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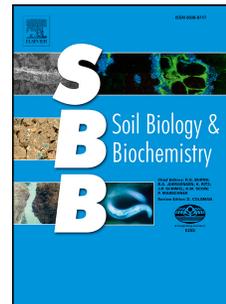
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1 **Abiotic and biotic drivers of microbial respiration in peat and its sensitivity**
2 **to temperature change**

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9 **Keywords:** soil respiration; temperature sensitivity; microbial biomass; peatland

10 **Type of paper:** Short Communication

11 **Highlights**

- 12 - Temperature increased respiratory activity until optimum temperature then declined
13 - More decomposed peat decreased the amount of microbes but not respiratory activity
14 - Q_{10} of aerobic respiration increased by 14 % at 35-40 cm than 5-10 cm peat layer
15 - Depth dependent Q_{10} in peat profile can be applied in modelling peat decomposition

16

17 **Abstract**

18 The effect of climate change on peatlands is of great importance due to their large carbon
19 stocks. In this study, we examined microbial biomass and effect of temperature and O₂
20 availability on soil respiration of surface and subsurface *Sphagnum* peat. The interactive
21 effect of biotic and abiotic factors significantly affects soil respiration. Increasing temperature
22 enhanced the microbial respiratory activity and thus the soil respiration, while there is a
23 temperature threshold. The more decomposed subsurface peat showed a lower CO₂
24 production due to less labile carbon and lower microbial biomass, but a higher temperature
25 sensitivity. Q₁₀ of aerobic respiration increased from 1.93 ± 0.26 in surface to 2.20 ± 0.01 in
26 subsurface peat. The linear relationship between Q₁₀ and depth in the uppermost 50 cm peat
27 section can be used to improve the estimation of CO₂ production in peat profiles.

28

29 Peatlands play a crucial role in global carbon cycle, with a storage of about 30 % of
30 global soil carbon (C) in 3 % of the earth's land surface (Gorham, 1991). However, global
31 climate change may alter the cold and wet conditions which favorable to their C sink function
32 (Page and Baird, 2016; Waddington and Roulet, 1996). Soil respiration, being an important
33 efflux of carbon dioxide (CO₂) from peatlands to the atmosphere, is largely controlled by
34 abiotic factors: temperature, soil moisture and O₂ availability (Szafranek-Nakonieczna and
35 Stepniewska, 2014; Wang et al., 2010). In addition, soil organic matter (OM) quality in terms
36 of the proportion of labile or complex C compounds (referred to high and poor quality
37 respectively; Dieleman et al., 2016), also affects respiration and temperature sensitivity. These
38 factors vary in vertical peat profile with temperature variability, O₂ availability and OM
39 quality decreasing with depth. Thus, in the context of climate change, it is crucial to
40 understand the response of soil respiration in different depths to realistic and expected
41 changes in temperature and water table depth (WTD) that determines O₂ availability. The
42 quality of OM is a key factor in the response of ecosystems to increase temperature.
43 Poor-quality OM decomposes slowly, resulting in lower CO₂ production, while it has been
44 reported to be more sensitive to temperature change (Conant et al., 2008b; Davidson and
45 Janssens, 2006). Effects of abiotic factors on CO₂ production in peat was frequently studied
46 (e.g. Hiltunen et al., 2013; Leifeld et al., 2012; Treat et al., 2014), while as the soil
47 respiration was regulated by the biological processes, the constrains are both abiotic, biotic
48 and interactive. To address this gap, we conducted a short-term incubation of peat from a site
49 presenting a sharp decrease of OM quality with depth to examine soil respiration under

50 various environmental conditions. Our objectives were to (1) determine the effect of
51 temperature, O₂ availability, OM quality and microbial biomass (MB) in regulating soil
52 respiration; (2) investigate the temperature sensitivity of peat decomposition at two different
53 degradation states.

