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Microbial iron(III) reduction during palsa collapse promotes greenhouse gas emissions before complete permafrost thaw

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1 ABSTRACT:

2 Reactive iron (Fe) minerals can preserve organic carbon (OC) in soils overlying intact
3 permafrost. With permafrost thaw, reductive dissolution of iron minerals releases Fe and OC
4 into the porewater, potentially increasing the bioavailability of OC for microbial
5 decomposition. However, the stability of this so-called rusty carbon sink, the microbial
6 community driving mineral dissolution, the identity of the iron-associated carbon and the
7 resulting impact on greenhouse gas emissions are unknown. We examined palsa hillslopes,
8 gradients from intact permafrost-supported palsa to semi-wet partially-thawed bog in a
9 permafrost peatland in Abisko (Sweden). Using high-resolution mass spectrometry, we found
10 that Fe-bound OC in intact palsa is comprised of loosely bound more aliphatic and strongly-
11 bound more aromatic species. Iron mineral dissolution by both fermentative and dissimilatory
12 Fe(III) reduction releases Fe-bound OC along the palsa hillslopes, before complete permafrost
13 thaw. The increasing bioavailability of dissolved OC (DOC) leads to its further decomposition,
14 demonstrated by an increasing nominal oxidation state of carbon (NOSC) and a peak in
15 bioavailable acetate (61.7 ± 42.6 mg C/L) at the collapsing palsa front. The aqueous Fe^{2+} released
16 is partially re-oxidized by Fe(II)-oxidizing bacteria but cannot prevent the overall loss of the
17 rusty carbon sink with palsa collapse. The increasing relative abundance and activity of Fe(III)-
18 reducers is accompanied by an increasing abundance of methanogens and a peak in methane
19 (CH_4) emissions at the collapsing front. Our data suggest that the loss of the rusty carbon sink
20 directly contributes to carbon dioxide (CO_2) production by Fe(III) reduction coupled to OC
21 oxidation and indirectly to CH_4 emission by promoting methanogenesis even before complete
22 permafrost thaw.

23

24 INTRODUCTION:

25 Climate change has enormous consequences for permafrost environments, causing rapid
26 changes in soil conditions (such as thermal and moisture regime, and aeration) with direct
27 consequences for organic (OC) destabilization¹. Permafrost soils store ~60% of the world's soil
28 OC in 15% of the global soil area^{2,3}. This preserved OC will become increasingly exposed to
29 microbial decomposition and thus can be released from the active layer to the atmosphere as
30 greenhouse gases (GHGs) such as carbon dioxide (CO₂) and methane (CH₄)⁴ or discharged by
31 drainage⁵. However, the magnitude of the release of this OC depends strongly on a large variety
32 of factors⁶, including the hydrology, soil parent material, organic matter content and the ability
33 of soil minerals to protect OC from degradation, which can regulate long-term preservation of
34 OC⁷.

35 Iron (Fe) minerals are known to stabilize organic carbon by sorption/co-precipitation and
36 protect it from degradation by generating OC-Fe associations that are more persistent in soils^{8,9},
37 ¹⁰⁻¹³. However, by providing a terminal electron acceptor for anaerobic respiration^{14,15}, Fe can
38 also enhance decomposition. The fate of Fe and associated OC determines Fe-OC aggregate
39 formation and ultimately accessibility for microbial decomposition^{15, 16,17}. Reactive Fe-OC
40 associations (defined as the solid Fe phases that are reductively dissolved by sodium
41 dithionite^{11,18,19}) have been shown to serve as an effective rusty carbon sink and to preserve OC
42 over geological timescales¹¹. Previously, it has been shown that reactive Fe-OC associations
43 can mainly be found in intact permafrost soils²⁰, but cannot preserve OC along a permafrost
44 thaw gradient, following complete permafrost thaw from tundra to bog to fen type wetlands¹⁹.
45 However, the stability of Fe-OC associations during transitional processes along permafrost
46 thaw gradients remain unstudied. The need to better understand the climate impact of
47 transitional processes in thawing permafrost was stated previously by Shelef *et al.*²¹ who

48 emphasize large uncertainty in permafrost carbon stocks (>200%) due to processes at collapsing
49 fronts. Indeed, methane dynamics can also strongly differ between end-members and
50 transitional thaw stages²².

51 With permafrost thaw, soils become water-logged and oxygen (O₂) limited, favoring reductive
52 dissolution of reactive Fe(III)¹⁹. Fe(III)-reducing microorganisms are able to use the reactive
53 Fe(III) as an electron acceptor for anaerobic respiration and, depending on its composition, the
54 associated OC as electron source, resulting in CO₂ and Fe(II) formation²². Thus, Fe(III)
55 reduction directly contributes to CO₂ emissions²³. Fe(III) reduction may also influence CH₄
56 emissions in thawing permafrost peatlands. On the one hand, Fe(III) reduction is
57 thermodynamically more favorable and thus could outcompete methanogenesis²⁴. On the other
58 hand, Fe(III) reduction leads to proton consumption which results in an increasing pH that could
59 favor methanogenesis²⁵. The complex balance of these processes that either suppress or promote
60 GHG emissions such as CO₂ and CH₄ highlights the need for a fundamental understanding of
61 microbial Fe metabolisms and their interactions with methanotrophs and methanogens, which
62 is currently lacking.

63 The release of previously Fe-associated OC into surrounding porewater following reductive
64 dissolution could lead to further microbial decomposition of OC and emission of GHGs such
65 as CO₂ and CH₄. Mineral-associated OC (MAOC) has been proposed to be comprised of low
66 molecular weight compounds of microbial (e.g. microbial polysaccharides, amino sugars,
67 muramic acid) and plant origin^{13,26-30} with low activation energies of MAOC for degradation by
68 microbes. Therefore, the release of MAOC with permafrost thaw is considered an important
69 driver of the composition of arctic surface waters and microbial respiration^{31,32}. Recent studies
70 described carboxylic-rich³³ and aliphatic Fe-bound OC in forest soils as more resistant during
71 reductive dissolution³⁴. In Siberian permafrost soils, hydrophobic, aromatic DOC was
72 preferentially sorbed by shallower, acidic soil horizons and correlated with an increasing

73 abundance of Fe oxides³⁵. The identity of Fe-bound OC in permafrost environments, however,
74 still remains unknown.

75 To understand the direct impact of the loss of this so called rusty carbon sink³⁶ on net GHG
76 emissions in thawing permafrost peatlands, it is essential to further determine (1) the
77 bioavailability of Fe-bound OC and released OC during permafrost thaw and (2) changes in the
78 present and active microbial community, particularly the Fe(III)-reducing bacteria which are
79 key players in reactive Fe mineral dissolution and their interplay with methanotrophs and
80 methanogens.

81 We followed the dynamic biogeochemical interactions of Fe-OC associations in the active layer
82 along collapsing palsa hillslopes, where palsas underlain by intact permafrost are collapsing
83 into partially-thawed, semi-wet bogs. Fe-OC associations were characterized in the solid phase
84 using selective extractions, scanning electron microscopy (SEM), nanoscale secondary ion
85 mass spectrometry (nanoSIMS), and Mössbauer spectroscopy, and the effect of palsa collapse
86 on porewater geochemistry and CO₂ and CH₄ fluxes was quantified. Reactive Fe-associated OC
87 and DOC in the porewater along the palsa hillslope were investigated at the molecular-level
88 with Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), and the
89 present and active microbial community was characterized using DNA- and RNA-based 16S
90 rRNA amplicon (gene) sequencing.

91 RESULTS & DISCUSSION:

92 **Greenhouse gas emissions promoted by microbial iron cycling in thawing permafrost**
93 **peatlands.** In the palsa and at the collapsing front (Figures S1 and S2), net CO₂ emissions
94 measured from static flux chambers were similar on average ($1.57\pm0.27 \mu\text{mol}/\text{m}^2/\text{s}$) and slightly
95 decreased in the bog to $1.12\pm0.51 \mu\text{mol}/\text{m}^2/\text{s}$ (Figure 1). Replicate analysis of CO₂
96 concentrations in automatic Eosense eosFD gas flux chambers showed similar CO₂ emissions
97 along the palsa hillslope (Figure S3). Net CH₄ emissions were very low in the palsa
98 ($0.003\pm0.001 \mu\text{mol}/\text{m}^2/\text{sec}$), significantly increased at the collapsing front to 0.025 ± 0.001
99 $\mu\text{mol}/\text{m}^2/\text{s}$ and then slightly decreased in the bog ($0.013\pm0.001 \mu\text{mol}/\text{m}^2/\text{s}$; Figure 1). Emission
100 rates of CO₂ and CH₄ in the palsa and the bog are in line with previous studies at Stordalen mire
101 ³⁷⁻³⁹, however, this is the first report of emissions at the collapsing front, where palsa is
102 collapsing into the bog.

103 Previous work demonstrated that highest reactive Fe and associated OC contents can be found
104 where the organic and mineral horizons meet, which we have termed the “transition zone”¹⁹.
105 DOC concentrations in the porewater of the transition zone were low in the intact palsa (Palsa
106 A, $57.97\pm16.49 \text{ mg}/\text{L}$). Porewater DOC significantly increased towards the collapsing front to
107 $207.65\pm168.16 \text{ mg}/\text{L}$ in the more collapsed palsa (Palsa B). Highest DOC concentrations were
108 found directly at the collapsing front ($535.75\pm131.45 \text{ mg}/\text{L}$) and then significantly decreased in
109 the bog (279.62 ± 113.14 to $206\pm80.28 \text{ mg}/\text{L}$) (Figure 1, Figure S1, Figure S4).

110 The aqueous Fe²⁺ concentrations show the same trend as the DOC (Figure 1). Aqueous Fe²⁺
111 concentrations in the palsa were lowest along the palsa hillslope (4.47 ± 3.16 to 22.62 ± 30.14
112 mg/L ; Figure 1) and significantly increased at the collapsing front. Highest aqueous Fe²⁺
113 concentrations were measured at the collapsing front ($153.24\pm40.14 \text{ mg}/\text{L}$) and significantly
114 decreased again at the two measured locations in the bog to 48.86 ± 11.43 and $82.43\pm47.93 \text{ mg}/\text{L}$

115 (Figure 1). Other elements such as dissolved phosphorous (P) also strongly correlated with the
116 aqueous Fe^{2+} pulse at the collapsing front, suggestive of mineral dissolution and release of
117 mineral-associated P (Figures S5- S6).

118 The release of OC and aqueous Fe^{2+} along the palsa hillslope was accompanied by an increase
119 in the relative 16S rRNA gene sequence abundance (DNA-based) of iron- and methane-cycling
120 microorganisms in the transition zone and mineral horizon from the palsa to the collapsing front
121 (Figure 1; Figure S7). Towards the collapsing front, Fe(III)-reducing bacteria increased from
122 0.41 ± 0.07 to $2.46\pm 0.34\%$ in the transition zone and from 0.21 ± 0.05 to $2.42\pm 0.27\%$ in the
123 mineral horizon (Figure 1). Fe(II)-oxidizing bacteria also increased from the palsa to the
124 collapsing front from 0.54 ± 0.26 to $2.33\pm 0.33\%$ in the transition zone and from 0.92 ± 0.58 to
125 $1.66\pm 0.44\%$ in the mineral horizon. Methanogens increased along the palsa hillslope from
126 0.42 ± 0.37 to $2.83\pm 0.26\%$ in the transition zone and from 1.40 ± 1.40 to $11.68\pm 3.12\%$ in the
127 mineral horizon. Methanotrophs increased from the palsa to the collapsing front from 0.90 ± 0.30
128 to $1.93\pm 0.09\%$ in the transition zone and from 0.58 ± 0.08 to $1.26\pm 0.29\%$ in the mineral horizon
129 (Figure 1). Along the palsa hillslope, the relative 16S rRNA gene sequence abundances of iron-
130 and methane-cycling microorganisms were stable in the organic horizon (Figure 1). The iron-
131 and methane-cycling microorganisms are described in detail in Figure 2. For estimated absolute
132 abundances of bacteria and archaea as well as the manually-compiled database used to identify
133 iron- and methane-cycling microorganisms and the whole microbial community see Figure 2
134 and SI (Figures S8 and S9 and Tables S1-S4).

