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Title: Microbial "hotspots" of organic matter decomposition in temperate peatlands are driven by local spatial heterogeneity in abiotic conditions and not by vegetation structure

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Abstract

Climate change is triggering rapid shifts in plant communities and alterations in soil abiotic conditions in peatlands, with cascading effects on belowground decomposers and ecosystem C turnover. However, elucidating the dominant causal relationships between plant communities, soil biota and C fluxes in these vulnerable ecosystems requires a better understanding of the spatial-temporal variability of abiotic and biotic drivers. In this study we investigated the effects of biotic (plant functional types, PFTs) and abiotic factors (soil temperature and soil moisture) in determining dynamic patterns of soil microbial community structure and C cycling. Four representative temperate peatland habitats were selected based on their peat forming vegetation – an Atlantic wet heathland, two active blanket bogs with herbaceous plants (*Molinia caerulea* and *Eriophorum angustifolium*), and a transition mire dominated by *Sphagnum* mosses located along an altitudinal gradient to include the natural variations in soil temperature and water content regimes. We found that peat microbial communities were more strongly linked to local abiotic conditions than to the dominant above-ground vegetation. Aerobic conditions and warmer temperatures accelerated fungal driven decomposition and CO_2 emissions under shrubs, whereas decreases in Gram−negative bacteria promoted increased C losses under *Molinia*. These findings suggest that small spatial differences in abiotic conditions can create local "hotspots" of organic matter decomposition. We propose that temperate peatlands should be considered as 'ecosystem sentinels' for climate change, acting as early-warning indicators of climate-carbon feedbacks.

Keywords: carbon, climate change, microbial communities, peatland habitats, plant functional type, spatio-temporal patterns

1. Introduction

The majority of the world's peatlands occur in boreal and temperate parts of the Northern Hemisphere where they cover around 3.5 million km^2 of land and store about 455 Gt of carbon (C), representing around 25% of all the soil C stored on earth (Moore, 2002). They are complex ecosystems, consisting of habitat mosaics containing plant species that form peat under high precipitation-low temperature climatic regimes that restrict decomposition, leading to carbon accumulation. Their plant communities are dominated by different functional types (PFTs) as defined by their growth forms (e.g. vascular woody plants, herbaceous forbs and graminoids and non-vacular plants including bryophytes; Dorrepaal, 2007). The PFTs supply a wide range of food sources (as litter and root exudates) to below-ground decomposers with cascading effects on ecosystem C turnover (De Deyn et al., 2008; Ward et al., 2015; Chen et al., 2016). In addition to nutrient inputs, the abiotic conditions are also key abiotic regulators of decomposer activities, with soil temperature and moisture determining anaerobic and aerobic processes (Cobb et al., 2017; Morton and Heinemeyer, 2019), and temperature defining the activation energy of biochemical reactions (Davidson and Janssens, 2006).

Consequently, climate change is expected to cause profound alterations in peatland hydrology that will increase rates of decomposition (Ise et al., 2008; Waddington et al., 2015). In addition, some projections forecast a functional shift in peatlands plant communities to favour vascular plants over mosses (e.g., Gallego-Sala and Prentice, 2013; Dieleman et al., 2015), which could exacerbate C losses (Walker et al., 2016; Robroek et al., 2016; Malhotra et al., 2020). As a result, concerns have risen about these critical C reservoirs becoming the largest natural global sources of C, with temperate peatlands being more likely to have a greater greenhouse gas contribution than their northern counterparts due to their longer and warmer growing seasons (Limpens et al., 2008; Teh et al., 2011).

When analysing the temperature sensitivity of peat C decomposition and potential feedbacks to climate change, the interactions between abiotic and biotic factors have been recognised as regulators of C cycling in these ecosystems (Briones et al., 2014; Armstrong et al., 2015; Juan-Ovejero et al., 2020). However, linking abiotic and biotic drivers of peatland C dynamics is challenged by the variability in plant-soil interactions even a small spatial scales. For example, in the particular case of peatlands, decomposition will vary through acrotelm and catotelm layers (Lunt et al., 2019), and as a result, the above- and below-ground phenologies are often unparallel (Schwieger et al., 2019). This could explain the contradictory responses reported in the literature, where certain PFTs have been found to strongly influence carbon dioxide (CO_2)

fluxes (Ward et al., 2013; Armstrong et al., 2015), whereas other studies concluded that abiotic factors are the main drivers of CO_2 production irrespective of PFTs (Preston et al., 2012; Haynes et al., 2015). Similarly, while some studies have detected correlative relationships between different PFTs and DOC (Armstrong et al., 2012), others have concluded that plant control on DOC release is indirect through their influence on soil fauna (Carrera et al., 2009; Juan-Ovejero et al., 2020).

Therefore, elucidating the dominant causal relationships between PFTs, soil biota and C fluxes in these ecosystems requires spatially and temporally extensive assessments of biotic and abiotic factors in field environments. Previous studies have shown that temporal variations of soil abiotic conditions across different PFTs result in profound alterations of soil mesofauna community structure as a consequence of their different ecophysiological adaptations to water table drawdown (Juan-Ovejero et al., 2019). However, there is a distinct lack of data on similar temporal changes in microbial community responses in such microhabitats, and the potential implications for the C sink/source function (see review by Zhong et al., 2020).