54 Peat samples were taken from a near soil surface layer (5-10 cm) and a subsurface layer
55 (35-40 cm) at four different *Sphagnum* locations about 20 m apart under *Sphagnum rubellum*
56 hummocks on April 2019 in La Gnette peatland (a *Sphagnum* acidic fen in France, Gogo et al.,
57 2011). The samples from these four locations were used as replicates. The two layers
58 corresponded to less and more decomposed peat respectively as the older and deeper litters
59 has been exposed to decay for longer time (properties described in Table 2; Hiltunen et al.,
60 2013). Eight collected samples were homogenized separately and stored at 4 °C for two weeks
61 before incubation. Subsamples of 10g from 5-10 cm depth and 30 g from 35-40 cm depth
62 were transferred into 250 mL jars, sealed and vacuumed, then flushed with pure nitrogen (N₂)
63 or air for anaerobic and aerobic incubation (16 for each condition including 2 replicates for
64 each of the 8 collected samples), respectively. The jars were incubated at constant temperature
65 in FitoClima 1200 incubator (Aralab) for 7 days. Each day, 5 mL gas was collected and CO₂
66 concentration was analyzed by LGR Ultra-Portable Greenhouse Gas Analyzer (Los Gatos
67 Research, Inc. CA) and replaced by same volume of N₂/air to maintain pressure. These
68 processes were reproduced every week under 7 temperatures between 4 and 28 °C, in 4 °C
69 step. The CO₂ production rate was calculated by the linear regression of CO₂ concentration
70 versus time. Temperature sensitivity (Q₁₀) of CO₂ production was determined following Lloyd

71 and Taylor, (1994).

72 Total carbon and nitrogen contents (TC, TN) of the eight collected samples were measured by
73 an elemental analyzer (Thermo-126 FLASH 2000 CHNS/O Analyzer). Microbial biomass of
74 the eight collected samples and samples after incubation was determined by the chloroform
75 fumigation extraction method (Jenkinson and Powlson, 1976). Water extractable organic
76 carbon (WEOC) corresponded to the organic carbon concentration of non-fumigated samples.
77 Normality of distribution, homogeneity of variance of data were tested, three-way ANOVA
78 was used to determine effect of the temperature, O₂ availability and OM quality on the CO₂
79 production rate. One-way ANOVA was used to determine the difference of soil properties and
80 Q₁₀.

81 CO₂ production rate/gram dry peat continuously increased with increasing temperature
82 (Fig 1a and b). Whereas CO₂ production rate/gram MB increased with elevated temperature
83 until 24 °C, then declined at 28 °C (Fig 1c and d), suggesting an optimum temperature
84 between these two temperatures. The contrary trend observed at 28 °C could be attributed to
85 the higher amount of MB at 28 °C than at 24 °C (43.3 % and 197.2 % higher in 5-10 cm,
86 186.6 % and 99.2 % higher in 35-40 cm under aerobic and anaerobic, respectively). Therefore,
87 temperature increased the microbial respiratory activity and thus the soil respiration rate, but
88 there is an optimum temperature between 24 and 28 °C. When above this threshold
89 temperature, the increasing soil respiration could be attributed to the larger MB amount.

90 Low O₂ availability restricts microbial activities (Yavitt et al., 1997). Our study
91 confirmed that aerobic condition enhanced soil respiration and this effect depends on

92 temperature (Fig. 2; Table 1). At 28 °C, anaerobic incubation reduced CO₂ production rate
93 compared with aerobic conditions (decrease of 25.5 % and 35.5 % for 5-10 and 35-40 cm,
94 respectively), while significant difference was only observed in 35-40 cm ($p < 0.001$). No
95 significant effect of O₂ availability was found at 4 °C

96 The decreasing C:N with depth (Table 2) suggested an increased decomposition degree,
97 as microbes consume C-rich OM while recycle N, resulting in higher relative N concentration
98 in more decomposed soil (Biester et al., 2014; Broder et al., 2012; Kuhry and Vitt, 1996).
99 Additionally, WEOC also declined with depth (Table 2), suggesting a decreased availability
100 of labile substrates (Biester et al., 2006; Kalbitz and Geyer, 2002). These results showed that
101 the gradient of decomposition degree is steep in our site. CO₂ production rate/gram MB was
102 higher for 35-40 cm than 5-10 cm at 16-24 °C under aerobic, while it was similar under
103 anaerobic incubation (Fig 1 c and d). This could be related to the decline of fungi to bacteria
104 ratio with peat depth found by Zocatelli et al (article in preparation) of our samples and in
105 other studies (Sjögersten et al., 2016). Each unit cell mass of fungi release less CO₂ than
106 bacteria due to the lower surface-to-volume ratio. Thus the lower relative abundance of fungi
107 in 35-40 cm leads to higher respiration rate/gram MB (Blagodatskaya and Anderson, 1998).
108 However, a lower MB was observed in 35-40 cm compared to 5-10 cm both before (Table 2;
109 $p = 0.08$) and after incubation (average of all incubation conditions: 0.80 ± 0.51 vs. 2.70 ± 1.41
110 $\text{mgC g}^{-1}\text{dw}$; $p < 0.001$). Therefore, these results suggested that the decreasing CO₂ production
111 rate with depth (Fig. 1a and b) was linked to the lower available labile C substrate and less
112 MB, but not the microbial respiratory activity.