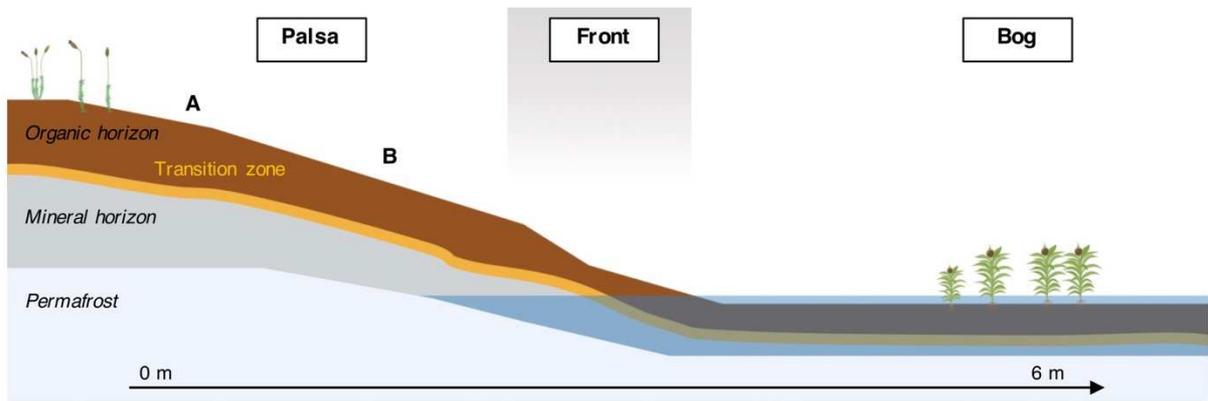
135 This data reveals that the so-called rusty carbon sink is already destabilized during palsa
136 collapse, even before complete permafrost thaw. Lateral flow by runoff of rain and/or melt
137 water^{40,41} in the transition zone between organic and mineral horizon, caused by bulk density
138 shifts (organic horizon: 0.03 ± 0.01 g/cm³ and mineral horizon: 0.84 ± 0.26 g/cm³)¹⁹, favors micro-
139 oxic conditions, as also described for other permafrost hillslopes⁴². These redox conditions

140 promote microbial reduction of reactive Fe(III) minerals coupled to carbon oxidation^{14,43}. This
141 results in a release of Fe and Fe-associated OC into the surrounding porewater and ultimately
142 contributes to a pulse of aqueous Fe²⁺ and DOC at the collapsing front – where we observed the
143 highest aqueous Fe²⁺ and DOC concentrations ever measured along the whole thaw gradient¹⁹.
144 The release of OC along the palsa hillslope results from multiple co-occurring processes. These
145 include the release of Fe-associated OC, changes in pH⁴⁴, plant community⁴⁵ (Figure S10), and
146 in microbial degradation of organic matter⁴⁶.

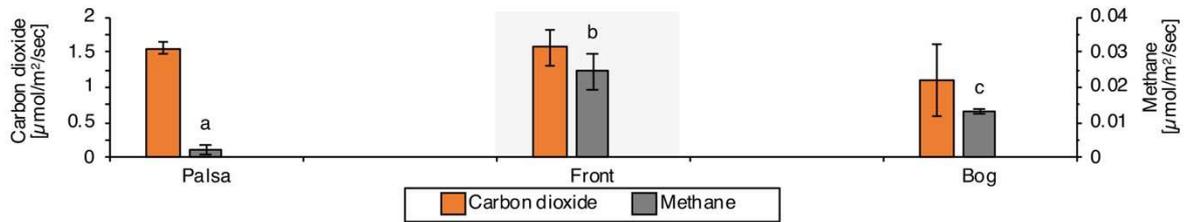
147 Although Fe(II)-oxidizers are present and active, they cannot prevent the overall loss of reactive
148 Fe and Fe-associated OC along the palsa hillslope. The CO₂ produced from degradation of
149 released carbon, including Fe-associated-OC, further stimulated methanogenic microorganisms
150 at the collapsing front. This CO₂ production was at least partially driven by Fe(III) reduction
151 coupled to carbon oxidation based on the increasing abundance of Fe(III)-reducing bacteria
152 along the palsa hillslope as has also been suggested for subalpine wetland soils⁴⁷.

153 Ultimately, the loss of this so called rusty carbon sink contributes to net GHG emissions of CO₂
154 and CH₄, directly by Fe(III) reduction coupled to carbon oxidation and indirectly by promoting
155 methanogenesis at the collapsing front.

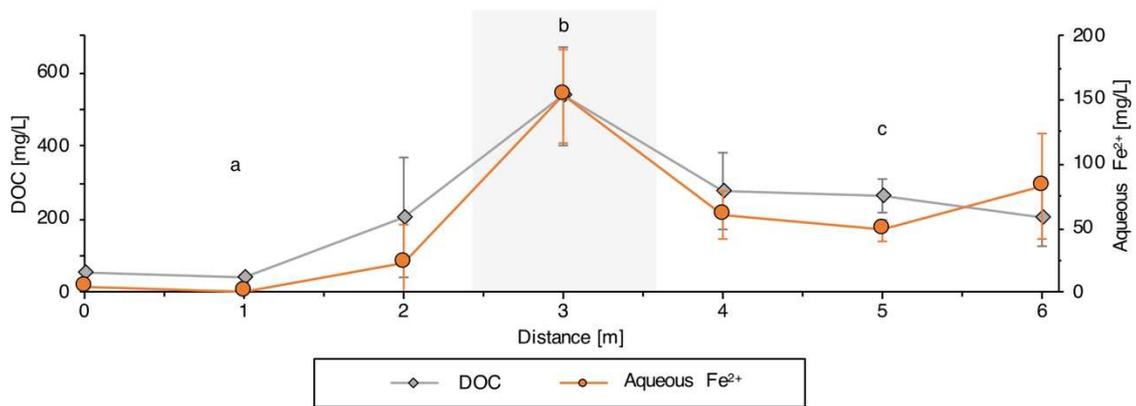
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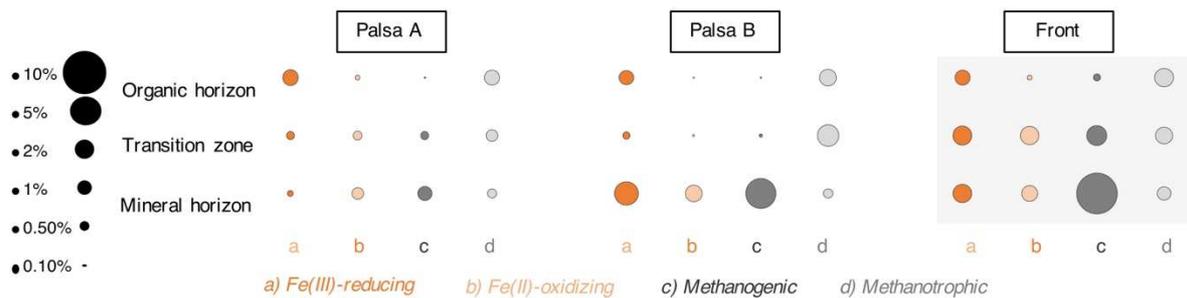
a Carbon dioxide and methane emissions along the palsa hillslope into bog



b Aqueous Fe²⁺ and DOC pulse along the palsa hillslope into bog



c Relative 16S rRNA gene sequence abundance of iron- and methane-cycling microorganisms from palsa to front



157

158 **Figure 1. Microbial iron cycling and carbon release as dissolved organic carbon (DOC),**

159 **carbon dioxide and methane emissions along a palsa hillslope. a, Carbon dioxide and**

160 **methane emissions along the palsa hillslope with highest emissions at the collapsing front. The**

161 reported values and error bars represent the average and standard deviation of measurements
162 collected on three days at three separate time points. b, Aqueous iron (Fe^{2+}) and DOC pulse
163 along the palsa hillslope at 30 cm depth with highest values at the collapsing front. Reported
164 values and error bars represent the average and standard deviation of eight palsa to bog
165 hillslopes sampled in June/July 2019 c, Relative 16S rRNA gene abundance of iron- and
166 methane-cycling strains along the palsa hillslope with highest abundances at the collapsing
167 front: a) Fe(II)-oxidizing, b) Fe(III)-reducing, c) methanogenic and d) methanotrophic. Small
168 letters above data mean significant differences ($P < 0.05$, one-way ANOVA: TukeyHSD test).

169

170 **Microbial iron- and methane-cycling communities during palsa collapse.** Along the palsa
171 hillslope, iron- and methane-cycling microorganisms increase in relative abundance, here
172 defined as DNA-based relative 16S rRNA gene abundance, and in potential activity, here
173 defined as RNA-based relative 16S rRNA abundance (Figure 2; for total microbial community
174 and replicate analysis see Figure S9 and S11, Table S1-S4).

175 Fe(III)-reducers, driving reactive Fe mineral dissolution and associated OC release, are found
176 in high abundance and potential activity along the palsa hillslope. From Palsa A to the
177 collapsing front, *Geobacter* spp., a classical Fe(III)-reducer²⁴, increased in relative abundance
178 from 0 to $1.55 \pm 0.30\%$ in the transition zone and to $1.62 \pm 0.18\%$ in the mineral horizon. The
179 potential activity of *Geobacter* spp. rose from 0 to $2.50 \pm 0.13\%$ in the transition zone and to
180 $4.75 \pm 1.07\%$ in the mineral horizon (Figure 2). *Clostridium* spp., a fermentative Fe(III)-
181 reducer⁵³, increased in relative abundance from 0 to $0.81 \pm 0.02\%$ in the transition zone and
182 $0.76 \pm 0.07\%$ in the mineral horizon (Figure 2). Potential activity of *Clostridium* spp. increased
183 from 0 to $2.31 \pm 1.15\%$ in the transition zone and to $1.23 \pm 0.22\%$ in the mineral horizon (Figure
184 2). *Rhodoferax* spp., known for dissimilatory Fe(III) reduction⁵², only appeared to be present

185 (1.98±1.51%) and potentially active (1.62±0.16%) in the mineral horizon of the more collapsed
186 palsa (Palsa B), close to the collapsing front (Figure 2). *Myxococcales* spp. showed highest
187 relative abundance from 1.67±0.15% in the intact palsa (Palsa A) to 1.30±0.23% at the
188 collapsing front and potential activity from 9.13±0.08 in the intact palsa to 7.03±2.08% at the
189 collapsing front in the organic horizon (Figure 2).

