al., 2003) and most of our results concur with this.
313 Hence, there are similarities in the drivers of functional microbial diversity across
314 distinctly different peatland types, in that they appear to be centred on indicators of carbon
315 substrate quality and surface vegetational constituents. Orwin et al. (2006) showed that
316 additions of carbon compounds of different chemical complexity to soil not only altered
317 the microbial community structure and carbon substrate utilisation patterns, but also
318 affected other ecosystem processes such as decomposition and plant growth. This was
319 mirrored in a study by Carney & Matson (2005). They studied soil microbial communities
320 displaying different substrate utilisation patterns which had been obtained from differing
321 experimental vegetation structures and found substantial differences in the ability of these

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consortia to decompose varying litter types. Kuzyakov and Bol (2006) demonstrated that additions of labile plant-derived compounds can accelerate the turnover of organic matter, which is a process described and debated as the 'priming effect (Mondini *et al.* 2006; Kuzyakov, 2006). Hamer & Marschner (2002; 2005) showed that the strength of the priming effect in forest, peat and arable soils depended on the nature of the added labile carbon source. Our data suggest that the influence of establishing surface vegetation on cutover peatlands on microbial CLPP is predominantly a function of the quantity of additional labile carbon rather than the composition of it.

The ultimate goal of ecosystem restoration can be either one or both of the following (Harris, 2003):

- Approximation to a target or reference system
- Maximisation of ecosystem efficiency with respect to its function

Harris (2003) advocated that the monitoring of the microbial community response is a more authentic and useful indicator of change than vegetation within a restored ecosystem as the vegetation is too easily manipulated within the framework of active peatland restoration efforts, e.g. by brush removal and *Sphagnum* re-introduction (Rochefort & Price, 2003). The difficulty in monitoring the success of peatland restoration is in finding comparable reference sites. This is exemplified in our study by the fact that the 'reference' site in Scotland was itself previously affected by peat extraction and was also therefore still in recovery. Within the Chaux d'Abel peatland, the intact reference is located close to the most advanced site of regeneration and may therefore be similarly affected through negative effects on the site hydrology. The use of a number of 'reference' sites, however, as in this study, may afford the possibility to compare trends in the recovery of these ecosystems. In all cases where significant site separation was observed, bare sites (where present) were separated from revegetated sites and the trend along the primary explanatory

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axis followed the predefined 'regeneration stages' (Fig. 3). In addition, the trend at the Scottish site for the microbial functional response to natural revegetation agrees with a similar trend in the taxonomic description and species diversity of the fungal populations which followed a similar trajectory with regeneration stage (Artz *et al.*, Submitted). Hence the functional attributes of peatland microbial diversity may mirror structural changes in the microbial community. Therefore, in response to our third aim (iii), the observed relationships between the functional microbial community response to peatland regeneration and the occurrence of particular sedge species indicate that it may be possible (with further research) to distinguish vegetational characteristics that are indicative of a return of the microbial ecosystem functioning. The dominant factors of location and horizon-specific differences, however, complicate such investigations.

Basiliko *et al.* (2005) recently showed data suggesting that climate-change induced variations in microbial respiration could substantially change the contribution to net ecosystem respiration and hence may induce changes in the net carbon balance of peatland ecosystems. Mitchell *et al*. (2003), for example, detected changes in the microbial community composition of peat under elevated carbon dioxide atmospheres. Degens *et al.* (2000) showed, for a range of organic soils, that decreases in the soil organic C reserves can reduce the catabolic potential (in their case defined as the evenness of the substrate utilisation patterns) of the soil microbial community, which impact on the rates of $CO_2$ emissions and decomposition. In the light of possible additional climatic pressures on regenerating peatland ecosystems, studies of the potential responses of the microbial community to altered substrate inputs may help us to focus restorative efforts in peatlands in a way that considers more than just the aboveground habitat.