113 The Q_{10} increased with depth in aerobic conditions, (Fig 1a and b, $p=0.014$) but not in
114 anaerobic condition ($p=0.072$). These results indicated that the more decomposed OM is more
115 sensitive to temperature change than labile ones, confirming previously reported results
116 (Conant et al., 2008b, 2008a; Davidson and Janssens, 2006). These results showed that the
117 combination of higher temperature and increase frequency of drought would generate most
118 favorable conditions for CO_2 production. This would stimulate soil respiration in subsurface
119 layer with more decomposed peat, especially this layer was only 40 cm apart from surface.
120 Such a stimulation of old peat decomposition could significantly increase the CO_2 emission to
121 the atmosphere with an increasing possibility of transforming this ecosystem into a net C
122 source.

123 Calculation of Q_{10} with a limited temperature range or insufficient points affects the
124 exponential fit and could cause large variations of results (e.g. Chen et al., 2010; McKenzie et
125 al., 1998; Waddington et al., 2001). In our study, a large temperature range (4-28 °C) with
126 reduced step (4 °C) was applied to get more reliable results. Our results were in the range of
127 those from different studies that showed Q_{10} of CO_2 production mostly ranged between 1-2.5
128 (65.9 %; Table S1 and Fig. S1). A linear increase of Q_{10} with peat depth was observed (Fig. S2,
129 $R^2=0.66$; $p=0.004$ without outliers). This relationship allows Q_{10} to be more finely adjusted in
130 models instead of using a constant value.

131 In conclusion, the effect of temperature, O_2 availability, substrate quality and their
132 interactions on soil respiration were identified (Table 1). Raised temperature, aerobic
133 condition and high OM quality significantly increased the release of CO_2 . These factors

134 regulate the respiratory activity or amount of MB with implications for peat decomposition.
135 Our study emphasized the importance of integrating environmental parameters, substrate
136 quality, and MB when evaluating the response of soil respiration to climate change. Q_{10} of
137 soil respiration was higher in more decomposed peat and showed a vertical variation. As an
138 important parameter in modeling carbon cycle of peatlands under global warming, the vertical
139 heterogeneity of Q_{10} should be taken into account to improve the estimation of CO_2
140 production in peat profiles.

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146 Mora-Gomez for the helpful suggestions.

147

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226

227 **Tables**

228 **Table 1** Effect of the organic matter (OM) quality, temperature, Aerobic/anaerobic condition
 229 and their interactions on CO₂ production rate ($\mu\text{gC g}^{-1} \text{dw h}^{-1}$). Significance levels of
 230 three-way ANOVA are expressed as *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ (n=8).

	CO ₂ production rate ($\mu\text{gC g}^{-1} \text{dw h}^{-1}$)
OM quality	***
Temperature	***
Aerobic/anaerobic condition	***
OM quality * Temperature	***
OM quality * Aerobic/anaerobic condition	
Temperature * Aerobic/anaerobic condition	*
OM quality * Temperature * Aerobic/anaerobic condition	

231

232 **Table 2** Physical, chemical and biological properties of peat from 5-10 cm and 35-40 cm
 233 layer (n=4, mean \pm SD). Significance levels of one-way ANOVA are expressed as *: $p < 0.05$,
 234 **: $p < 0.01$, ***: $p < 0.001$.