190 This microbial community analysis further indicates that the rusty carbon sink is lost by
191 dissimilatory and fermentative Fe(III) reduction. Dissimilatory Fe(III) reduction is conducted
192 along the palsa hillslope by abundant and active Fe(III)-reducing microorganisms such as
193 *Geobacter* spp., *Rhodoferrax* spp. and *Myxococcales* spp. (Figure 2; see also absolute
194 abundances in Figure S8 and replicate core analysis in Figure S15)^{48,49}. *Myxococcales* spp. are
195 not only capable of Fe(III) reduction, but also e.g. polysaccharide and protein degradation⁴⁶.
196 *Geobacter* spp. and *Rhodoferrax* spp. represent classical Fe(III)-reducing microorganisms, that
197 are well studied in different environments²³ with *Rhodoferrax* spp. also being described at other
198 permafrost sites¹⁴. Fermentative Fe(III) reduction is probably performed by *Clostridium* spp.
199 who might use the present DOC as carbon and energy source.

200 The abundant and active Fe(III)-reducing bacteria are accompanied by less relatively abundant
201 and probably less active Fe(II)-oxidizers. *Gallionella* spp. had a relative abundance of
202 0.82±1.16% in the present microbial community and 1.42±1.92% in the active community of
203 the mineral horizon of the more collapsed palsa (Palsa B). *Sideroxydans* spp. increased in their
204 relative abundance from below detection to 1.42±0.21% in the transition and to 1.08±0.34% in
205 the mineral horizon. Other *Gallionellaceae*, besides *Gallionella* spp. and *Sideroxydans* spp.,
206 were equally distributed in their relative abundance along the palsa hillslope from 0.54±0.26%
207 in the transition zone and 0.86±0.55% in the mineral horizon of the intact palsa (Palsa A) to
208 0.90±0.12% in the transition zone and 0.58±0.09% in the mineral horizon at the collapsing
209 front. The activity of the other *Gallionellaceae* was probably highest at the collapsing front

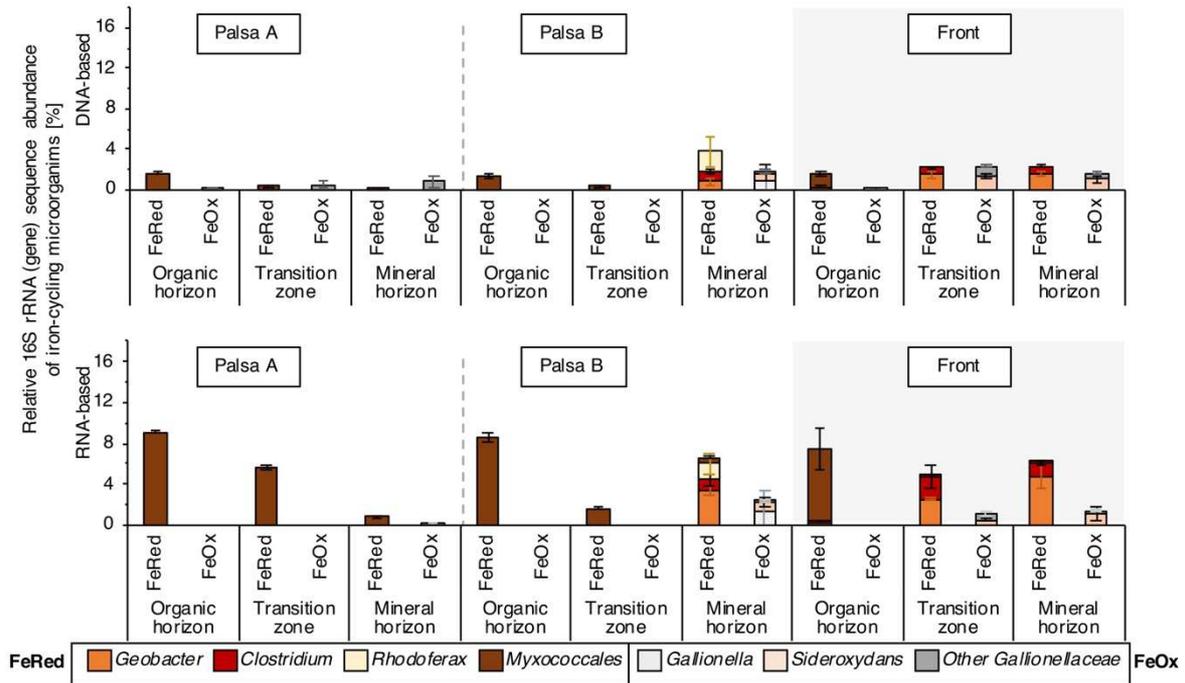
210 with $0.53\pm 0.24\%$ in the transition zone and $0.35\pm 0.07\%$ in the mineral horizon. The classical
211 Fe(II)-oxidizing bacteria^{48,49} such as *Gallionella* spp. and *Sideroxydans* spp., observed to be
212 present and potentially active in this system were already described in arctic ponds⁵⁰. In this
213 setting, these cannot sustain or reform the rusty carbon sink during palsa collapse (Figure 2).

214 The increasing relative 16S rRNA (gene) abundance (DNA- and RNA-based) of classical
215 Fe(III)-reducing bacteria is accompanied by an increase in the relative abundance of
216 methanogenic microorganisms, mainly *Methanobacterium* spp. These significantly increased
217 in their relative abundance in the transition zones from $0.25\pm 0.24\%$ in the intact palsa (Palsa
218 A) to $2.05\pm 0.14\%$ at the collapsing front. In the mineral horizon, they rose in their relative
219 abundance from $1.15\pm 1.22\%$ in the intact palsa (Palsa A) to $10.07\pm 2.84\%$ at the collapsing front
220 (Figure 2). Along the palsa hillslope, only a slight increase in potential activity of
221 *Methanobacterium* spp. was observed in the transition zone from 0 to $0.14\pm 0.05\%$ and in the
222 mineral horizon from 0 to $1.91\pm 0.85\%$ (Figure 2). Other methanogens belonging to
223 *Bathyarchaeia* also increased in relative abundance along the palsa hillslope from $0.17\pm 0.13\%$
224 to $0.71\pm 0.12\%$ in the transition zone and from $0.25\pm 0.18\%$ to $1.45\pm 0.24\%$ in the mineral
225 horizon. Methanotrophs, such as *Roseiarcus* spp. and other *Beijerinckiaceae* (i.e.
226 *Methylobacterium* spp. or *Methylocystis* spp.) had an equal relative abundance in the
227 community present along the palsa hillslope (i.e. DNA-based) and had its highest potential
228 activity in the palsa closest to the collapsing front (Palsa B; $12.55\pm 0.30\%$).

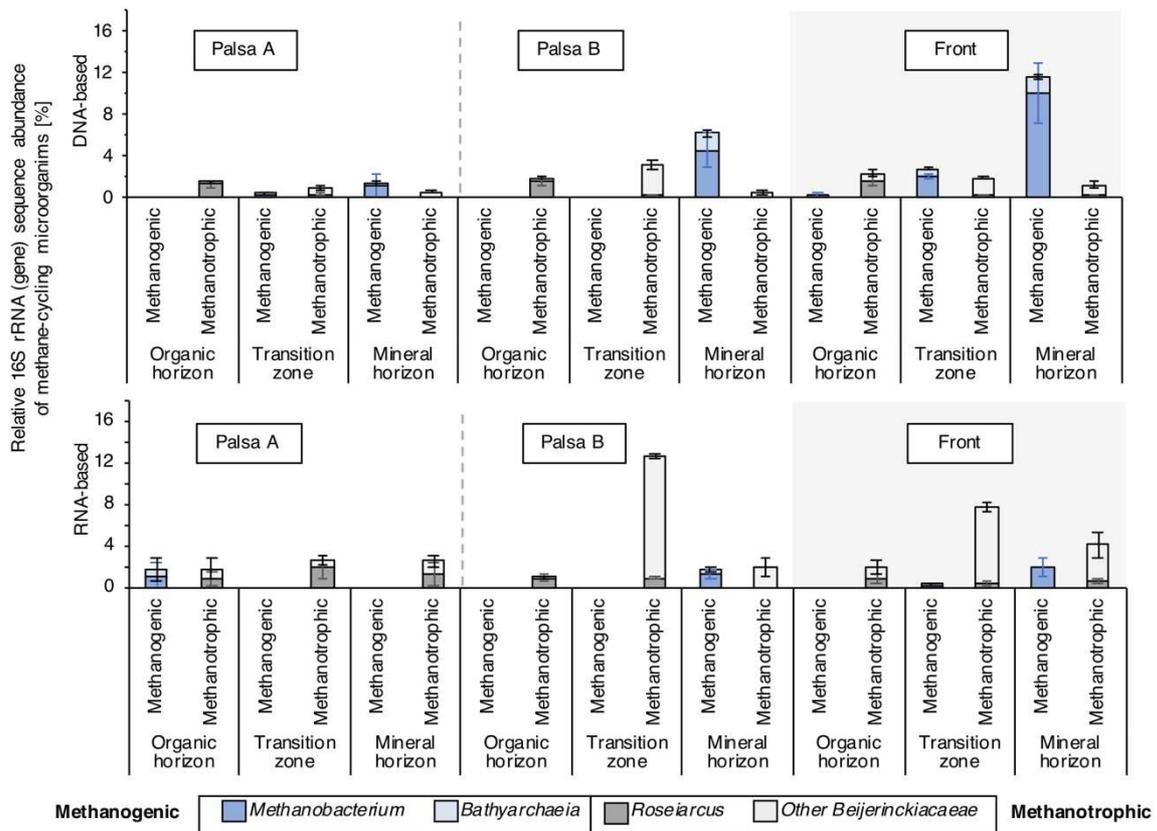
229 Acetate, formed along the palsa hillslope and accounted for up to 61.70 ± 42.56 mg C/L (10.33%
230 of the total DOC) at the collapsing front (Figure 4). It is expected that this stimulates Fe(III)
231 reduction coupled to acetate oxidation and leads to CO₂ formation by Fe(III)-reducing bacteria
232 such as *Geobacter* spp., known to metabolize acetate²³. The potential for reductive acetogenesis
233 from CO₂ by *Bathyarchaeia* was previously suggested⁵¹. Our MetaCyc ontology predictions
234 showed a high potential for acetoclastic methanogenesis (Figure S12), but contradictory to this,

235 we only saw a high relative abundance of hydrogenotrophic methanogens such as
236 *Methanobacterium* spp. This could be explained by the higher thermodynamic favorability of
237 Fe(III) reduction coupled to acetate oxidation as compared to acetoclastic methanogenesis. H₂
238 and CO₂, partially produced by fermentation and Fe(III) reduction by e.g. *Clostridium* spp., can
239 be used by hydrogenotrophic methanogens and lead to CH₄ emissions at the collapsing front.
240 The CH₄ is partially oxidized back to CO₂ by methanotrophs as shown by Perryman *et al.*²² who
241 described highest methane oxidation rates for palsa at the transition between palsa and bog (here
242 referred to as the collapsing front).
243 Our data clearly shows a co-existence of microbial iron- and methane-cycling microbial
244 communities during palsa collapse, which ultimately cause GHG emissions and effect the
245 balance between CO₂ and CH₄ emissions even before complete permafrost thaw.

a Iron-cycling microorganisms along the palsa hillslope



b Methane-cycling microorganisms along the palsa hillslope



246

247

248

Figure 2. Relative 16S rRNA (gene) abundance of abundant (DNA-based) and likely active (RNA-based) iron (Fe)- and methane-cycling microbial communities along the palsa

249 **hillslope (Palsa A, Palsa B, Front).** a, Iron-cycling microorganisms show an increasing
250 relative 16S rRNA (gene) abundance (DNA- and RNA-based) along the palsa hillslope with
251 highest abundances in the transition zone and mineral horizon at the collapsing front. b,
252 Methane-cycling microorganisms are increasing in relative 16S rRNA (gene) abundance along
253 the palsa hillslope. Reported values and error bars represent the average and standard deviation
254 of triplicate analysis of each soil horizon along the palsa hillslope. Replicate cores show similar
255 relative 16S rRNA (gene) abundance of abundant (DNA-based) and potentially active (RNA-
256 based) Fe- and methane cycling microbial communities along the palsa hillslope (Figure S11,
257 Table S1-S4).