In this study, we aimed to disentangle the effects biotic (PFTs) and abiotic drivers (soil microclimatic conditions) on temperate peatland microbial community structure and C cycling. We selected four representative temperate peatland habitats based on their peat forming vegetation (Atlantic wet heathland (*Erica mackayana* and *Calluna vulgaris*), two active blanket bogs with herbaceous plants (*Molinia caerulea* and *Eriophorum angustifolium*), and a transition mire dominated by *Sphagnum* mosses) located at different elevations to include the natural altitudinal gradient in soil temperature and water content regimes (Bragazza et al., 2015). We hypothesized that distinct microbial communities will be associated with different PFTs (i.e., vascular vs. non-vascular), irrespective of their spatial location, in agreement with other studies linking peatland habitats to specific microbial taxa (Chroňáková et al., 2019). However, based on microbial responses to abiotic factors (e.g., Bragazza et al., 2015; Kumar et al., 2019), we also hypothesised that greater seasonal variations in temperature and moisture will determine changes in microbial community structure over time disregarding PFT. Finally, in addition to microclimatic conditions, litter quality differences among PFTs also drive microbial decomposition processes and accordingly, we expected a higher C turnover under a greater supply of more decomposable plant litter. *Sphagnum* mosses and shrubs have large concentrations of high molecular weight polyphenolic compounds they are very resistant to microbial attack (Hattenschwiler and Vitousek, 2000; Fenner and Freeman, 2011). Similarly, the cotton-grass *Eriophorum angustifolium* produces litter that is low in nutrient content than other vascular species and hence, its decomposition rates are similar to those of shrubs (Trinder et al.,

2008). In contrast, the graminoid *Molinia caerulea* is a fast growing grass that produces nutrient-rich litter (Certini et al., 2015; Kaštovská et al., 2018), proving a much greater supply of labile C to decomposers. Since previous modelling exercises have shown that C exports in these systems are abiotically mediated via direct and indirect effects on the mesofauna populations (Juan-Ovejero et al., 2020), we assessed if abiotic factors are also the major drivers of microbial decomposition, while above-ground vegetation composition acts as secondary modifier.

2. Materials and Methods

2.1. Peatland habitats

The study area is located in "Serra do Xistral'' (NW of the Iberian Peninsula) within the Atlantic Biogeographical Region. Data from the nearest meteorological station (Fragavella 43° 27" 16.56'' N, 7° 26" 46.5'' W; 710 m a.s.l.) indicate that the area is characterised by an oceanic climate, with a mean annual temperature of 10.5 °C (ranging from 6.0 °C in February to 16.0 °C in August) and annual rainfall of 1533 mm in the 17 years prior to sampling. Similar temperature records were observed during the two years of study (2016 and 2017). However, 2017 was drier than 2016, with 25% less precipitation falling throughout the year (as result of the contrasting extreme rainfall values recorded in January of both years and the low precipitation records observed in July, September and October of 2017 compared with 2016; Fig. S1).

Four different peatland habitats with functionally different plant communities (*sensu* Dorrepaal, 2007) were selected. Two of them were active blanket bogs (Nat-2000 7130) with herbaceous vascular plants: one dominated by the common cotton grass *Eriophorum angustifolium* and the endemic species of the Iberian NW *Carex durieuii* belonging to the Cyperaceae family (sedges) (43° 30' 12'' N, 7° 33' 02'' W; 970 m a.s.l.) and the other by the deciduous *Molinia caerulea*, a true grass belonging to the Poaceae family (43° 27' 36'' N, 7° 34' 12'' W; 960 m a.s.l.). The other two habitats were located in a valley (43° 26' 56'' N, 7° 33' 61'' W; 714 m a.s.l.): an Atlantic wet heathland Nat-2000 4020) where *Erica mackayana* but also *Calluna vulgaris* (woody vascular plants) colonize the drier fringes, and a transition mire (Nat-2000 7140) represented by pioneer communities associated with the existence of areas that receive a certain inflow of water, on which discontinuous tapestries of *Sphagnum* spp. are established (non-vascular) together with other hygrophilic plants (e.g. *Drosera* sp*., Rynchospora alba*). The selection is also justified by the amount of exhaustive background information in the form of flora inventories and habitat maps that is available (e.g. Izco Sevillano and Ramil-Rego, 2001; Ramil-Rego and Izco, 2003; Rodríguez-Guitián et al., 2009; Cillero et al., 2016).

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147 *2.2. Field sampling*

148 Intact peat samples were collected every two months at each peatland habitat during 2016 and
149 2017 (January to November; 12 samplings in total).

150 On each sampling occasion, to determine soil moisture at each habitat ten intact soil cores (PVC
151 pipes, 10 cm diameter x 10 cm depth) were randomly taken and oven-dried at 105 °C for 48 h
152 or until constant weight on re-weighing. Another subsample of fresh soil from each core was
153 freeze-dried and sieved (< 2 mm) and the total C and nitrogen contents determined by means
154 of a LECO elemental analyser (CN-2000, LECO Corp., St Joseph, MI).

155 Hourly soil temperature was recorded at 5 cm soil depth in each habitat for the duration of the
156 study using a temperature data logger (UA-002-08 HOBO). Due to temporal data acquisition
157 failures, 8% of temperature data were gap filled by triangulating temperature data from the
158 three nearest meteorological stations (for full details of the extrapolation procedure see Juan-
159 Ovejero et al., 2019).

160 Soil respiration was measured by inserting five PVC cylindrical collars (10 cm diameter × 10 cm
161 depth) into the soil (to a depth of 8 cm and approximately 2 cm remaining above the soil surface)
162 at each habitat on the first sampling occasion (January 2016), which remained in place for the
163 entire investigated period. We did this to avoid an overestimation of the soil CO_2 efflux
164 associated with perturbations due to the insertion of the PVC collars (Heinemeyer and
165 McNamara, 2011; Jovani-Sancho et al., 2017). We measured respiration rates ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)
166 every two months (since March 2016) from all cores using a LI-8100 automated soil CO_2 flux
167 system (LI-COR Biosciences, Lincoln, Nebraska, USA) connected to a 10 cm survey chamber.

168 For DOC determinations, three additional intact soil cores of smaller size (PVC pipes, 5.5 cm
169 diameter × 10.5 cm depth) were also collected at each habitat on each sampling occasion. Soil
170 samples were leached by immersion in 200 ml of distilled water and draining under gravity
171 (Anderson and Ineson, 1982). The leachates were filtered (FilterLab® No. 1252, 7–9 µm pore
172 size) and frozen until analysis. Total dissolved organic C in the microbial extracts and leachates
173 was measured with a Shimadzu Total Organic Carbon Analyser (TOC-5000A) equipped with an
174 autosampler ASI-V. The pH of the soil solutions was also measured using a Crison micropH 2000
175 and combination electrode.