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**Table 1.** Sample location and site descriptions for vegetation and time since abandonment.

| Peatland | Location elevation) | 10-y average air temp. (°C) | Site code | Dominant vegetation, listed in order of abundance | Age of plant community (y) | Plant cover (%) | Regeneration stage[a] | Average annual total respiration (mg $CO_2$ $m^{-2}$ $h^{-1}$) | Average annual watertable (cm) |
|---|---|---|---|---|---|---|---|---|---|
| Chaux d'Abel, Jura Mountains, Switzerland | 47°10 N, 6°57' E, 1040 m | 6.4 | A | *S. fallax, P. strictum, P. commune, Eriophorum spp.* | >29 | Complete | E | 664 | 16 |
| | | | B | *Same species, intermediate between A and C* | >42 | Complete | A | 789 | 14 |
| | | | C | *S. fallax, P. strictum, E. vaginatum, Vaccinium spp.* | >51 | Complete | A | 880 | 16 |
| | | | D | *S. magellanicum, S. fuscum, S. rubellum, Vaccinium spp.*(no survey data) | Intact | Complete | I | ND [b] | ND |
| Baupte, France | 49°17 N, 1°21' W, 20 m | ND | A | *Bare peat, no vegetation* | 5-10 | 0 | B | 599 | 61 |
| | | | B | *E. angustifolium, Hypnacaea* | 5-10 | 79 | E | 326 | 55 |
| Aitoneva, Finland | 62°12' N, 23°18 E, 156 m | 4.2 | A | *Eriophorum vaginatum* [d] | 10 | 80 | E | 386 | -10 [c] |
| | | | B | *Eriophorum vaginatum* [d] | 10 | 24 | E | 241 | 10 |
| | | | C | *Carex rostrata* [d] | 10 | 42 | E | 220 | -30 |
| | | | D | *C. rostrata, S. fallax* [d] | 10 | Complete | E | 70 | -18 |
| | | | E | Bare peat, no vegetation [d] | 10 | 0 | B | 57 | 1 |
| Le Russey, Jura Mountains, France | 47°18' N, 6°79' E, 867m | 7.7 | A | Bare peat | >4 | 0 | B | 65 | 5 |
| | | | B | *Sphagnum fallax, E. angustifolium, E. vaginatum (rare)* | >22 | Complete | E | 282 | 14 |
| | | | C | *S. fallax, E. angustifolium, E. vaginatum, Calluna vulgaris* | >21 (<40) | Complete | E | 419 | 14 |
| | | | D | ND (no survey data) | Intact | Complete | I | ND | ND |
| Middlemuir Moss, Scotland, United Kingdom | 57°36' N, 2°9' W, 110m | 8.0 | A | Mostly bare, isolated *E. vaginatum*, *Campylopus. introflexus* | <5 | 5 | B | 50 | 27 |
| | | | B | *S. cuspidatum, S. auriculatum, E. vaginatum* | 5-10 | Complete | E | 117 | -1 |
| | | | C | *E. angustifolium, S. auriculatum, S. cuspidatum* | 5-10 | Complete | E | 168 | -1 |
| | | | D | *Sphagnum spp., C. vulgaris, Deschampsia flexuosa* | >50 | Complete | A | 296 | 11 |

[a] B – bare, E – early, A – advanced, I – Intact. [b] ND - not determined. [c] Negative average watertable indicates site with periodic flooding. [d] Survey data only available as averages.

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**Table 2.** Variance decomposition of the effects of 'peatland', 'site', 'core' and 'horizon depth' on peat microbial CLPP, at the larger spatial scale.

| Component | Variable tested | Covariable (also defines permutation in blocks) | Degrees of freedom (DF) | Whole plots represent | Variance explained (%)[a] |
|---|---|---|---|---|---|
| Peatland (P) | P | - | 4 | S | 26.0 *** |
| Site (S) | S | P | 16 | C | 2.3 * |
| Core (C) | C | S | 50 | H | 2 % $^{NS}$ |
| Horizon depth (H) | H | C | 225 | None (unrestricted design) | 11.7 *** |
| | | **Total variance explained** | | | **40.0** |

[a] Estimated using MonteCarlo permutation testing (999 permutations) in RDA within blocks defined by the co-variables: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ and $^{NS}$ not significant. N/A - not applicable.