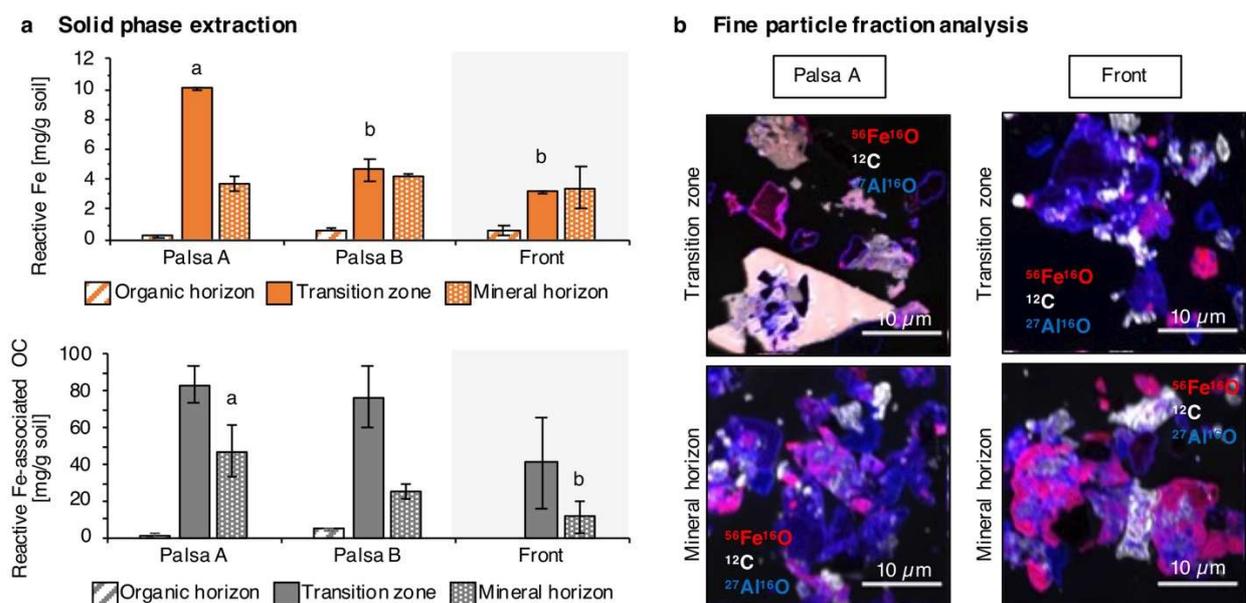
258

259 **Release of bioavailable iron-associated organic carbon during palsa collapse.** To
260 investigate if the loss of the rusty carbon sink also directly contributes to net GHG emissions
261 by releasing bioavailable, previously Fe-bound organic carbon, into the porewater we
262 determined the quantity and identity of Fe-bound OC in the solid phase (defined as dithionite
263 extractable OC) and of the released OC in the porewater. Dithionite did not affect the identity
264 of extractable OC and did not lead to molecular artifact formation (see SI, Table S5).

265 Highest reactive Fe concentrations (defined as Fe reductively dissolved by sodium dithionite
266 and control corrected by leachable Fe, see Methods) were found in the transition zone of the
267 most intact palsa (10.04 ± 0.07 mg reactive Fe per g soil; Figure 3). Towards the collapsing front,
268 reactive Fe in the transition zone between the organic and mineral horizons significantly
269 decreased to 3.22 ± 0.06 mg per g soil at the front (Figure 3). Absolute values are listed in Table
270 S6 and replicate core analysis can be seen in Figure S13. The amount of reactive Fe-associated
271 OC (OC dissolved after reductive dissolution of reactive Fe minerals by sodium dithionite and
272 control corrected by leachable OC, see Methods) also decreased from the palsa to the bog in

273 the transition zone (83.69 ± 10.04 and 76.60 ± 16.89 mg Fe-associated OC per g soil in the palsa
 274 to 40.88 ± 10.76 mg per g soil in the bog) (Figure 2). In the organic horizons along the palsa
 275 hillslope, reactive Fe and Fe-associated OC abundance was the lowest in the soil profile with
 276 average values of 0.49 ± 0.25 mg reactive Fe per g soil and 2.08 ± 2.47 mg Fe-associated OC per
 277 g soil (Figure 3). In the mineral horizons from the palsa to the collapsing front, reactive Fe was
 278 very stable (average 3.81 ± 0.38 reactive Fe per g soil), whereas Fe-associated OC slightly
 279 decreased from 47.21 ± 14.30 mg Fe-associated OC per g soil in the palsa to the collapsing front
 280 which had only 11.60 ± 8.54 mg Fe-associated OC per g soil (Figure 3). The highest content of
 281 Fe-associated OC was found in the most intact palsa along the palsa hillslope. This is supported
 282 by the strong spatial associations of OC with Fe minerals in the fine fraction observed by
 283 nanoSIMS analysis in the transition zone in this core (Figure 3; see replicate analysis of intact
 284 palsa core “Palsa A” in Figures S1, S14-S15). The transition zone and mineral horizons at the
 285 collapsing front showed organic-free, co-existing Fe and aluminum (Al), suggestive of Fe-
 286 bearing clays (Figure 3). This is also supported by Mössbauer spectroscopy (Figure S16, Table
 287 S7) and by previous observations with extended X-ray adsorption fine structure (EXAFS)¹⁹.

288



289

290 **Figure 3. Reactive iron (Fe) and associated organic carbon (OC) from intact palsa to the**
291 **collapsing front in a, the bulk soil and b, in the fine particle fraction.** a, Reactive Fe and Fe-
292 associated organic carbon in the solid phase decrease from the intact palsa (Palsa A) towards
293 the collapsing front. Reactive Fe values are the average of sodium dithionite citrate duplicate
294 extractions, control corrected by sodium chloride bicarbonate extractable Fe (leachable Fe). Fe-
295 associated OC values are the average of sodium dithionite citrate extractions, control corrected
296 by subtraction of the citrate background and the sodium chloride bicarbonate extractable OC
297 (leachable OC) (see Methods). Error bars of reactive Fe represent a combined standard
298 deviation of sodium chloride bicarbonate extractable Fe and sodium dithionite citrate
299 extractable Fe. Errors of the Fe-associated carbon represent a combined standard deviation of
300 the citrate blank, sodium chloride bicarbonate extractable OC and sodium dithionite citrate
301 extractable OC. Different small letters above bars mean significant differences ($P < 0.05$, one-
302 way ANOVA: TukeyHSD test). b, High spatial resolution analysis of Fe-OC associations by
303 nanoSIMS in the fine fraction of the soil, displayed as $^{12}\text{C}^-$ (white), $^{56}\text{Fe}^{16}\text{O}^-$ (red) and $^{27}\text{Al}^{16}\text{O}^-$
304 (blue) overlaid in a composite image. For the two end-members, Palsa A and collapsing front,
305 four particles of the fine fractions of each layer were analyzed by correlative SEM and
306 nanoSIMS, all showing the same spatial distribution of Fe, C and Al as shown by the four
307 representatives (Figure S14-S15).

308

309 FT-ICR-MS analyses showed that, in the intact palsa, the reactive Fe-associated OC had a
310 higher relative abundance of aliphatic species than the reactive Fe-associated OC at the
311 collapsing front (Figure 4, un-processed van Krevelen diagrams in Figure S17). This more
312 aliphatic-like fraction could represent amino sugar-like, carbohydrate-like and lignin-like
313 compounds (O/C range: 0.3 to 0.6, H/C range: 1.0 to 1.5)^{52,53}. A higher fraction of aromatics
314 was associated with reactive Fe phases at the collapsing front compared to the organics bound

315 by reactive Fe in the intact palsa (Figure 4). In general, it should be noted that the amounts
316 (mg/g) of reactive Fe-associated OC are decreasing along the palsa hillslope (Figure 3 and Table
317 S5). The higher relative abundance of the more aliphatic compounds associated with reactive
318 Fe in the intact palsa is lost during reductive dissolution to the surrounding porewater along the
319 palsa hillslope, thus the aliphatic fraction most likely contributes to the aqueous Fe²⁺ and DOC
320 pulse at the collapsing front (Figure 4). Loosely bound OC (salt extractable) appeared in lower
321 quantities and showed less defined but similar identity of organic fractions to the reactive Fe-
322 associated OC (Figure S18, Table S5 and S7).

323 Porewater extracted from the same soil interface (transition zone), where the rusty carbon sink
324 is lost along the palsa hillslope (Figures 1 and 2), contained a higher relative abundance of more
325 aliphatic species and more aromatic species compared to porewater extracted at the collapsing
326 front (Figure 4; un-processed van Krevelen diagrams in Figure S17). At the collapsing front, an
327 increased relative abundance of organic molecules, potentially representing tannin-like
328 compounds (O/C range: 0.5 to 0.9, H/C range: 0.5 to 1.4)^{52,53}, is observed (Figure 4 ; Figure
329 S17). The more aliphatic species had a lower relative abundance in the DOC at the collapsing
330 front, whereas a higher relative abundance of more aromatic species was observed (Figure
331 4). This could indicate decomposition processes that occur in the palsa hillslope porewater that
332 yield smaller organic compounds, uptake by native microbes, assimilation of organic carbon
333 into biomass and/or further metabolism, and ultimately emissions of GHGs by microbial
334 respiration. Porewater analysis along a replicate palsa hillslope showed the same identity of
335 aliphatic and aromatic species in intact palsa and at the collapsing front (Figure S19).

336 Further decomposition of DOC along the palsa hillslope is supported by an increasing nominal
337 oxidation state of carbon (NOSC) of the DOC in the porewater at the transition zone from the
338 palsa towards the collapsing front from 0.12±0.04 to 0.24±0.04 (Figure 4). As the DOC
339 becomes more oxidized, the NOSC increases at the collapsing front. This is in line with an

340 increasing average molecular weight (MW) from 591.24 ± 7.70 in palsa to 614.80 ± 0.40 at the
341 collapsing front (Figure 4 b and Figure S20). NOSC values slightly decreased in the bog to
342 0.20 ± 0.02 due to the overall loss of organic carbon mainly as CO_2 and, consequently,
343 enrichment of less decomposed and more reduced DOC in the porewater. The double-bond
344 equivalents (DBE, the number of rings plus double bonds to carbon, calculated from the neutral
345 elemental composition⁵⁴), remained stable along the palsa hillslope (0.39 ± 0.08). The DBE
346 along the palsa hillslope showed lower values than previously reported for bog and fen⁴⁴,
347 indicating that bog and fen DOC is overall more unsaturated compared to DOC released along
348 the palsa hillslope.