176 Another set of three soil cores of the same size as before (PVC pipes, 5.5 cm diameter x 10.5 cm
177 deep) were also taken from each peatland habitat on each sampling occasion, and frozen at -20

°C. These were subsequently freeze-dried (Christ alpha 1-4 LD Plus) and then sieved to 2 mm. Stones and roots were removed and the remaining soil was ball milled (Fritsch Planetary Mill Pulviresette 5) to a fine powder. Bulked subsamples of the 0-10 cm freeze-dried ground soil (≈ 1 g dry weight) were used for PLFA analyses to determine the microbial community structure under each system.

2.3. PLFA profiling

PLFA biomarkers were extracted as part of the total lipid extract of freeze-dried soil samples using a modified Bligh-Dyer extraction (White et al., 1979). Identification of PLFA's was carried out on a GC (Agilent Technologies 6890) fitted with a mass selective detector (Agilent technologies 5973). The straight-chain saturated fatty acids (14:0, 15:0, 16:0, 18:0 and 17:1ω8) were considered to be general bacterial markers (Willers et al., 2015). The terminal and mid-chain branched fatty acids 15:0i, 15:0a, 16:0i, 17:0i and 17:0a were used as indicators of Gram−positive bacteria (Whitaker et al., 2014) together with the branched saturated br17:0 and br18:0 (Seifert et al., 2011) and the methyl branched saturated fatty acid 7Me-17:0 (Willers et al., 2015). Cyclopropyl saturated (7 cyclic 17:0 and 7,8 cyclic C19:0) and monounsaturated fatty acids (16:1ω7, 16:1ω7, 18:1ω5 and 18:1ω7) were used as indicators of Gram−negative bacteria (Rinnan and Baath, 2009). The fatty acids 18:2ω6,9 was taken as indicator of fungi (Kaiser et al., 2010). Due to the poor correlation between 18:2ω6,9 and 18:1ω9 that makes the latter biomarker a poor indicator of fungi (Frostegård et al., 2011), this and two other monounsaturated fatty acids (16:1 and 19:1) were assigned to the “unspecific microbial biomarkers” category. Each identified PLFA was quantified as μg g^{-1} dwt soil. Total microbial biomass was taken as the sum of all identified PLFA's (n = 23). See also Table S1 for the full list of PLFA markers used for taxonomic microbial groups and microbial indicators.

2.4. Statistical analyses

Data were checked for normality and homogeneity of variances using the Kolmogorov–Smirnov and Levene’s tests, respectively, and transformed where necessary before running parametric analyses. We first tested for significant differences in microbial biomarker abundances between different PFTs across the whole study as well as per sampling date using ANOVA (Generalised Linear Model or GLM) followed by the Tukey's Studentized range tests. In addition, we used linear regression analyses to detect any potential relationships between the concentrations of

the different PLFA biomarkers and the two independent variables (soil water content and soil temperature values) across PFTs. Both types of analyses were performed using SAS system v9.3 (SAS Institute, Cary, NC, USA, 2011).

Since biological responses to changes in the environment are nonlinear but unimodal, we also used Detrended Canonical Correspondence Analysis (DCCA) to identify the best set of response variables that explain the observed temporal patterns of variation in microbial community structure (ter Braak, 1986). Therefore, we analysed the relationships between the microbial communities and the environmental gradients in abiotic soil properties and C transformations (soil respiration and DOC exports) at each PFT. For these analyses, we combined existing data from 2016 and 2017 that showed a crucial role of direct and indirect effects of abiotic factors on the release of gaseous and aqueous C across the PFT's at our field sites (Juan-Ovejero et al., 2020; see also Table S2). The ordination result is displayed as a triplot, showing the optimum distribution of the microbial groups (points) along these environmental gradients (arrows) and PFTs as "centroids" (i.e. the (weighted) mean of response variables at a particular habitat). We further checked the variance inflation factor among selected variables to test the independence of the variables in the ordination space. Finally, the statistical significance of the relationship between the species and the whole set of environmental variables was tested using Monte Carlo permutation test. DCCA analyses were performed using the CANOCO software for Windows v4.5 (ter Braak and Šmilauer, 2002).

3. Results

3.1. Microbial community structure under different PFTs

Total PLFA biomarker abundance was significantly higher in the peat samples from the Atlantic wet heathland and the *Sphagnum* site (152.8±5.3 and 140.6±5.4 μg g^{-1}, respectively) than from the two blanket bogs (*Eriophorum*: 108.7±3.7 and *Molinia*: 105.4±4.2 μg g^{-1}; Table 1 and Fig. 1).

However, microbial community structure was very similar across habitats, with bacteria being the most dominant group relative to total abundance (79-80%; Fig. 1), and fungi representing the smallest proportion (< 3%; Fig. 1). As a result, the Fungal:Bacteria (F:B) ratio was low across all four peatland habitats (0.02-0.03). Among the bacterial groups, Gram−negative biomarkers were significantly more abundant (35.9-40.1% of total PLFAs) than Gram−positive ones (23.6-28.5% of total PLFAs), and with general bacterial markers accounting for 16.2-18.3% of total

PLFA concentrations (Fig. 1). Consistently with total PLFA concentrations, these three PLFA groupings showed significantly lower values at the two blanket bogs (Fig. 1 and Table 1).

Further support for this clear distinction between upland and lowland valley bottom areas was found in the PLFA profiles (Fig. S2), which indicated that the concentrations of up to nine biomarkers were significantly higher in the samples from the two valley habitats than from the two blanket bogs, including the most abundant bacterial fatty acids (palmitic acid-C16:0, pentadecanoic acid-C15:0i, C18:1ω7, and 7,8Cy-C19:0; > 10 $\mu g\ g^{-1}$).

3.2. *Abiotic regulation of microbial communities*

The temporal changes in microbial community structure were more noticeable during the warmer periods observed from May to September (Fig. 2), especially in 2017 when significantly warmer temperatures were recorded at the two valley habitats (15.8 ^{o}C on average) than at the two upland ones (13.7 ^{o}C) and resulted in significant increases in total PLFA concentrations at the four habitats. This finding was supported by the significant positive relationship between total PLFA concentrations and soil temperature ($p= 0.0104$; Fig. S3a), which was not detected in the case of soil moisture.