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**Table 3:** Variance decomposition of the effects of 'site', 'core' and 'horizon depth' on peat microbial CLPP at the smaller spatial scale of individual peatlands.

| Peatland | Component | Variable tested | Covariable (also defines permutation in blocks) | DF | Whole plots represent | Variance explained (%)[a] |
|---|---|---|---|---|---|---|
| CH | Site (S) | S | N/A | 3 | C | 19.5 *** |
| | Core (C) | C | S | 8 | H | 1.8 $^{NS}$ |
| | Horizon depth (H) | H | C | 36 | None (unrestricted) | 20.4 *** |
| | | | **Total variance explained** | | | **39.9** |
| | | | | 3 | | |
| FR | Site (S) | S | N/A | 8 | C | 23.7 *** |
| | Core (C) | C | S | 36 | H | 1.8 $^{NS}$ |
| | Horizon depth (H) | H | C | | None (unrestricted) | 36.4 *** |
| | | | **Total variance explained** | | | **60.1** |
| SC | Site (S) | S | N/A | 3 | C | 13.9 *** |
| | Core (C) | C | S | 8 | H | 1.5 $^{NS}$ |
| | Horizon depth (H) | H | C | 36 | None (unrestricted) | 23.4 *** |
| | | | **Total variance explained** | | | **37.3** |
| FI | Site (S) | S | N/A | 4 | C | 12.7 *** |
| | Core (C) | C | S | 10 | H | 1.3 $^{NS}$ |
| | Horizon depth (H) | H | C | 45 | None (unrestricted) | 26.0 *** |
| | | | **Total variance explained** | | | **38.7** |
| FB | Site (S) | S | N/A | 1 | C | 6.0 $^{NS}$ |
| | Core (C) | C | S | 4 | H | 8.9 $^{NS}$ |
| | Horizon depth (H) | H | C | 18 | None (unrestricted) | 43.2 *** |
| | | | **Total variance explained** | | | **43.2** |

[a] Estimated using MonteCarlo permutation testing (999 permutations) in RDA within blocks defined by the co-variables: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ and $^{NS}$ not significant.

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**Table 4**. Summary of mean, SD and range of various physico-chemical and biological properties of peat samples from 5 European peatlands.

| Variable | Abbreviation | CH | | FR | | SC | | FI | | FB | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Range | Mean | Range | Mean | Range | Mean | Range | Mean | Range |
| **Physico-chemical** | | | | | | | | | | | |
| Total carbon (%) | TC | 48.1 | 27-57 | 52.3 | 46-57 | 53.0 | 43-60 | 53.8 | 39-61 | 52.4 | 48-55 |
| Total nitrogen (%) | TN | 2.1 | 1-4.9 | 1.9 | 0.7-2.8 | 1.5 | 0.9-2.0 | 1.3 | 0.5-2.4 | 2.4 | 2.0-2.9 |
| C/N ratio | C/N | 27 | 10-55 | 29.4 | 18-65 | 37.7 | 23-64 | 44.1 | 22-108 | 21.6 | 18-27 |
| Soluble organic carbon (µg C g dry peat$^{-1}$) | SOC | 1056 | 277-2130 | 828 | 194-2117 | 1418 | 304-1969 | 1102 | 665-1807 | 613 | 370-1030 |
| Soluble organic nitrogen (µg N g dry peat$^{-1}$) | SON | 299 | 139-661 | 244 | 64-425 | 193 | 17-1455 | 160 | 44-389 | 99.8 | 52-157 |
| C/N ratio (solubles) | Sol C/N | 3.95 | 1-8.6 | 4 | 0.6-9.6 | 11.5 | 2.2-35.9 | 8.3 | 2.7-15 | 6.3 | 4.2-10.3 |
| Level of humification | PS-COO | 1.88 | 0.95-3.44 | 1.56 | 1.06-2.74 | 1.56 | 0.77-3.39 | 1.49 | 0.85-3.99 | 0.72 | 0.57-0.91 |
| **Microbiological** | | | | | | | | | | | |
| Microbial biomass C (mg C l$^{-1}$) | C (mic) | 234 | 25-586 | 184 | 25-576 | 92 | 2.8-414 | 42 | 6-87 | 32.4 | 1.7-140 |
| Microbial biomass N (mg N l$^{-1}$) | N (mic) | 36.5 | 1-95 | 25.2 | 1.3-83 | 16.7 | 0.6-82.6 | 5.3 | 0.3-19 | 5.5 | 0.6-16.7 |
| Microbial biomass C:N | C/N (mic) | 13.3 | 2.2-62.8 | 21.2 | 2.8-118.4 | 32.3 | 1.3-226.0 | 23.3 | 0.9-149.9 | 9.9 | 2.0-26.4 |