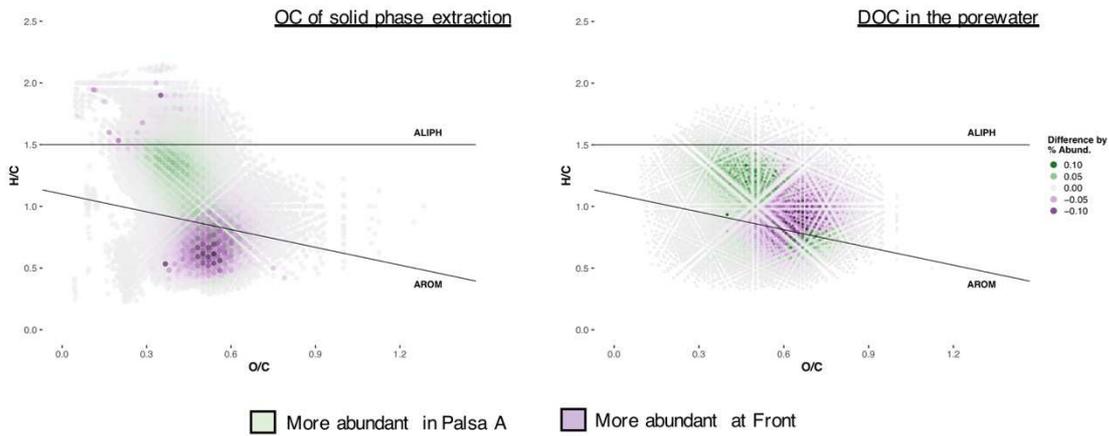
349 The further decomposition of released organic carbon contributes to acetate formation (Figure
350 4) at the collapsing front, probably by pyruvate fermentation, indicated by MetaCyc ontology
351 predictions (Figure S12). Along the palsa hillslope, acetate in the porewater at the transition
352 zone between organic and mineral horizons significantly increased (unpaired *t*-test, $N = 8$, $\alpha =$
353 0.05 , $p = 0.0024$) from 6.24 ± 0.34 mg C/L (3.56% of the total DOC) in the palsa to 61.70 ± 42.56
354 mg C/L (10.33% of the total DOC) at the collapsing front, the highest acetate concentrations
355 observed across the whole thaw gradient¹⁹. Further into the bog, the acetate concentrations
356 significantly decreased from 15.13 ± 6.53 to 6.10 ± 1.44 mg C/L.

357 Previous studies at Stordalen mire focused on the soil organic carbon quantity and identity as
358 well as on dissolved organic matter composition (DOM) and DOC transport along the thaw
359 gradient. These analyses have highlighted shifts towards faster decomposition from partially-
360 thawed bog to fully-thawed fen with an increasing proportion of carbon released as CH_4 ^{40,44,55}.
361 Processes occurring at the transition between palsa and bog had not been studied, thus enhanced
362 production of acetate and its promotion of methanogenesis at this transition has been
363 overlooked.

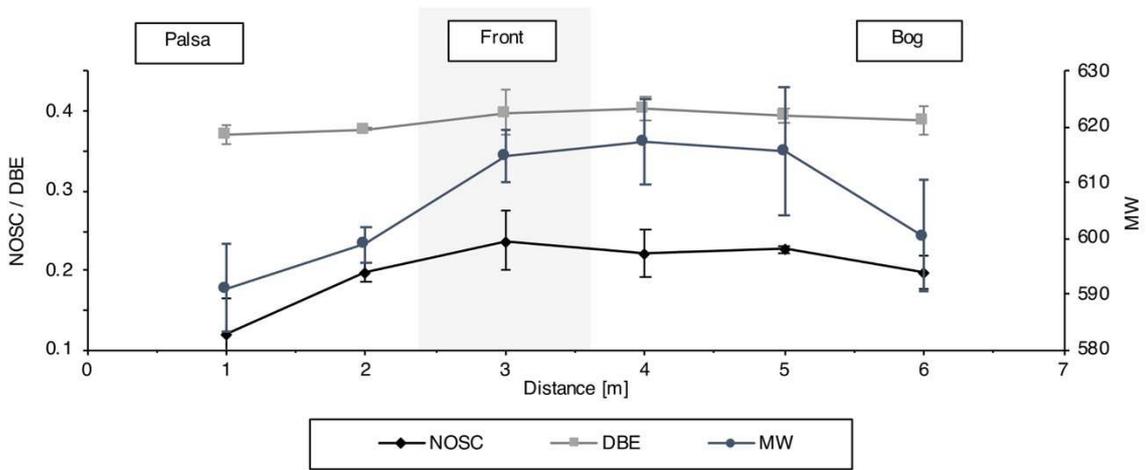
364 Our data showed that reactive Fe at the redox boundary between organic and mineral horizons
365 can bind aliphatic organic carbon, probably by downward cycling of DOM (defined as
366 continuous sorption and precipitation of DOM, as well as of microbial processing, desorption
367 and dissolution proportions of more recent plant-derived compounds^{56,57}) which is released
368 during reductive dissolution into the surrounding porewater. Lower molecular weight
369 compounds, aliphatic compounds or compounds poor in carboxyl functional groups show lower
370 binding strength to Fe minerals than higher molecular weight compounds, aromatics, or
371 compounds rich in carboxyl functionalities⁵⁷. This is also supported by the leachable OC
372 extractions (same ionic strength and pH as the sodium dithionite extraction; Figure S18). Thus,
373 these compounds are not protected from microbial degradation along the palsa hillslope. The
374 previously Fe-associated aliphatic fraction becomes more bioavailable to microorganisms when
375 it is released from mineral associations⁵⁷. This likely contributes to promotion of microbial
376 growth and respiration of DOM during permafrost thaw^{56,58-60}. Relative to aromatic compounds,
377 aliphatic compounds are expected to be even more labile to microorganisms^{56,59,60}, which is
378 supported by the overall loss of this more aliphatic carbon fraction in the porewater at the
379 collapsing front (Figure 4) with only minor quantities of aromatic organic molecules remaining
380 preserved by reactive Fe minerals after palsa collapse (Figure 3 and Figure 4). Kawahigashi *et*
381 *al.* showed that aromatic DOC was preferentially retained by mineral horizons in Siberian
382 tundra containing reactive Fe³⁵.

383 Our data clearly suggests that the loss of this rusty carbon sink directly contributes to high DOC
384 concentrations along the palsa hillslope and provides a bioavailable organic carbon source that
385 stimulates microbial respiration and promotes GHG emissions.

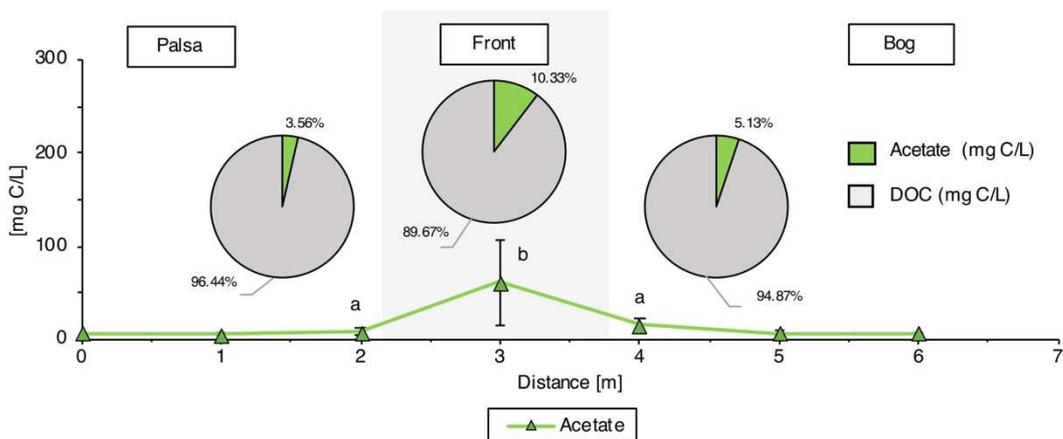
a Fate of reactive Fe associated organic carbon and released organic carbon into the porewater



b Redox properties and molecular weight of released organic carbon into the porewater along the palsa hillslope



c Acetate formation along the palsa hillslope



386

387 **Figure 4. Bioavailability of reactive iron (Fe)-associated organic carbon (OC) released**

388 **along the palsa hillslope. a, Composition of reactive Fe-associated OC and OC released into**

389 **the porewater. Fe-bound carbon in palsa soils, underlain by intact permafrost, is comprised of**

390 more aliphatic species (class 1, green) and more aromatic species (class 2, purple). This is lost
391 with reductive dissolution into the porewater. Towards the collapsing front into the bog, the
392 remaining Fe-associated OC fraction (purple) is comprised of less bioavailable organic
393 compounds which are likely associated with clay minerals (common in Palsa A, Front and Bog).
394 Dissolved OC, which is only found in Palsa A, is enriched in more aliphatic compounds (green).
395 Towards Front and Bog, only more aromatic species (purple) remain (Figures S17-S19, Table
396 S5). B, Redox properties and molecular weight of released organic carbon into the porewater
397 along the palsa hillslope (Figure S20). Reported values and error bars represent the average and
398 the range of duplicate porewater analysis along two palsa hillslopes (Figures S1 and Figure S4).
399 a, Acetate formation along the palsa hillslope. Following further decomposition of the dissolved
400 OC, highly bioavailable acetate [mg C/L] is formed which then is again used to further reduce
401 present reactive Fe(III) to Fe²⁺ coupled to acetate oxidation and CO₂ formation. Reported values
402 and error bars represent the average and standard deviation of 8 palsa to bog hillslopes, sampled
403 in June/July 2019 (Figures S1 and Figure S4). Different small letters above data mean
404 significant differences (P<0.05, one-way ANOVA: TukeyHSD test).

405

406 **Implications for the carbon cycle in thawing permafrost peatlands.** There is a substantial
407 need to piece together carbon sources and sinks in thawing permafrost environments to better
408 understand and quantitatively predict the overall climate impact of permafrost thaw⁶¹. One such
409 carbon sink or source are Fe-OC associations³⁶, which sequesters organic carbon in intact
410 permafrost soils²⁰ but releases it with complete permafrost thaw¹⁹. Our data now showed that
411 the release of the OC from the rusty carbon sink turns the OC into a source of labile DOC, CO₂
412 and CH₄ even before permafrost-supported palsas have completely collapsed. With increasing
413 abrupt thaw, occurring in 20% of the permafrost zone, new active hillslope features are formed⁶²
414 and thus could speed up the loss of the rusty carbon sink in currently intact permafrost

415 environments. Newest estimates showed that collapsing fronts will occupy 3% of abrupt thaw
416 terrain by 2300, but could emit one-third of abrupt thaw carbon losses⁶². Ultimately, interlinked
417 processes of iron- and carbon cycling in thawing permafrost environments need to be integrated
418 into existing climate models to better understand and predict GHG emissions of thawing
419 permafrost areas and thus better estimate its overall climate impact. For this, it is crucial to
420 further determine co-occurring Fe(III) reduction rates and CO₂ and CH₄ production rates
421 following Fe mineral dissolution.

422

423 METHODS

424 **Site information.** Stordalen Mire (68 22' N, 19 03' E) is a subarctic peatland in northern
425 Sweden underlain by discontinuous permafrost. The mire consists of three distinct sub-habitats:
426 (1) palsa (intact permafrost) with ericaceous and woody plants; (2) ombrotrophic peatland or
427 bog (intermediate thaw) with *Sphagnum spp.*, sedges and shrubs and (3) minerotrophic peatland
428 or fen (fully thawed) with sedges, mainly *Eriophorum spp.*⁴⁵ (Figure S1). Generally, palsas and
429 bogs are only fed by precipitation and melt water and have more acidic surface waters (pH ~4).
430 Fens are fed by surface water and groundwater, and maintain slightly acidic to alkaline pH⁴⁰.
431 The areal extent of intact palsa across Stordalen mire has declined significantly since 1970 due
432 to progressive warming in the Arctic, while fen habitats have expanded⁶³. It is also predicted
433 that the whole mire might be free of permafrost as early as 2050^{64,65}.