	5-10 cm	35-40 cm	<i>p</i>
Water content (%)	85.17 \pm 3.00	86.09 \pm 3.10	
C:N	97.44 \pm 13.29	21.94 \pm 1.29	***
WEOC (mg C g ⁻¹ dw)	1.02 \pm 0.14	0.54 \pm 0.09	**
Microbial biomass C (mg C g ⁻¹ dw)	2.97 \pm 1.36	1.39 \pm 0.70	

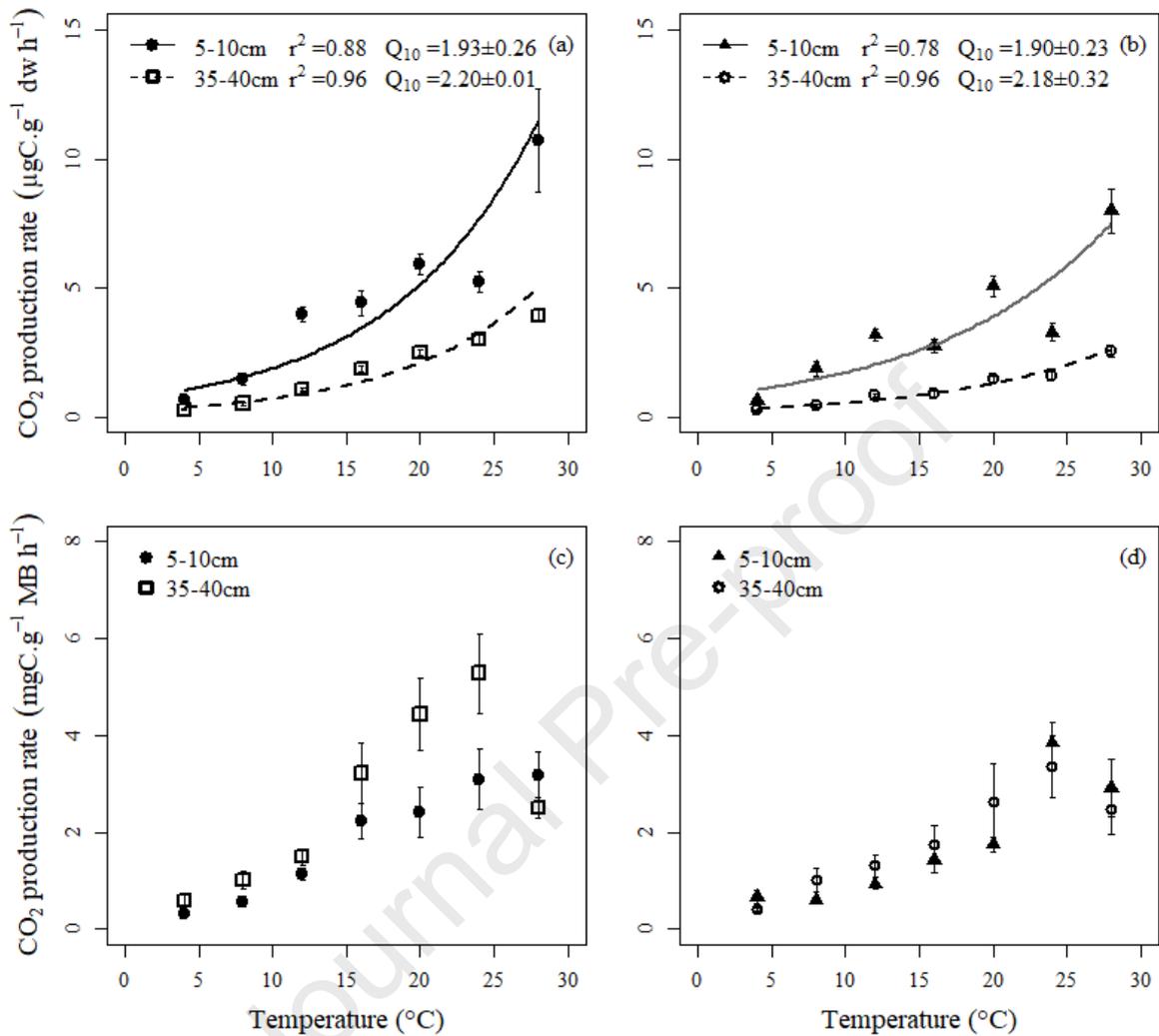
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237 **Figures**