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**Table 5.** Effect of sample-specific environmental variables on sample variance at the larger spatial scale. The main significant hierarchical variance components (none, P and C, respectively, see Table 2) were used as covariates in partial RDA.

| Level in hierarchy | Alternative environmental variable | % variance explained by individual variables | % variance explained after forward selection |
|---|---|---|---|
| 'Peatland' | 10-year average air temperature | 4.2 *** | 4.2 *** |
| | | **Total variance explained** | **4.2** |
| 'Site' | Cover of *Sphagnum cuspidatum* (%) | 1.6 * | 1.6 * |
| | Cover of *Sphagnum angustifolium* (%) | 0.7 ** | NS |
| | Cover of *Carex nigra* (%) | 1.4 ** | 0.8 * |
| | Cover of *Eriophorum vaginatum* (%) | 1.3 ** | NS |
| | Cover of *Eriophorum angustifolium* (%) | 1.0 *** | 1.0 * |
| | Cover of *Erica tetralix* (%) | 1.0 * | NS |
| | Cover of *Betula nana* (%) | 0.7 * | NS |
| | Cover of *Molinia caerulea* (%) | 0.5 * | NS |
| | % bare peat | 1.2 ** | 1.2 ** |
| | Average annual total respiration | NS | NS |
| | Minimum age of plant community | 1.1 * | 1.1 * |
| | Average annual watertable | 0.6 * | 0.6 * |
| | | **Total variance explained** | **6.4** |
| 'Horizon depth' | Total C | 2.5 ** | 1.7 *** |
| | Total N | NS | 1.0 * |
| | C:N ratio | 3.0 *** | 2.5 *** |
| | Soluble C | NS | NS |
| | Soluble N | NS | 1.4 ** |
| | C/N ratio (solubles) | 3.7 *** | 5.0 *** |
| | Level of decomposition (PS-COO) | 4.7 *** | 4.7 *** |
| | Microbial biomass C | 2.2 ** | NS |
| | Microbial biomass N | 2.7 ** | 3.8 *** |
| | Microbial biomass C:N | 1.6 ** | NS |
| | | **Total variance explained** | **30.5** |

[a] Level of significance determined by Monte-Carlo permutation testing (999 repeats): *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ and NS not significant.

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**Table 6.** Effect of alternative environmental variables[a] on sample variance of CLPP within the 'site' and 'horizon' level of each individual peatland.

| **Peatland** | **CH** | **FR** | **SC** | **FI** | **FB** |
|---|---|---|---|---|---|
| **Variable** | | | | | |
| **'Site' level** | | | | | |
| Cover of *Sphagnum cuspidatum* (%) (S-cus) | NS | N/A | 1.6 * | NS | NS |
| Cover of *Sphagnum angustifolium* (%) (S-ang) | N/A | N/A | N/A | 2.1 ** | NS |
| Cover of *Sphagnum fallax* (%) (S-fal) | N/A | NS | N/A | 5.9 *** | NS |
| Cover of *Carex nigra* (%) (C-nigr) | 9.4 *** | NS | N/A | N/A | NS |
| Cover of *Eriophorum vaginatum* (%) (E-vag) | 3.6 *** | 10.9 *** | NS | 4.4 ** | NS |
| Cover of *Eriophorum angustifolium* (%) (E-ang) | N/A | 1.6 * | NS | N/A | NS |
| Cover of *Erica tetralix* (%) (E-tet) | N/A | N/A | 10.6 *** | N/A | NS |
| Cover of *Betula nana* (%) (B-nana) | 6.2 * | N/A | N/A | N/A | N/A |
| Cover of *Molinia caerulea* (%) (M-caer) | 4.4 *** | NS | NS | N/A | N/A |
| Cover of *Vaccinium oxycoccus* (%) (V-oxy) | NS | 2.4 * | N/A | N/A | NS |
| Minimum age of plant community (age) | NS | 9.8 * | NS | N/A. | NS |
| Water table (WT) | 2.1 * | NS | 2.5 * | NS | NS |
| **Total variance explained (%)** | **25.7** | **24.7** | **14.7** | **12.3** | **NS** |
| **'Horizon' level** | | | | | |
| Total C | NS | NS | NS | NS | NS |
| Total N | NS | NS | NS | NS | NS |
| C:N ratio | NS | 5.1 * | NS | NS | NS |
| SOC | NS | NS | NS | 3.2 * | NS |
| SON | NS | 14.2 *** | NS | 21.5 *** | NS |
| Solubles C/N | NS | 7.6 *** | NS | NS | NS |
| Level of decomposition | 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 annual total respiration | NS | NS | NS | NS | NS |
| **Total variance explained (%)** | **7.8** | **43.5** | **41.2** | **24.6** | **46.7** |