434 **Gas measurements.** To measure CO₂ emissions along the palsa hillslope, two eosense
435 instruments (eosFD Forced Diffusion chamber in conjunction with the eosLink-FD software,
436 EOSENSE INC, Dartmouth, Canada) were installed (Figures S1 and S2): (1) at the top of the
437 palsa hillslope (spot: Palsa A) and (2) at the transition to bog (spot: Front). The collar was
438 situated in a flat location and inserted to near full depth. A centimeter of space was left to aid
439 in installation of the eosFD itself as well as collar retrieval. The collar area was cleared of any
440 rocks or debris, larger vegetation was removed or avoided. The eosFD was deployed in the
441 installed collar. The collars were deployed at least 24 hours prior to the start of the eosFD
442 measurement collection to avoid disturbance-related fluxes in the early portion of the data
443 collection. The eosFD samples gases from the atmospheric and soil cavities within the device.
444 Briefly, gas is pulled from the atmospheric cavity to the sensor for 20 seconds to purge the
445 sensor cavity, then sampled every 10 minutes for five samples. Gas is then pulled from the soil
446 cavity for 20 seconds, then pulsed every 10 minutes for five samples. Forced diffusion flux is
447 calculated as follows:

448
$$\frac{V}{A} * \frac{\partial C}{\partial t} = F_s - D \left(\frac{\Delta C}{L} \right)$$

449 (volume/surface area scaled rate of change in flux rate equal to the flux from the soil surface
450 (Fs) minus the difference in concentration, ΔC (scaled by both the path length L and the
451 diffusivity of the interface (membrane), D)).

452 The change in the flux rate over the timespan of the concentration measurements (around 60
453 seconds) is assumed to be zero (steady state):

454
$$\frac{V}{A} \frac{\partial C}{\partial t} = 0$$

455 This assumption results in a linear dependence with the path length and interface (membrane)
456 diffusivity being constant and represented by a single coefficient, G:

457
$$F_s = G \Delta C$$

458 Furthermore, carbon dioxide and methane emissions along the palsa hillslope were measured
459 in triplicate using plastic chambers sealed with a rubber stopper (Figures S1 and S2), as
460 described previously⁶⁶. The metal frames were pushed into the ground at least 24 hours before
461 the measurements to avoid collecting gas emissions from the soil during installation. Again
462 rocks, debris and larger vegetation was avoided. Deionized water was used in the frames to seal
463 off the chambers from ambient air. Gas chamber samples were collected with a gas-tight syringe
464 (1100TLL 100 mL Gastight, Hamilton, Reno, NV, USA) and directly transferred into evacuated
465 12 mL exetainer vials⁶⁷ until analyzed. The sampling was done every 5 min for a total period
466 of 30 mins in duplicates for palsa and front and in triplicates for bog. All gas samples from the
467 field and standard gases used for calibration were measured with a gas chromatograph (Hewlett
468 Packard, 5890 Series II) equipped with an electron capture detector (⁶³Ni-ECD).

469 **Sample collection.** In July 2019, cores were taken along three palsa hillslopes (Figure S1 and
470 Figure S7), gently collapsing into bog, following the expected hydrological flow described
471 previously⁴⁰. A Humax corer of 50 cm length and 3-cm diameter with inner liners was used to
472 sample the active layer¹⁹. The cores for mineral analysis were directly split after sampling under
473 100% N₂ atmosphere in a glove bag and subsamples stored at -20°C until analysis. The cores
474 for microbial community analysis were split directly in the field, immediately frozen with liquid
475 nitrogen and stored at -80°C until further processing. As previously described¹⁹, the cores were
476 split into three soil horizons based on texture and color changes: (1) A peat or organic horizon,
477 followed by (2) a transition zone between the organic-rich and mineral-rich layer and (3) a
478 mineral horizon.

479 In July and September 2019, porewater samples were collected from 30 and 60 cm depth below
480 the peat surface along the palsa hillslope (8 transects, Figure S1 and Figure S4) using a luer
481 lock syringe connected to a lysimeter with an effective pore size of 2.5 microns (Simpler Luer-
482 Lock Micro Samplers, Model 1910LL, Soilmoisture Equipment Corp., Santa Barbara, CA).
483 Prior to use, syringes and lysimeters were rinsed 10 times with 50 mL MilliQ water and air
484 dried. Syringe filters (0.22 μm, PES, Merck™ Steritop™, Millipore) were pre-rinsed with 120
485 mL MilliQ water each to avoid leaching residuals of the filters. The syringes were flushed three
486 times with N₂ and sealed till further use. Syringe filters (0.22 μm) were flushed three times with
487 N₂ and placed into a SCHOTT bottle with N₂ atmosphere till further use. The lysimeters were
488 installed in the soil, pre-flushed by pulling porewater with a syringe and the first 2 mL
489 discharged. Immediately afterwards, the N₂ flushed syringes were unsealed, nitrogen gas
490 pushed out, and then tightly connected to the installed lysimeter. To avoid direct sunlight
491 exposure, syringes were covered with white cotton bags during the time of porewater extraction.
492 After 3-4 hours, the samples were filtered through a 0.22 μm syringe filter into stoppered, N₂

493 flushed glass vials, wrapped in aluminum foil to avoid any sunlight exposure. The first 1 mL
494 filtrate from each sample was disposed. The samples were stored at 4°C till further analysis.

495 **Selective iron and carbon extractions.** To extract reactive Fe and associated OC, selective
496 dissolutions were conducted as described previously¹⁹. Briefly, a sodium bicarbonate (0.11 M)
497 sodium dithionite (0.27 M) trisodium citrate (0.27 M) solution was used to reductively dissolve
498 reactive Fe and associated organic carbon. As described in Lalonde *et al.*¹¹, a sodium
499 bicarbonate (0.11 M) sodium chloride (1.85 M) solution was used as a control experiment to
500 distinguish between Fe and OC readily desorbed (leachable OC) and organic carbon associated
501 with reactive Fe and only dissolved during reductive dissolution with dithionite. The citrate
502 background in the extract also needs to be subtracted to receive the reactive Fe-associated OC.
503 Thus, only the control corrected values are discussed:

$$504 \text{ Reactive Fe} = \text{Fe}(\text{dithionite citrate}) - \text{Fe}(\text{sodium chloride}) \quad (1)$$

$$505 \text{ Reactive Fe} - \text{associated OC}$$

$$506 = \text{DOC}(\text{dithionite citrate}) - \text{DOC}(\text{blank citrate}) - \text{DOC}(\text{sodium chloride}) \quad (2)$$

507 For each soil horizon (organic horizon, transition zone, mineral horizon), 0.3 g dry soil was
508 weighed into 10 mL glass vials with 6.25 mL extractant and N₂ headspace. After 16 hours at
509 room temperature on a rolling shaker, samples were centrifuged at room temperature for 10 min
510 at 5300 g. The supernatant was decanted and further analyzed for total Fe and DOC.

511 **Geochemical analysis.** To determine total Fe and Fe(II), porewater or extract was acidified in
512 1 M HCl and quantified spectrophotometrically in triplicates with the ferrozine assay⁶⁸. DOC
513 was measured in triplicates with a total organic carbon analyzer (High TOC II, Elementar,
514 Elementar Analysensysteme GmbH, Germany). Inorganic carbon was removed by acidification
515 with 2 M HCl addition prior to analysis. High performance liquid chromatography (HPLC;
516 class VP with refractive index detector [RID] 10A and photo-diode array detector SPD-M10A

517 VP detectors; Shimadzu, Japan) was used to determine the fatty acid concentrations. To further
518 quantify other elements in the porewater (i.e. phosphorous and sulfur) the samples were
519 acidified in 1% (v/v) HNO₃ and analyzed in triplicates by inductively coupled plasma mass
520 spectrometry (ICP-MS/MS Agilent 8900). A flow injection analysis (FIA) instrument equipped
521 with a dialysis membrane for removal of Fe to prevent side reactions during measurement (Seal
522 Analytical, Germany) was applied for quantification of NH₄⁺, NO₃⁻ and NO₂⁻ concentrations.

523 **Correlative SEM and nanoSIMS analysis.** The free particles of the fine fraction of each
524 organic horizon, transition zone and mineral horizon in cores Palsa A (referred to intact palsa),
525 Palsa B (referred to more collapsed palsa) and Front (referred to collapsing front) along the
526 palsa hillslope were analyzed using correlative SEM and nanoSIMS, as described
527 previously^{19,69,70}. Briefly, subsamples of each layer (1 mg) were dispersed in anoxic deionized
528 water and gently shaken to obtain the free organo-mineral particles. All larger particles and
529 aggregates were allowed to settle. A drop of 100 μl of the suspension was placed on a silica
530 wafer and dried in an anoxic glovebox (N₂ atmosphere). Finally, samples were sputter-coated
531 with ~30 nm Au/Pd conductive layer using a Bal-Tec SCD005 sputter coater (Baltec GmbH,
532 Germany). To characterize the organo-mineral particles of the fine fraction by size and
533 crystallinity and identify representative particles, a field emission scanning electron microscope
534 (FE-SEM; Jeol JSM-6500F), equipped with secondary electron detector, was used prior to
535 nanoSIMS analysis. The acceleration voltage was set to 5 kV, with a working distance of 10
536 mm. The nanoSIMS analysis were performed at the Cameca nanoSIMS 50L of the Chair of
537 Soil Science (TU München, Germany). As described previously¹⁹, a primary ion beam (~2 pA)
538 was set at a lateral resolution ~100 nm and scanned over the samples with ¹²C⁻, ¹⁶O⁻, ¹²C¹⁴N⁻, ³¹P⁻,
539 ³²S⁻, ²⁷Al¹⁶O⁻ and ⁵⁶Fe¹⁶O⁻ secondary ions collected using electron multipliers.

540

541 **Mössbauer spectroscopy.** The soil samples for ^{57}Fe Mössbauer spectroscopy were collected
542 under the protection of 100% N_2 . Samples from three thaw stages were measured, including
543 Palsa A, Bog and Fen (both wetland cores obtained by a previous campaign see Patzner *et al.*¹⁹)
544 of transition zone and mineral horizon (Figure S16 and Table S7). The samples were dried
545 anoxically before loading into a Plexiglass holder. The prepared samples were stored anoxically
546 at -20°C until measurement. Mössbauer spectroscopy was performed in a standard transmission
547 setup (Wissel, Wissenschaftliche Elektronik GmbH), and absorption spectra were collected at
548 77 and 6 K controlling with a closed-cycle cryostat (SHI-850-I, Janis Research Co). The spectra
549 were calibrated with $\alpha^{57}\text{Fe}^0$ foil at 295 K, and fitted using the Voigt Based Fitting (VBF)⁷¹
550 routine in the Recoil software (University of Ottawa, Canada). Results are shown in the Figure
551 S16 and Table S7.

552 **TOC and TN analysis.** As described previously¹⁹, total organic carbon (TOC) and total organic
553 nitrogen (TN) were quantified by an Elementar vario El (Elementar Analysysteme, GmbH,
554 Germany). Soil samples were dried at 60°C until no further weight loss was observed. The dried
555 soils were ground and acidified with 16% HCl to remove the inorganic carbon. After washing
556 with deionized water, followed by drying, the TOC and TN content was analyzed. Results of
557 C/N ratios are shown in the SI (Figure S10).