The two blanket bogs consistently showed the lowest abundance of Gram−positive bacteria and the habitat dominated by *Erica* and Sphagnum mosses the highest values during the two investigated years (Fig. 2). Interestingly, and for most of 2016, the peat under *Sphagnum* had concentrations of this bacterial group that were more similar to those recorded at the two blanket bogs than to those of the heathland (Fig. 2). This was related to higher soil moisture contents being measured at these three sites compared to the heathland (Fig. 2); however, the negative relationship between Gram−positive bacteria and soil water content was only marginally significant ($p = 0.0567$; Fig. S3b). The abundance of Gram−negative bacteria showed a more variable pattern over time at all four habitats (Fig. 2), and the two blanket bogs were typically associated with lower concentrations of this PLFA grouping (Figs. 2a,b). However, under *Sphagnum* mosses, significantly lower abundances of Gram−negative bacteria were observed in September of both years (Fig. 2d) that were mainly driven by decreases in the concentrations of the monosaturated fatty acid C16:1ω7 in response to increases in soil water content ($p < 0.0001$; Fig. S3c).

More marked abundance fluctuations with time were observed in the case of the fungal biomarker C18:2ω6,9, and even more so in the case of the two valley habitats (Figs. 2c,d). Across

the whole investigated period, the highest fungal abundance was observed in the drier and warmer soils from the *Erica* site (Fig. 2c), when compared with the other three habitats (Figs. 2a,b,d). These rapid responses to changes in local abiotic conditions can be attributed to the strong negative relationship between fungi and soil moisture ($p < 0.0001$; Fig. S3d).

3.3. Above-ground vegetation, below-ground microbial communities and C cycling

The output from the canonical multivariate analysis (Fig. 3) revealed the existence of positive relationships between PFTs, certain microbial PLFA groupings and indicators, and C turnover at these four peatland habitats. The first ordination axis explained 50.2% of the species-environment relation variance and was significant (Monte Carlo test: F-ratio = 8.452, P-value = 0.032). It confirmed the similarities between the two the valley habitats based on the microbial community structure, by showing the highest bacterial dominance, and more specifically Gram−negative bacteria, than the two upland habitats (*Molinia* and *Eriophorum*).

The second canonical axis accounted for 26.2% of the variance and revealed that the *Erica* site, and to a less extent the *Molinia* habitat, could be differentiated from the other two peatland habitats in terms of microclimatic conditions and C transformations. Accordingly, the warmer and drier peat soils at the heathland, with the highest abundance of fungi and Gram−positive bacteria, emitted more C as CO_2, whereas the soils under *Molinia* grasses with higher F:B and Gpos:Gneg ratios were exporting C mainly as dissolved organic carbon (DOC). This contrasted with the wetter soils under *Eriophorum* and *Spagnum* mosses that produced less acidic soil solutions and retained more C (i.e., higher C:N ratio and lower C release; Fig. 3).

4. Discussion

4.1. Linking habitat properties to below-ground microbial community structure

The two-year field study showed that microbial communities were more strongly linked to local soil abiotic conditions than to the dominant above-ground vegetation. These results contradict previous studies concluding that different vascular plants are inhabited by unique microbial communities (Chroňáková et al., 2019), but agree with those observations in tropical peatlands where contrasting plant communities supported similar microbial communities (Girkin et al., 2020).

The four peat soils investigated here had very similar edaphic characteristics (low bulk density, high C content, low soil pH), but the peat under mosses had higher porosity (with the majority being macropores) than the other three peat soils (Juan-Ovejero et al., 2019). This means that water is able to move more freely within the peat matrix under the non-vascular plant community but is more efficiently retained under the vascular vegetation, creating localised differences in hydrology. In addition, the location of study sites at different altitudes provides an additional set of microclimatic conditions that shape these habitats. Accordingly, the two blanket bogs located at 960-970 m a.s.l. are subjected to more frequent precipitation and upslope fogs (Ramil-Rego et al., 2017), whereas the two habitats at the lowest elevation experienced slightly warmer soil temperatures and more variable patterns in soil moisture due to a greater microtopographical heterogeneity (i.e., the *Erica* heath colonises the drier hummocks and hence, are more disconnected from the water table, whereas the transition mire consisted of wetter flat lawns that are occasionally inundated).

Because of these microclimatic differences, a greater local spatial dissimilarity in microbial community structure was expected across investigated sites. A shift in soil microbial community structure with altitude has been previously observed, with fungi being less abundant at higher elevations (Bragazza et al., 2015). Accordingly, we also found an increasing abundance of fungi with improved soil oxygenation, which can be explained by the sensitivity of fungi to anoxic conditions (Jaatinen et al., 2007; Peltoniemi et al., 2009; Kwon et al. 2013; Lamit et al., 2017). Fungal communities were low at all four investigated sites compared to other PLFA biomarkers, in particular when compared to bacteria, in agreement with previous observations (Briones et al., 2014); however, those habitats that experienced more often drier spells created more favourable conditions for their communities (Bragazza et al., 2015; Girkin et al., 2020).

The greatest bacterial dominance at the investigated sites is typical of temperate peatlands (Gilbert and Mitchell, 2006; Andersen et al., 2013; Briones et al., 2014; Chroňáková et al., 2019). Both Gram−positive and Gram−negative as well as general bacterial PLFA biomarkers were significantly more abundant in the peats under *Erica* and *Sphagnum* than in the two blanket bogs, which is in agreement with the suggestion that their abundance tends to decrease along the minerotrophic-ombrotrophic gradient (Jaatinen et al., 2007). Prokaryotes have been observed to respond more to local edaphic properties associated to specific habitats than fungi (Chroňáková et al., 2019), with pH, N and water table being the most influential factors controlling their communities (Waldrop et al., 2012; Kaštovská et al., 2018; Tian et al., 2019). Due to the great similarities in soil pH and N content across our investigated sites, microclimatic conditions might have played a more determinant role in structuring soil bacteria communities

under the different PFTs. The marked temporal variability shown by bacterial abundances during the investigated period indicates that their populations are strongly influenced by intra- and inter-annual fluctuations in soil temperature and moisture. Accordingly, the observed negative relationship between peat water content and Gram−negative bacteria has been previously reported (Balasooriya et al., 2008), whereas warmer peat temperatures seemed to decrease the abundance of Gram−positive bacteria (Bragazza et al., 2015), which suggest a better adaptability of the latter group to anaerobic soil conditions (e.g. Actinomycetes, the most abundant Gram-positive group are facultative anaerobes). However, their consistently greater abundance at the warmest and driest site during the investigated period does not support this latter conclusion. Furthermore, it has been suggested that the abundance of monounsaturated and saturated PLFAs in peat samples are indicative of the presence of aerobic and anaerobic eubacteria, respectively (Sundh et al., 1997) and, in our samples, monosaturated PLFAs were the most abundant biomarkers (46%), suggesting that aerobic bacteria dominated bacterial community composition at these sites.