[a] Only significant variables determined using forward selection are shown. Significance determined by Monte-Carlo permutation testing (999 repeats): *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, NS not significant. N/A - not applicable, where this species was not described as part of the plant community.

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**Figure legends**

**Fig. 1.** Community level physiological profiles obtained from the regeneration gradient at Middlemuir Moss, Scotland. As well as variation in the CLPP between the different stages of regeneration (sites), a general decrease in the amount of substrate utilisation with depth, from the surface horizons (Horizon 3 and 4) to the catotelm layers (Horizons 6 and 8) can be observed. Sites are indicated as follows: Site A (open bars), Site B (grey bars), Site C (hatched bars) and Site D (filled bars).

**Fig. 2.** Ordination plot of the results of PCA of the CLPP dataset. The sample distribution shows a clear effect according to peatland. Samples from each peatland are shown as open circles (CH), upward open triangles (FB), downward open triangles (FI), filled triangles (FR) and filled squares (SC).

**Fig. 3.** Ordination plot of the effects of alternative explanatory variables in RDA on CLPP data from each individual peatlands at the 'site' level. Effects of environmental variables which contribute significantly (see Table 6, also for abbreviations used) to explaining CLPP variance are shown as projected arrows. Sites shown are as described in Table 1 for each peatland, respectively, and are depicted as follows in the graphs: Site A (open circles), Site B (downward open triangles), Site C (upward open triangles), Site D (filled squares) and Site E (FI only, filled circles). Loadings of substrates are shown as crosses. N.B. Missing values in the environmental dataset lead to averaging of the CLPP data for such data (e.g. vegetation at FI reported as averages only, see Table 1; hence only one corresponding CLPP point).