558 **Microbial community analysis.** Total RNA and DNA was extracted using the PowerSoil®
559 RNA and DNA isolation kit as described by the manufacturer (MO BIO Laboratories, Carlsbad,
560 CA, USA), with the following modifications: 2-3 g of soil was used from each soil horizon; 10
561 min bead-beating; centrifugation steps at maximal speed (7000 x g) at 4°C ; and longer
562 incubation times at -20°C (1.5 h). RNA and DNA were eluted in 50 μl RNase/DNase-Free
563 water. RNA and DNA concentrations were determined using a Qubit® 2.0 Fluorometer with
564 RNA and DNA HS kits (Life Technologies, Carlsbad, CA, USA). Subsequent DNA digestion

565 and reverse transcription reactions were performed using a Reverse Transcriptase (Invitrogen,
566 Life Technologies) as described previously by Otte *et al.*, 2018⁴⁸. Quantitative PCR (qPCR)
567 specific for the 16S rRNA (gene) of bacteria and archaea was performed as described
568 previously⁴⁸. Microbial 16S rRNA (genes) were amplified using primers 515F and 806R⁷².
569 Quality and quantity of the purified amplicons were determined using agarose gel
570 electrophoresis and Nanodrop (NanoDrop 1000, Thermo Scientific, Waltham, MA, USA).
571 Subsequent library preparation steps (Nextera, Illumina) and sequencing were performed by
572 Microsynth AG (Switzerland) using the 2 × 250 bp MiSeq Reagent Kit v2 on an Illumina MiSeq
573 sequencing system (Illumina, San Diego, CA, USA). From 10,112 to 396,483 (average
574 113,374) read pairs were generated per sample in three separate sequencing runs on the same
575 MiSeq machine, resulting in total in 8.6 million read pairs. Quality control, reconstruction of
576 16S rRNA (gene) sequences and taxonomic annotation was performed with nf-core/ampliseq
577 v1.1.2^{73,74} with Nextflow v20.10.0⁷⁵ using containerized software with singularity v3.4.2⁷⁶. Data
578 from the three sequencing runs were treated initially separately by the pipeline using the option
579 “multipleSequencingRuns” and ASV tables were merged. Primers were trimmed, and
580 untrimmed sequences were discarded (< 25%, on average 7.7%) with Cutadapt v2.6⁷⁷. Adapter
581 and primer-free sequences were imported into QIIME2 version 2019.10.0⁷⁸, processed with
582 DADA2 version 1.10.0⁷⁹ to eliminate PhiX contamination, trim reads (before the median
583 quality drops below 35, i.e. position 230 in forward reads and 174 in reverse reads), correct
584 errors, merge read pairs, and remove PCR chimeras; ultimately, in total 9,576 amplicon
585 sequencing variants (ASVs) were obtained across all samples. Alpha rarefaction curves were
586 produced with the QIIME2 diversity alpha-rarefaction plugin, which indicated that the richness
587 of the samples had been fully observed. A Naive Bayes classifier was fitted with 16S rRNA
588 (gene) sequences extracted with the PCR primer sequences from the QIIME compatible, 99%-
589 identity clustered SILVA v132 database⁸⁰. ASVs were classified by taxon using the fitted

590 classifier⁸¹. 225 ASVs that classified as chloroplasts or mitochondria were removed, totalling
591 to < 7% (average 0.6%) relative abundance per sample, and the remaining 9,351 ASVs had
592 their abundances extracted by feature-table (<https://github.com/qiime2/q2-feature-table>).

593 Pathways, i.e. MetaCyc ontology predictions, were inferred with PICRUSt2 version 2.2.0-b
594 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States)⁸² and
595 MinPath (Minimal set of Pathways)⁸³ using ASVs and their abundance counts. Inferring
596 metabolic pathways from 16S rRNA amplicon sequencing data is certainly not as accurate as
597 measuring genes by shotgun metagenomics, but it yields helpful approximations to support
598 hypotheses driven by additional microbiological and biogeochemical analyses⁸².

599 **FT-ICR-MS analysis.** Soil extracts and DOM in the porewater were analyzed with FT-ICR
600 MS to identify and monitor compositional changes in the mineral-associated organic carbon
601 fraction and the mobile, DOC fraction. All of the samples were prepared for FT-ICR-MS
602 analysis by solid phase extraction (SPE) under N₂ atmosphere (glove bag) following the
603 procedure described by Dittmar *et al.*, 2008⁸⁴ and Li *et al.*, 2016⁸⁵ with some modifications. In
604 brief, 1 g, 3 mL Bond Elut PPL cartridges (part#12102148, Aglient Technologies, Santa Clara,
605 CA, USA) were conditioned with 5 mL of HPLC grade methanol (Sigma-Aldrich, Rehovot,
606 Israel), followed by 5 mL of 0.01 M HCl. Each sample was acidified to pH ~2.5 and then loaded
607 onto the SPE columns, loading volume was adjusted to load a total of 0.5 mg C based on the
608 TOC content. After sample loading, the SPE cartridges were rinsed with 5 mL of 0.01 M HCl
609 followed by drying with N₂ for 3-5 mins. Finally, the samples were eluted with 1 mL of HPLC
610 grade methanol and stored in airtight amber sample vials wrapped in aluminum foil at 4°C.
611 There was no additional dilution of the samples performed prior to analysis by negative ion
612 electrospray ionization.

613 The samples were analyzed with a custom-built FT-ICR mass spectrometer, equipped with a
614 21T superconducting solenoid magnet and a modular software package for data acquisition
615 (Predator)⁸⁶. Sample solution was infused via a microelectrospray source⁸⁷ (50 μm i.d. fused
616 silica emitter) at 500 nL/min by a syringe pump. Typical conditions for negative ion formation
617 were: emitter voltage, -3.0 kV; S-lens RF level, 45%; and heated metal capillary temperature,
618 350 °C. Ions were initially accumulated in an external multipole ion guide (1-5 ms) and released
619 m/z -dependently⁸⁸. Ions were excited to m/z -dependent radius to maximize the dynamic range
620 and number of observed mass spectral peaks (32-64%)⁸⁹, and excitation and detection were
621 performed on the same pair of electrodes⁹⁰. The dynamically harmonized ICR cell in the 21 T
622 FT-ICR is operated with 6 V trapping potential^{91,92}. Time-domain transients (100 time-domain
623 acquisitions for all experiments) of 3.1 seconds were acquired with the Predator data station
624 that handled excitation and detection only, initiated by a TTL trigger from the commercial
625 Thermo data station⁹³. Mass spectra were phase-corrected⁹⁴ and internally calibrated with 10-
626 15 highly abundant homologous series that span the entire molecular weight distribution (~150
627 to 1300 m/z) based on the “walking” calibration method⁹⁵. Experimentally measured masses
628 were converted from the International Union of Pure and Applied Chemistry (IUPAC) mass
629 scale to the Kendrick mass scale⁹⁶ for rapid identification of homologous series for each
630 heteroatom class⁹⁷. Peaks with signal to noise ratios greater than 6 times the noise at the baseline
631 root-mean-square (rms) noise at m/z 500 were exported to custom software (PetroOrg©) for
632 additional formula and elemental composition assignment⁹⁸. All assigned formulas were part of
633 a ≥ 3 peak carbon series and had less than ± 0.3 ppm mass error. A LOD of 6σ was considered
634 sufficient to minimize ionization difference effects between samples, and therefore biasing by
635 large numbers of low abundance peaks. To further identify macro compositional shifts, analysis
636 of differences between samples was performed only on peaks with $\geq 20\%$ difference in relative
637 abundance. Additionally, modified aromaticity index (ModAI) was calculated according to

638 Koch&Dittmar⁸⁴ and nominal oxidation state of carbon (NOSC) was calculated according to La
639 Rowe&Van Cappellen⁹⁹. Data processing post formula assignment was performed with
640 RStudio utilizing R software (V4.0.3).

641 **Statistical analysis.** The geochemical parameters were checked with the test of homogeneity.
642 Then a one-way analysis of variance (ANOVA) was used to identify differences in the
643 geochemical parameters along the palsa hillslope, combined with a post-hoc test to identify
644 significant differences between the different sampling spots along the palsa hillslope (from
645 palsa to collapsing front to bog). Based on Gloor *et al.*¹⁰⁰ no statistical analysis (such as e.g.
646 one-way ANOVA or unpaired t-test) were chosen for the compositional data obtained by 16S
647 rRNA Amplicon (gene) sequencing.

648

649 DATA AVAILABILITY

650 The data that support the finding of this study are included in a compressed Source Data file at
651 Zenodo (doi link will be included after acceptance of the manuscript) and raw sequencing data
652 was deposited at the Sequence Reads Archive (SRA, reviewer link included
653 <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA725216>, final link will be included after
654 acceptance of the manuscript). All FT-ICR MS data (raw files and calibrated peak lists) are
655 available at the Open Science Framework (link will be included after acceptance of the
656 manuscript). Other data are included in the Supplementary Information. Pre-processed data are
657 available upon request.

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661 **Author Contributions:**

662 The original hypothesis was formulated by M.S.P., C.B. and A.K. M.S.P, C.B. and A.K.
663 designed the project, interpreted the data and wrote the manuscript. M.S.P, C.B. and M.L.
664 collected the samples. M.S.P and M.L gathered the data presented in the main text. A.M.
665 conducted the FT-ICR-MS measurements and contributed to the data interpretation. T.B. and
666 R.Y. contributed to the data analysis and interpretation. Z.Z. performed the Mössbauer
667 spectroscopy and helped interpreting the results. H. J helped collecting the porewater samples
668 and data interpretation. C.H. and C.W.M., together with M.S.P., collected, analyzed and
669 interpreted the nanoSIMS data. D.S. processed the amplicon sequencing data and, together with
670 S.K., helped with interpretation of the microbial community results. T.S. contributed to project
671 design and data interpretation. All authors contributed to the preparation of the manuscript and
672 have given approval to the final version of the manuscript.