4.2. Linking microbial community structure to C fluxes across different habitats

Because the four dominant plant species differed in their litter quality, we anticipated higher decomposition rates under vascular plants than under mosses, in agreement with previous studies (Ward et al., 2013; Walker et al., 2016), but more so under graminoids than under sedges and shrubs, due to higher N and lower polyphenolic contents in the litters (Ward et al., 2009, 2015; Bragazza et al. 2013) and enhanced microbial priming effects (Dieleman et al., 2017). Our results partly confirmed these findings with more DOC released from the peat under *Molinia*, and the highest respiration rates measured under *Erica*. This can be attributed not only to a more favourable abiotic environment for microbial activities (i.e. warmer temperatures and oxic conditions) at the Atlantic heathland, but also to the fact that the association of ericoid mycorrhizas to the hair roots of ericaceous shrubs can increase the supply of labile C to decomposers (Trinder et al., 2008). Furthermore, it has been shown that, in peatlands, increased aerobic conditions favour CO_2 over DOC as a metabolic end product (Freeman et al., 2004) and that increased oxygen concentrations in the rhizosphere also remove the enzymatic latch preventing C decomposition (Freeman et al., 2001, 2004; Fenner and Freeman, 2011; Dunn and Freeman, 2018). From this, it is possible to anticipate that the expansion of shrubs in peatlands might not prevent microbial decomposition as suggested by some studies (Wang et al., 2015; Ward et al. 2015).

Interestingly, the larger C exports from shrub and graminoid dominated systems were also associated with increased abundances of fungi and Gram−positive bacteria under shrub and to higher F:B and Gpos:Gneg ratios under *Molinia*, suggesting that these three microbial groups and their relative abundances play a critical role in peatlands C cycling. Under shrubs increased peat aeration led to a greater abundance of fungi relative to bacteria, and warmer temperatures to a higher abundance of Gram−positive bacteria, whereas under graminoids the higher values of these two ratios were caused by the overall decrease in total bacterial abundances, but more specifically by the decreasing abundance of Gram−negative bacteria. Fanin et al. (2019) suggested that Gpos:Gneg ratio has potential as a useful indicator of the relative C availability for soil bacterial communities in organic soils and accordingly, this ratio increases with decreasing labile C availability. This is because Gram−positive and Gram−negative bacteria use older and labile C compounds, respectively (Börjesson et al., 2012; Balasooriya et al., 2014). Indeed, the Gram−positive and Gram−negative bacteria distinction overlaps with that of oligotrophic-copiotrophic; however, in this study, higher Gpos:Gneg ratios did not correlate well with higher C:N ratios.

On the other hand, it has been shown that the anteiso fatty acids promote a more fluid membrane structure than the iso fatty acids, and that the bacteria producing these fatty acids modify their iso:anteiso ratio in response to temperature and pH stress (Zhang and Rock, 2008), and anaerobic conditions (Weijers et al., 2006). The Gram−positive bacteria recorded in this study showed higher values of the iso:anteiso ratio at the shrub and graminoid dominated habitats than at the other two sites (with the lowest values being measured under *Sphagnum* mosses; results not shown), indicating that no substantial amounts of anteiso fatty acids were necessary for their growth at the two former habitats. Since soil temperature and pH cannot explain these differences, less aerobic conditions is the most likely factor driving these responses.

Conclusions

Research to find common mechanisms that shape the diversity of above- and below-ground plant-soil organisms have shown that community structure is governed by many interacting factors (Bardgett and van der Putten, 2014). In temperate peatlands, local abiotic factors (such as microtopography, soil temperature and pH, water and pore space availability, etc.) and differences in local plant communities are expected to have a strong influence on soil communities and C cycling. Despite the high heterogeneity in the peatland habitats included in

our study, we did not find that peat botanical origin was the main driver structuring microbial communities, in contradiction with other studies (Girkin et al., 2020). Instead, changes in the local abiotic environment, even at small spatial scales (namely, peat temperatures and aeration), exerted a stronger influence on microbial community composition and temporal shifts in their relative dominance. However, we could not confirm the contrasting relationships between Gram−positive and Gram−negative with altitude (Bragazza et al., 2015; Kumar et al., 2019), nor between Gram−negative bacteria and labile C availability (Balasooriya et al., 2014; Lyons and Lindo, 2020), as observed patterns were better explained by their different ecological requirements and stress tolerance to environmental changes.

Importantly, our results confirmed that certain microbial indicators, such as the F:B and Gpos:Gneg ratios, are reliable proxies for C transformations in peatlands (Briones et al., 2014; Fanin et al., 2019); however, careful interpretation of the changes in the abundances of both fraction terms is required. While aerobic conditions and warmer temperatures accelerate fungal driven decomposition and CO_2 emissions, decreases in Gram−negative bacteria might trigger increased C losses in the soil solution, and hence creating local "hotspots" of organic matter decomposition. Since it has been suggested that lowered water tables may pose more serious risks to temperate peatlands than warmer temperatures under projected future climate changes (Urbanová et al., 2013; Morton and Heinemeyer, 2019; Tiang et al., 2020), we propose that these high sensitive systems should be considered as 'ecosystem sentinels' for climate change-mediated impacts on the C cycle.

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688

689 **Table 1.** Results from ANOVA for the temporal changes in PLFA biomarker abundance (μg g^{-1}
690 dwt soil) at the investigated peatland habitats (Atlantic wet heath-*Erica mackayana*, transition
691 mire-*Sphagnum* mosses, blanket bog-*Molinia caerulea* and blanket bog-*Eriophorum*
692 *angustifolium*) during the investigated period (field sampling every two months in 2016 and
693 2017). Significance multivariate test on each factor and the interactions is Tukey's Studentized
694 range test.