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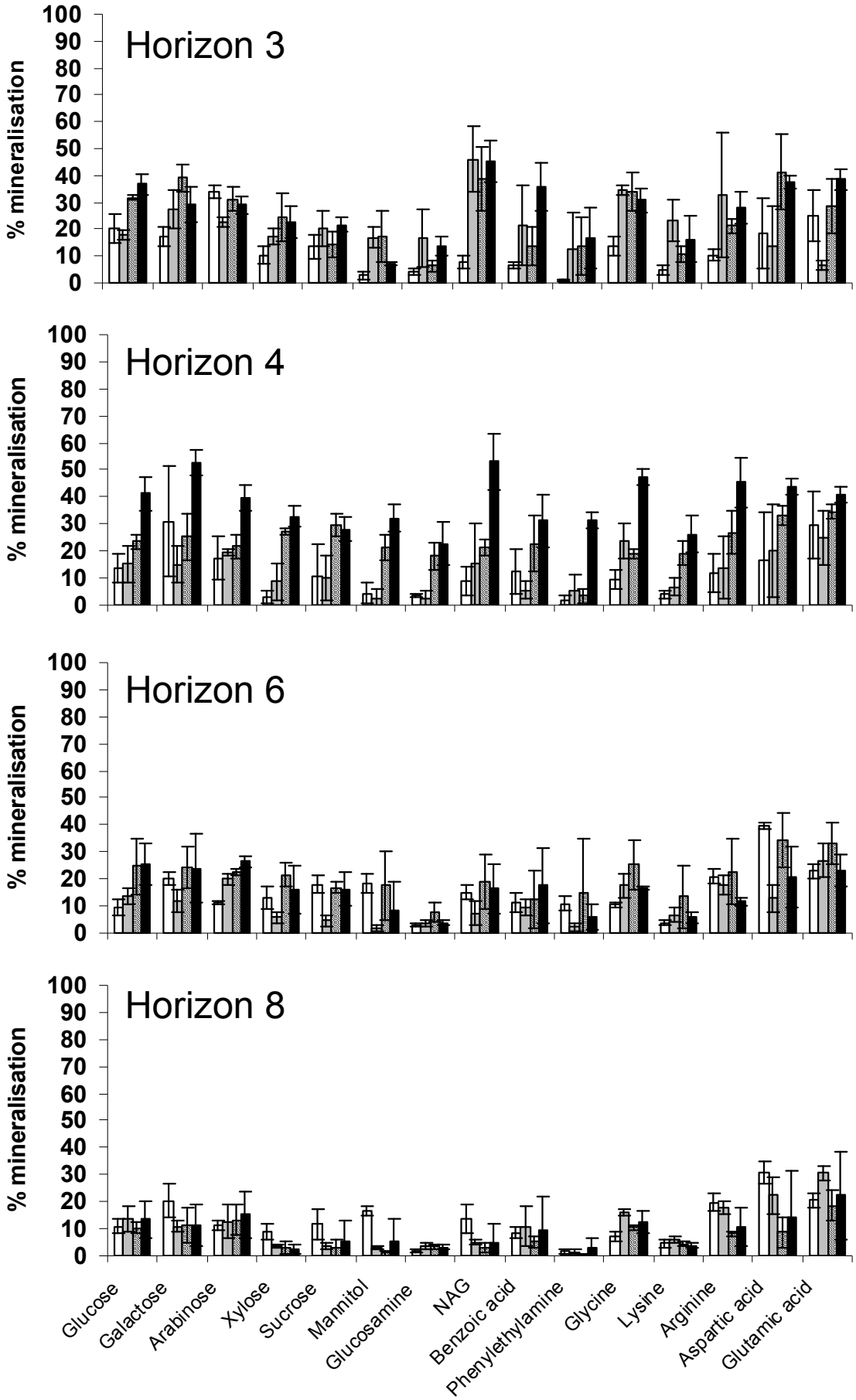


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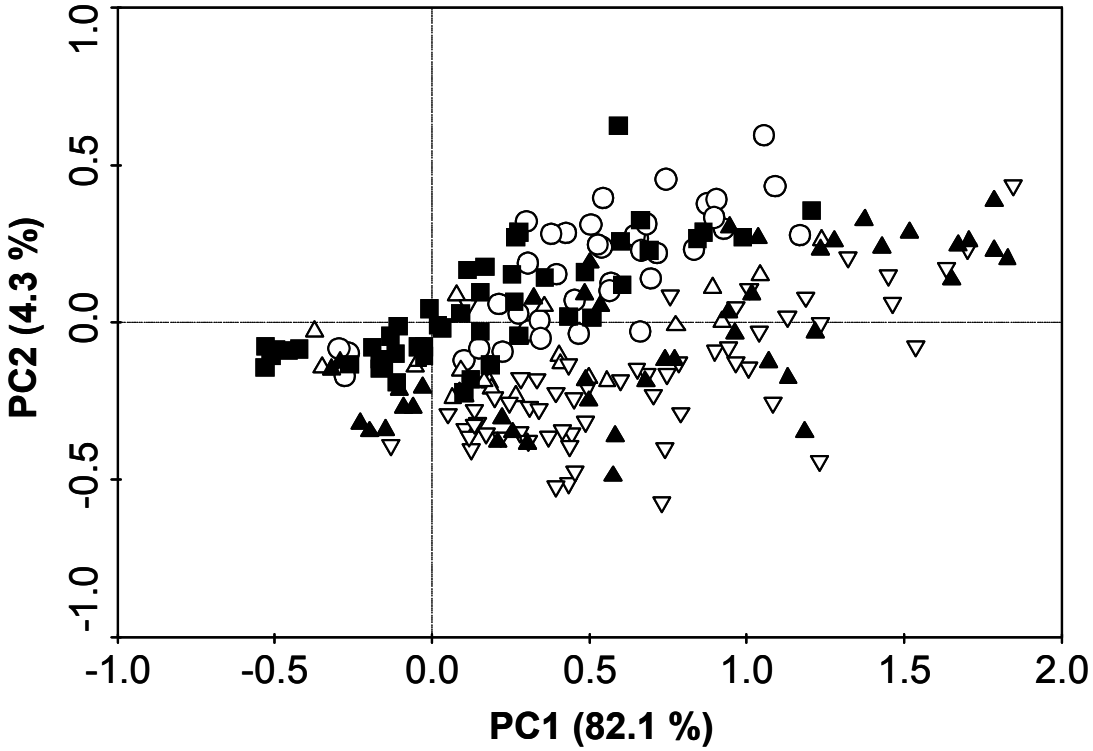