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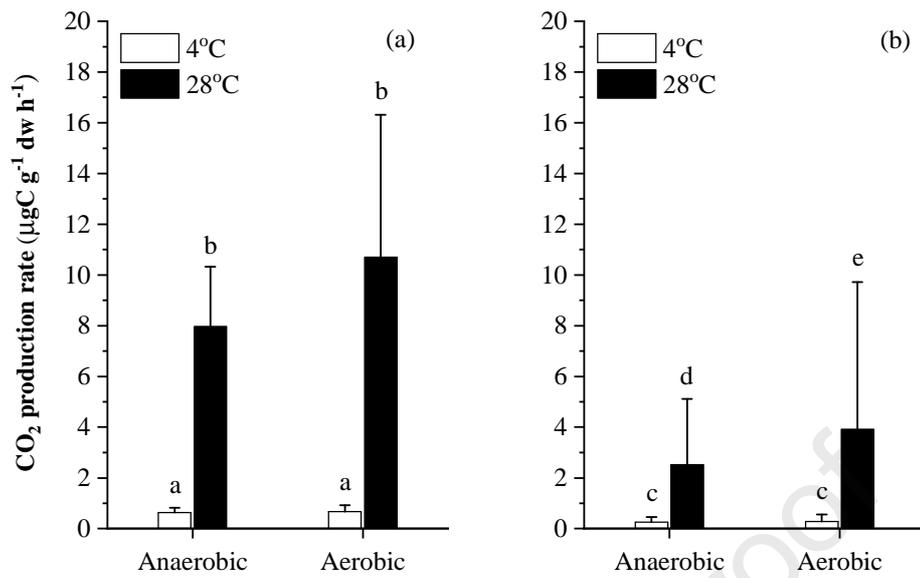


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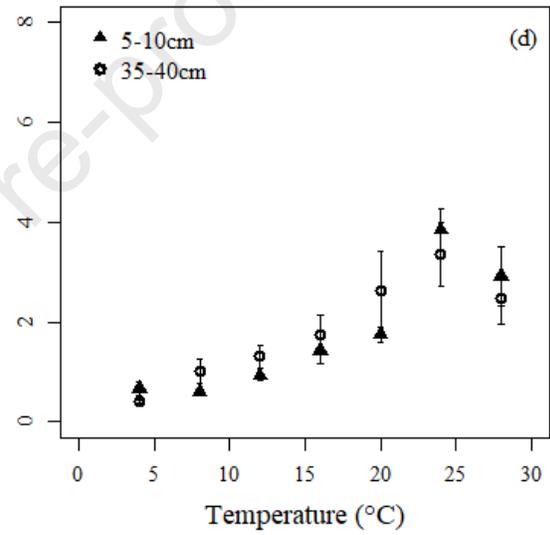
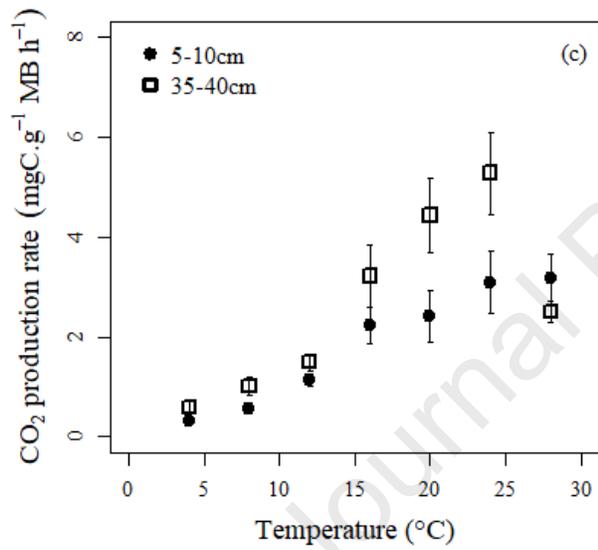
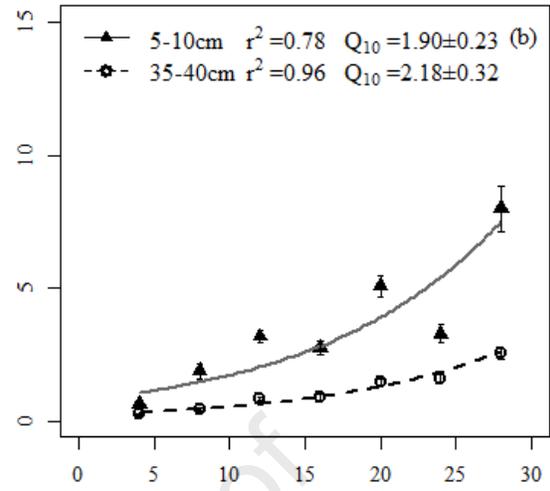
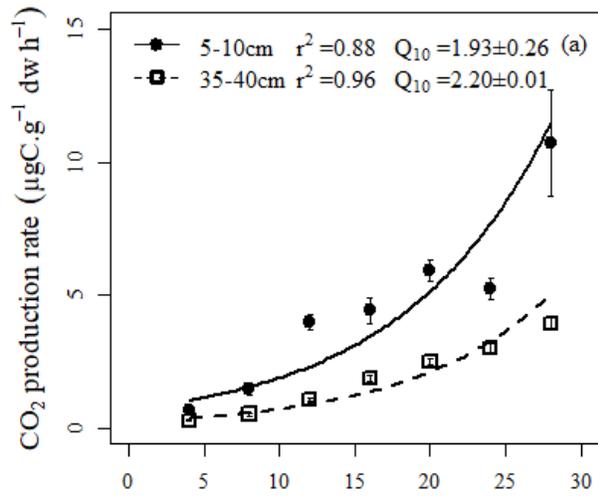
240 **Fig. 1** CO₂ production rate (µgC g⁻¹ dw h⁻¹) under (a) aerobic and (b) anaerobic conditions;241 and CO₂ production per gram microbial biomass (mgC g⁻¹ MB h⁻¹) under (c) aerobic and (d)