Source	*DF*	*F*	*P*
Total PLFA			
YEAR	1	62.73	<0.0001
MONTH	5	9.86	<0.0001
HABITAT	3	56.17	<0.0001
YEAR*HABITAT	3	3.56	0.0171
MONTH*HABITAT	15	1.77	0.0495
YEAR*MONTH*HABITAT	20	3.72	<0.0001
Fungi			
YEAR	1	0.05	0.8232
MONTH	5	7.59	<0.0001
HABITAT	3	99.16	<0.0001
YEAR*HABITAT	3	0.59	0.6244
MONTH*HABITAT	15	2.48	0.0041
YEAR*MONTH*HABITAT	20	4.11	<0.0001
Bacteria			
YEAR	1	68.81	<0.0001
MONTH	5	13.56	<0.0001
HABITAT	3	66.02	<0.0001
YEAR*HABITAT	3	2.87	0.0405
MONTH*HABITAT	15	2.07	0.0181
YEAR*MONTH*HABITAT	20	3.27	<0.0001
Gbacteria			
YEAR	1	64.5	<0.0001
MONTH	5	13.02	<0.0001
HABITAT	3	74.29	<0.0001
YEAR*HABITAT	3	4.96	0.0030
MONTH*HABITAT	15	2.23	0.0100
YEAR*MONTH*HABITAT	20	5.92	<0.0001
Gram positive			
YEAR	1	109.2	<0.0001
MONTH	5	16.48	<0.0001

HABITAT	3	110.92	<0.0001
YEAR*HABITAT	3	5.16	0.0024
MONTH*HABITAT	15	2.25	0.0094
YEAR*MONTH*HABITAT	20	3.81	<0.0001
Gram negative			
YEAR	1	37.44	<0.0001
MONTH	5	11.93	<0.0001
HABITAT	3	38.25	<0.0001
YEAR*HABITAT	3	1.52	0.2132
MONTH*HABITAT	15	3.26	0.0002
YEAR*MONTH*HABITAT	20	3.42	<0.0001
Unspecific			
YEAR	1	26.03	<0.0001
MONTH	5	2.74	0.0235
HABITAT	3	3.01	0.0340
YEAR*HABITAT	3	2.43	0.0700
MONTH*HABITAT	15	4.34	<0.0001
YEAR*MONTH*HABITAT	20	5.91	<0.0001
Fungal:Bacteria ratio			
YEAR	1	0.94	0.3336
MONTH	5	7.93	<0.0001
HABITAT	3	80.21	<0.0001
YEAR*HABITAT	3	0.77	0.5156
MONTH*HABITAT	15	2.39	0.0057
YEAR*MONTH*HABITAT	20	3.85	<0.0001
G+ve:G-ve ratio			
YEAR	1	12.11	0.0008
MONTH	5	6.84	<0.0001
HABITAT	3	38.05	<0.0001
YEAR*HABITAT	3	5.63	0.0013
MONTH*HABITAT	15	8.84	<0.0001
YEAR*MONTH*HABITAT	20	10.16	<0.0001

695

696 **Figure legends**

697 **Figure 1.** Box plot charts show the median and quartiles (25th and 75th) of PLFA concentrations
698 assigned to functional groups (fungi, general bacterial markers, Gram−positive bacteria,
699 Gram−negative bacteria, unspecific) together with averaged total PLFA concentrations (upper
700 horizontal lines) in the peat samples collected at the four peatland habitats dominated by
701 different functional plant types (PFTs). Different letters indicate significant differences between
702 PFTs (upper case) and between PFTs per PLFA grouping (lower case).

703 **Figure 2.** Temporal changes in soil temperatures (^{o}C) and averaged soil moisture (%) recorded
704 on each sampling occasion during the investigated period, from January 2016 to December 2017
705 (upper charts), together with the average abundance of Gram−positive bacteria, Gram−negative
706 bacteria and fungal biomarkers (bottom charts) at each peatland habitat dominated by different
707 functional plant types (PFTs): a) *Molinia*, b) *Eriophorum*, c) *Erica* and d) *Sphagnum*. Asterisks
708 indicate significant differences between Gram−positive bacteria, Gram−negative bacteria per
709 sampling time.

710 **Figure 3.** Detrended Canonical Correspondence Analysis (DCCA) triplot of microbial groupings
711 and indicators (small black filled circles), environmental (arrows) and categorical variables
712 (squares filled with different patterns to indicate peatland type (i.e. blanket bog, wet heathland
713 and transition mire)) for the soil samples collected during the whole investigated period.
714 Abbreviations: Total PLFAs (total PLFA), Total bacterial PLFAs (TBacteria); fungi PLFA (Fungi);
715 Gram−positive bacterial PLFAs (Gpositive); Gram−negative bacterial PLFAs (Gnegative); General
716 bacterial PLFAS (Gbacteria); Non-specific PLFAs (Unspecific), fungal to bacteria ratio (FB ratio),
717 Gram−positive to Gram−negative ratio (G+:G- ratio), soil temperature (Soil T), soil moisture
718 (Moisture), pH of the soil solution (pH leachates), carbon content (Carbon), CO_2 production
719 (CO_2), dissolved organic carbon (DOC), ratio of C to N (C/N).