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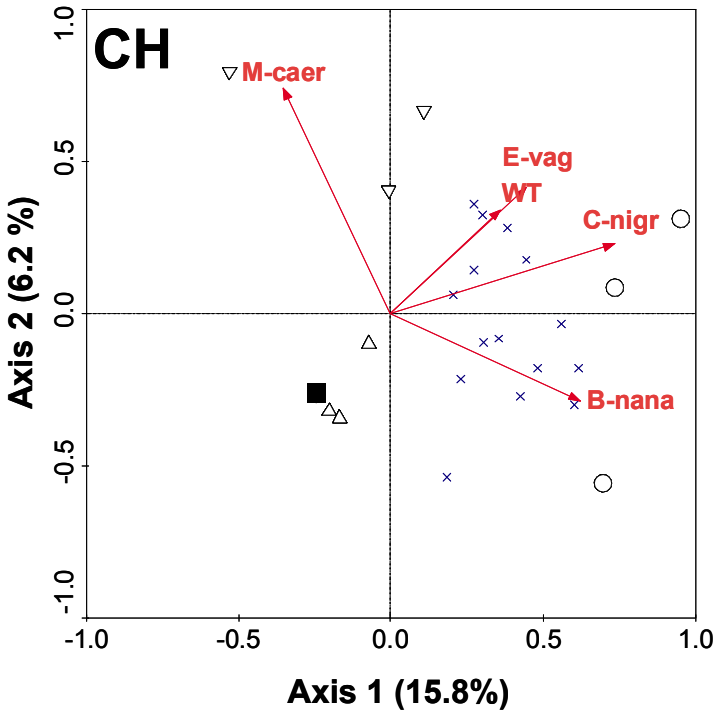