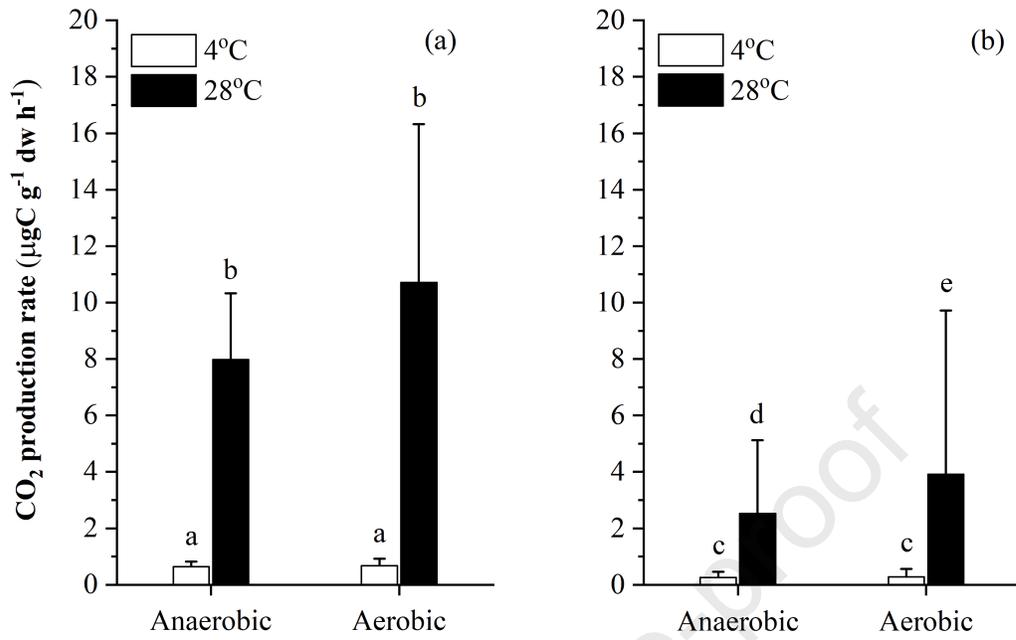
242 anaerobic conditions as a function of temperature for peat from 5-10 cm and 35-40 cm layer.

243 The lines in panels a and b correspond to the model fitted to the measurements.



244
245 **Fig. 2** CO₂ production rate (μgC g⁻¹ dw h⁻¹) of peat from (a) 5-10 cm layer and (b) 35-40 cm
246 layer incubated at 4 and 28°C during 7 days incubation under anaerobic and aerobic
247 conditions. Different letters represent significant differences by ANOVA in each panel and
248 error bars represent the standard error.





Highlights

- Temperature increased respiratory activity until optimum temperature then declined
- More decomposed peat decreased the amount of microbes but not respiratory activity
- Q_{10} of aerobic respiration increased by 14 % at 35-40 cm than 5-10 cm peat layer
- Depth dependent Q_{10} in peat profile can be applied in modelling peat decomposition

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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