720

Figure 1

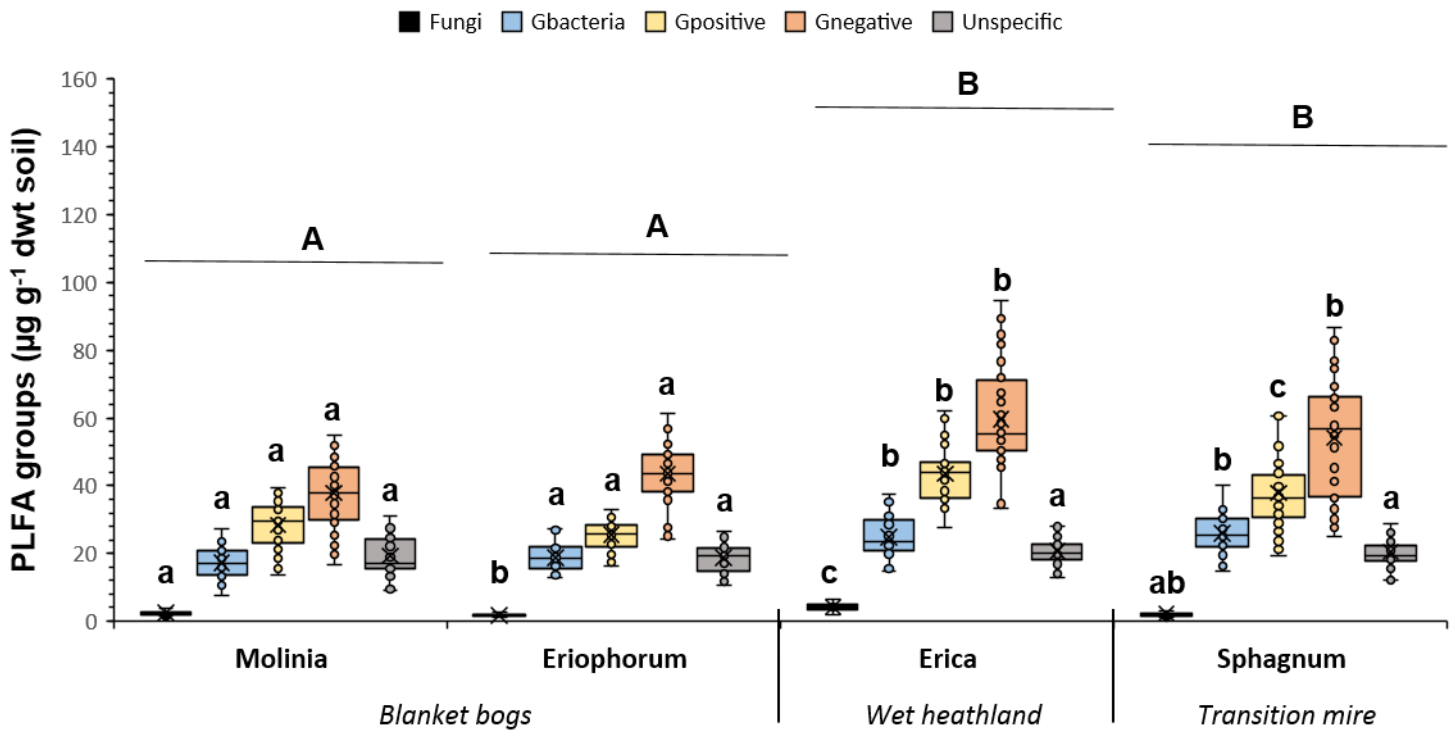
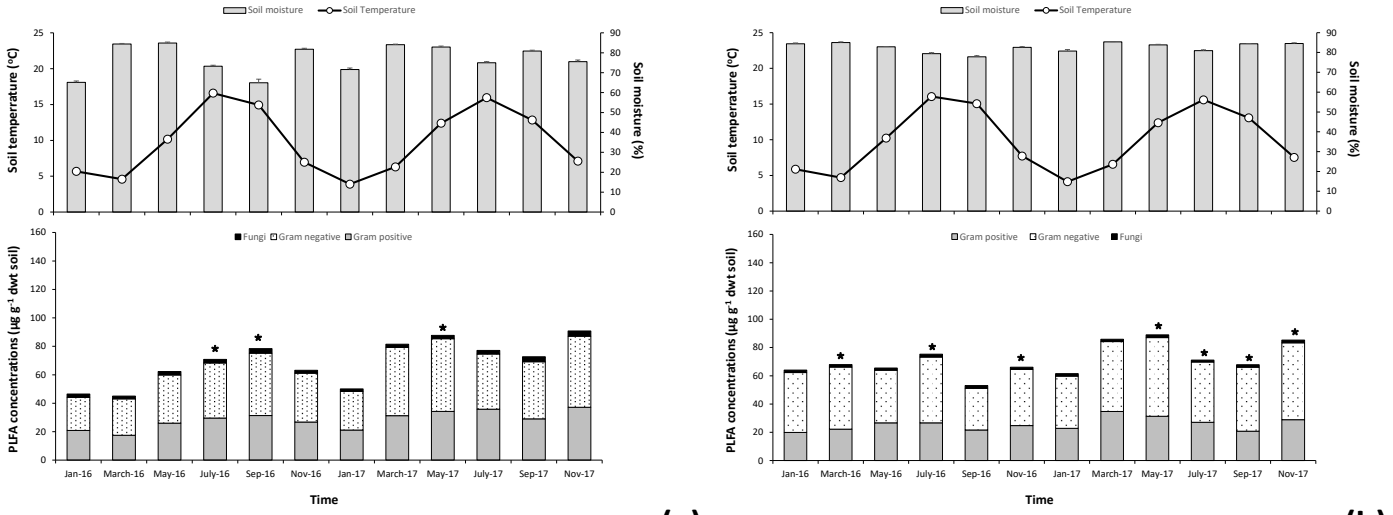

Fungi
Gbacteria
Gpositive
Gnegative
Unspecific
PLFA groups (µg g-1 dwt soil)
160
140
120
100
80
60
40
20
0
A
A
B
B
a
a
a
a
a
b
a
a
a
a
c
b
b
b
a
ab
b
c
b
a
Molinia
Eriophorum
Erica
Sphagnum
Blanket bogs
Wet heathland
Transition mire

Figure 2


Soil moisture
Soil Temperature
Soil temperrature (°C)
Soil moisture (%)
Fungi
Gram negative
Gram positive
PLFA concentrations (µg g-1 dwt soil)
Jan-16
March-16
May-16
July-16
Sep-16
Nov-16
Jan-17
March-17
May-17
July-17
Sep-17
Nov-17
Time
(a)
(b)