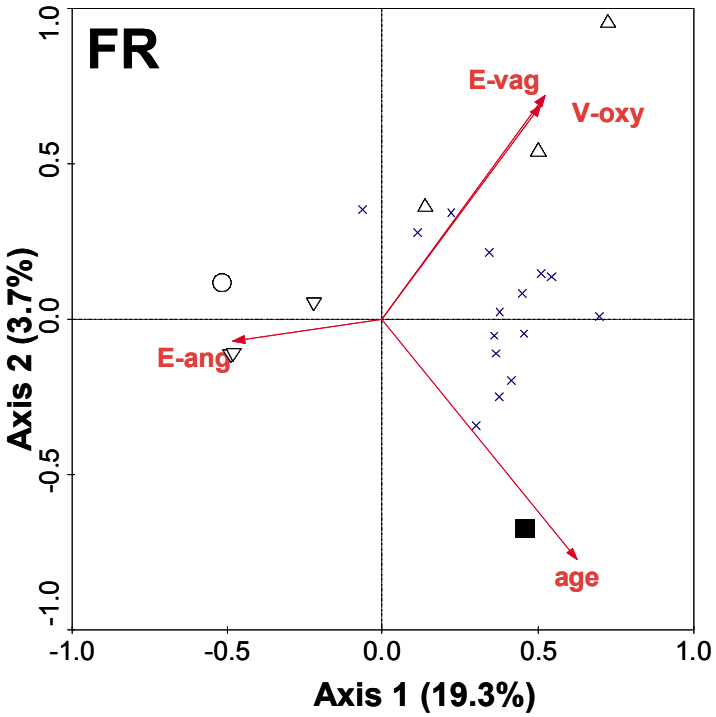


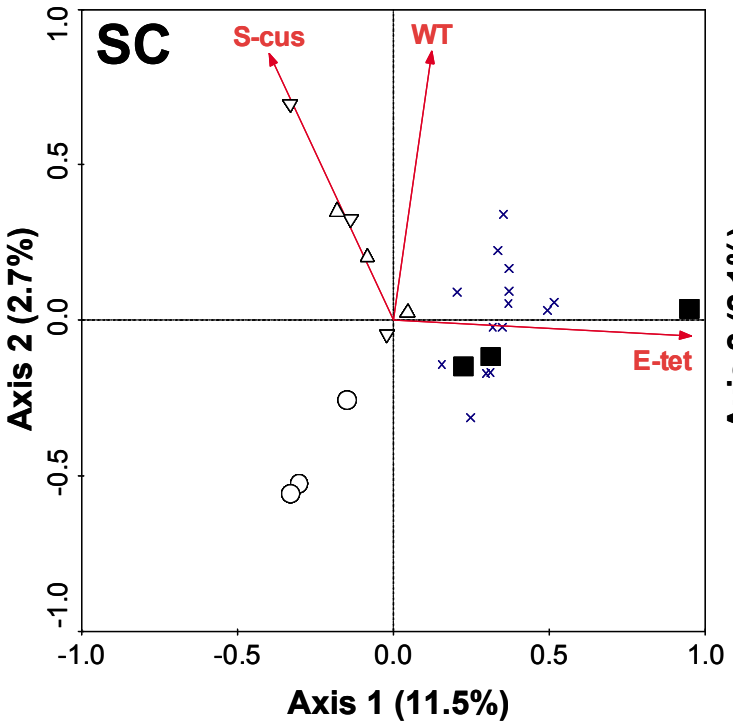


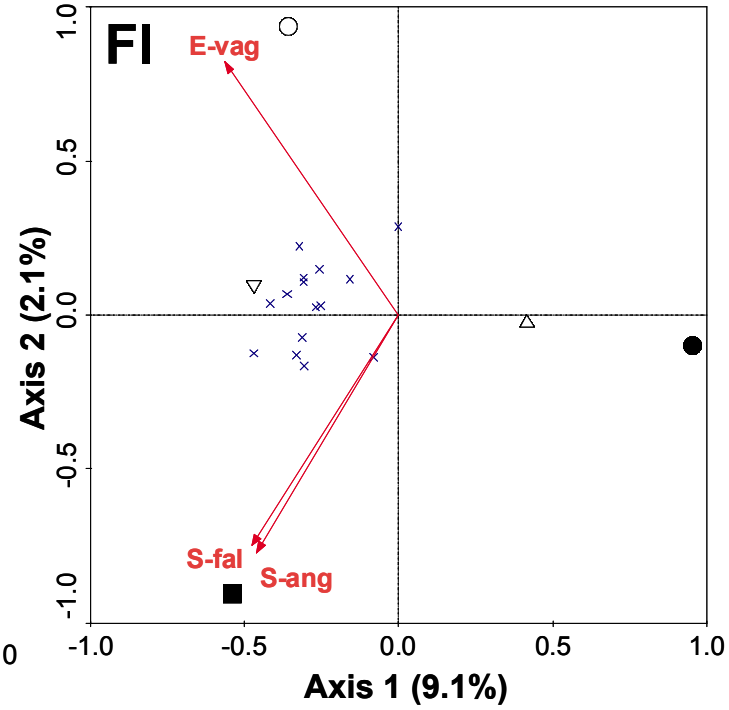


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