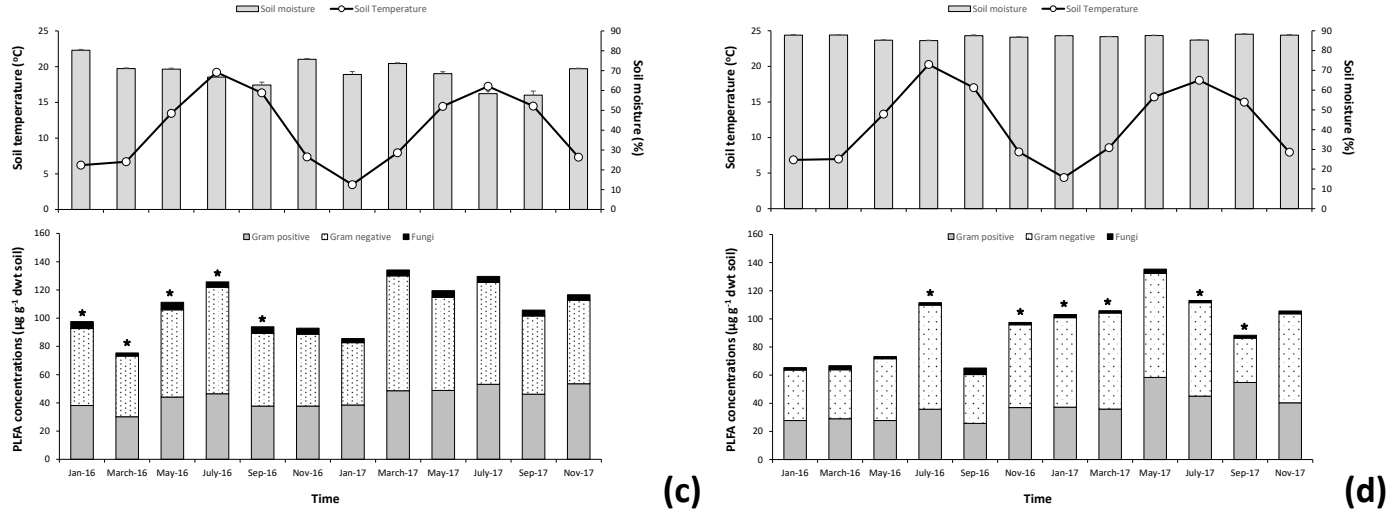
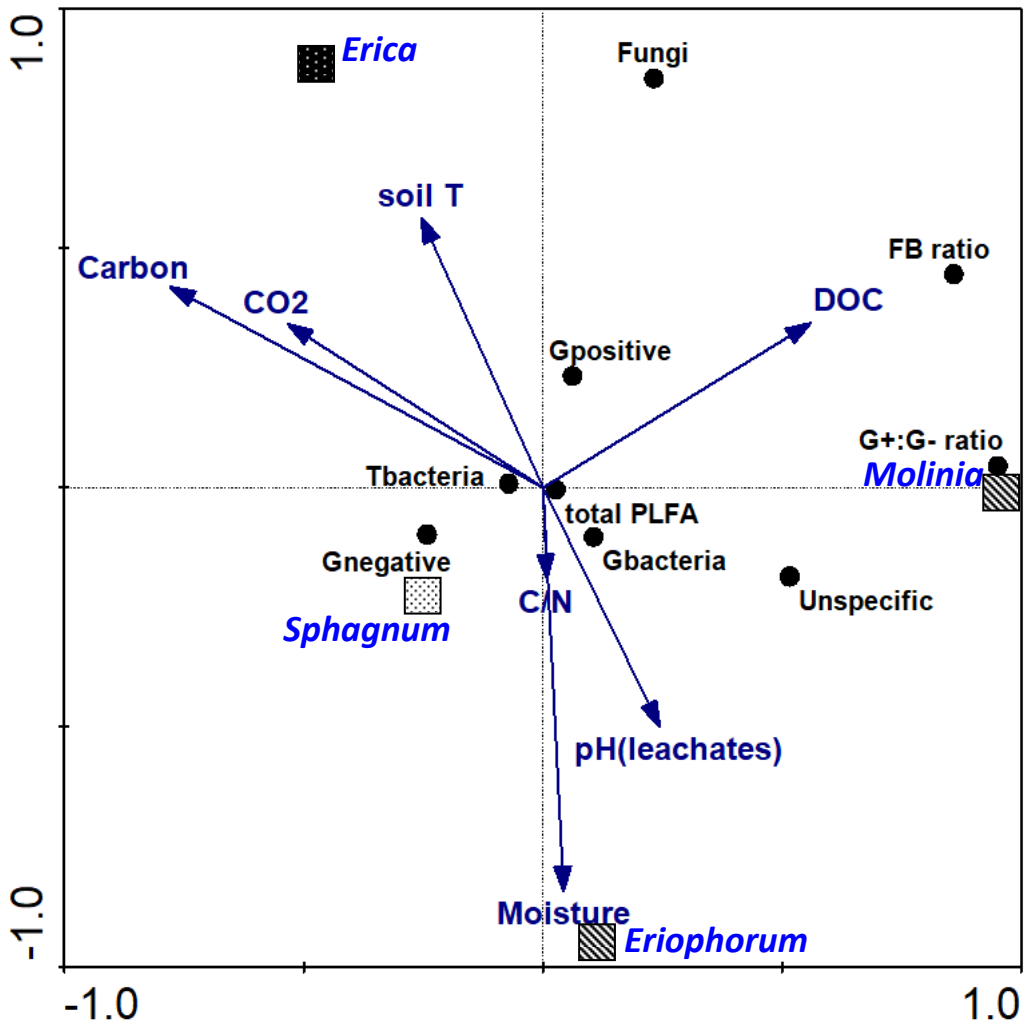

Soil moisture
Soil Temperature
Soil temperrature (°C)
Soil moisture (%)
Gram positive
Gram negative
Fungi
PLFA concentrations (µg g-1 dwt soil)
Jan-16
March-16
May-16
July-16
Sep-16
Nov-16
Jan-17
March-17
May-17
July-17
Sep-17
Nov-17
Time
(c)
(d)

Figure 3


1.0
-1.0
-1.0
1.0
Erica
Fungi
soil T
Carbon
CO2
FB ratio
DOC
Gpositive
G+:G- ratio
Molinia
Tbacteria
total PLFA
Gnegative
Gbacteria
Unspecific
C/N
Sphagnum
pH(leachates)
Moisture
Eriophorum