673 **Notes:**

674 The authors declare no competing interests.

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936

Supplementary Information for

937 **Microbial iron(III) reduction during palsa collapse promotes greenhouse**
938 **gas emissions before complete permafrost thaw**

939

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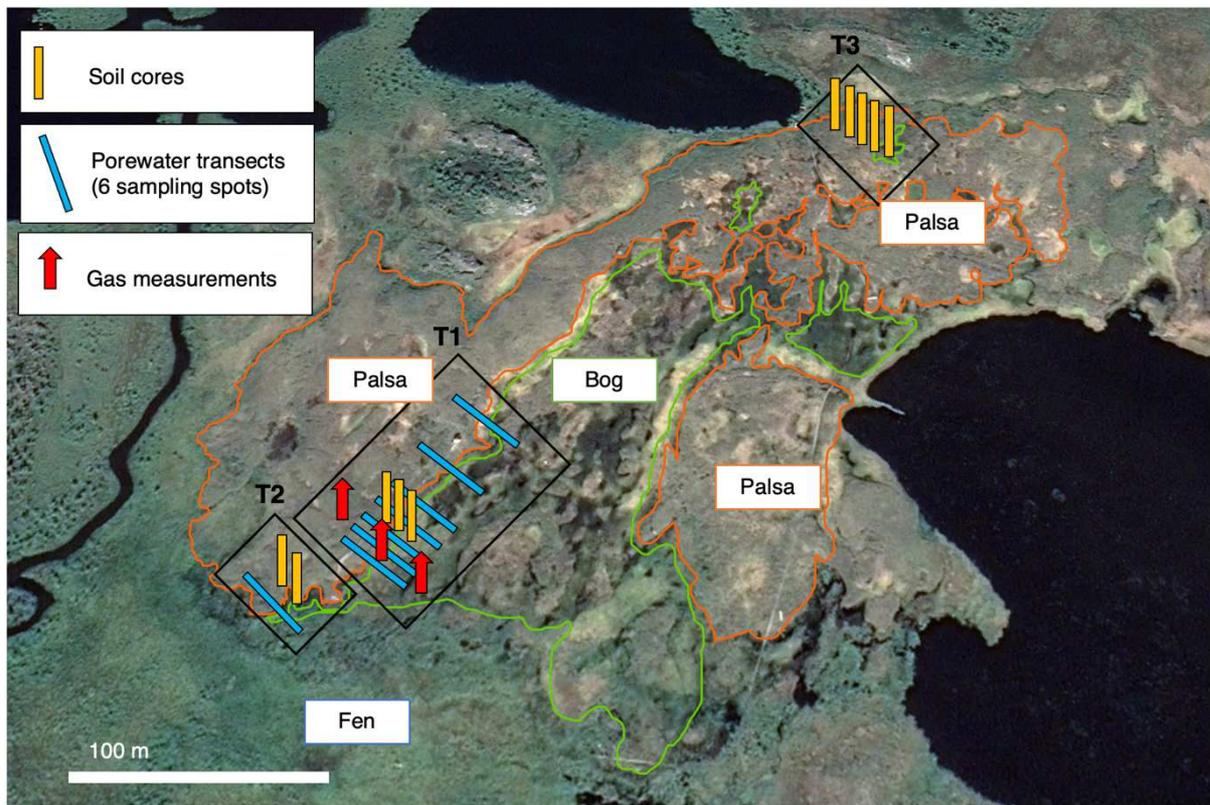
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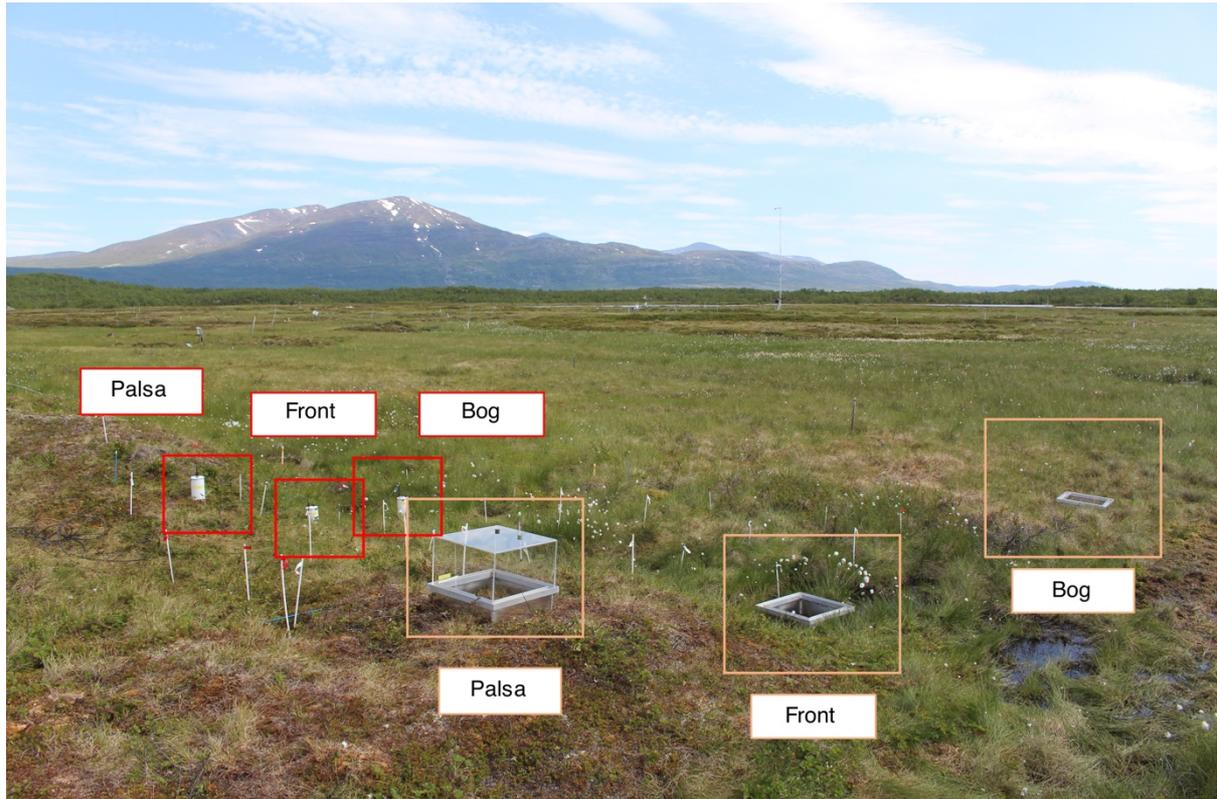


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941

942 **Figure S1. Field site and sample collection.** Soil cores (yellow), porewater samples (blue) and
 943 gas samples (red) were taken in three transects (T1, T2 and T3) along palsa hillslopes into bog
 944 at Stordalen mire, Abisko (Sweden). Background picture was taken by GoogleEarth in 2019.

945

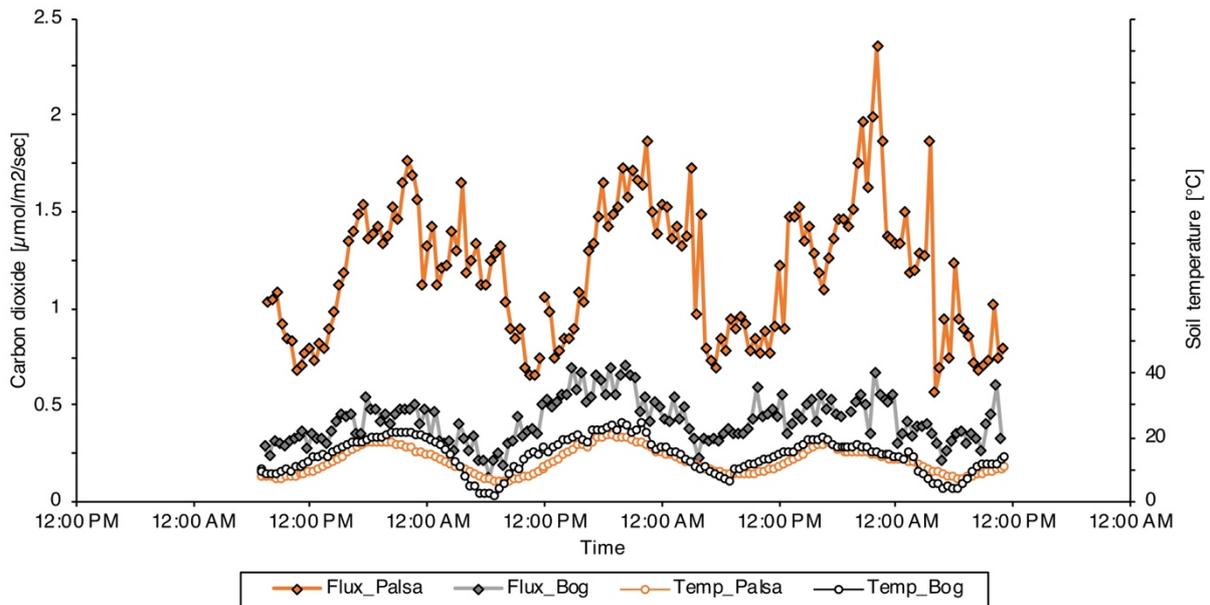


946

947

948 **Figure S2. Gas sampling along palsa hillslopes.** In transect 1 (see also Figure S1), Eosense
949 gas chambers (eosFD Forced Diffusion chamber in conjunction with the eosLink-FD software,
950 EOSENSE INC, Dartmouth, Canada) (red) were installed to measure carbon dioxide emissions
951 along the palsa hillslope (68°21'18.70"N, 19° 2'38.00"E). Additional gas chambers with plastic
952 chambers, sealed with a rubber stopper, and metal frames (orange) were installed to obtain
953 replicate carbon dioxide and additionally methane emissions along the palsa hillslope into the
954 bog area.

955

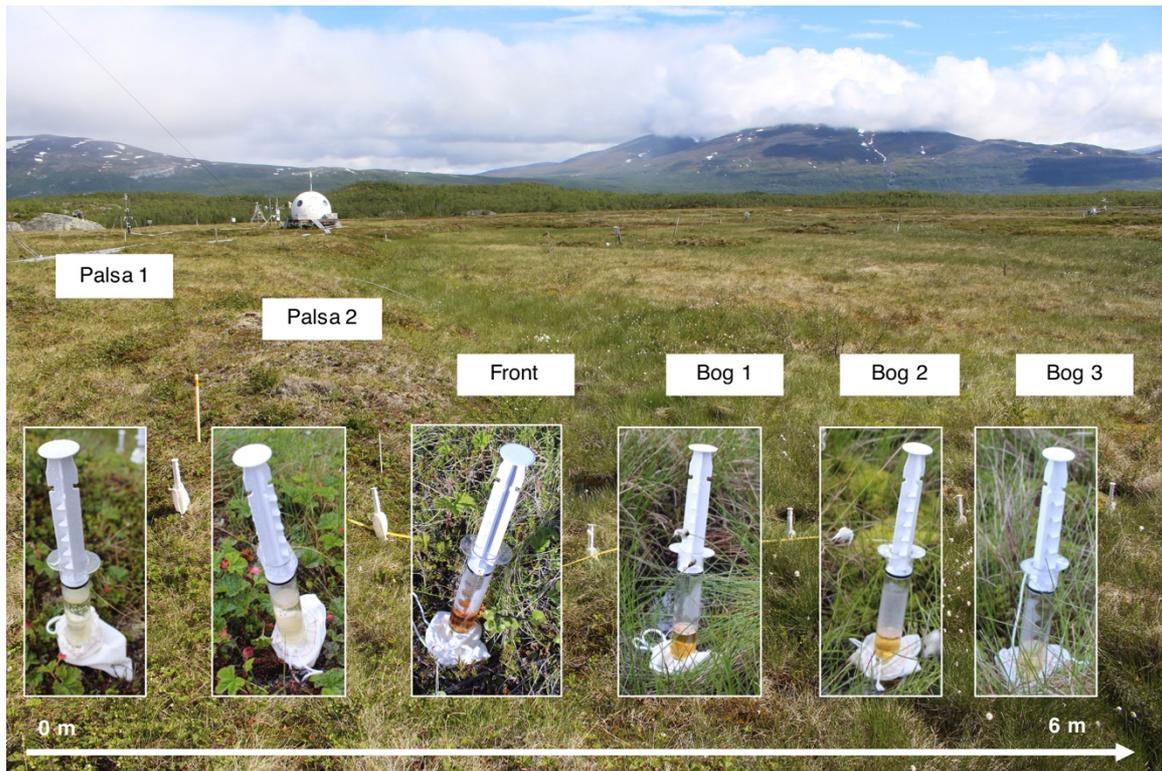


956

957

958 **Figure S3. Carbon dioxide emissions along the palsa hillslope (palsa and bog).** Eosense gas
 959 chambers (eosFD Forced Diffusion chamber in conjunction with the eosLink-FD software,
 960 EOSENSE INC, Dartmouth, Canada) were installed along the palsa hillslope and analysis
 961 performed from the 8th of July to 10th of July 2019. Unfortunately, the second Eosense
 962 instrument at the collapsing front (shown in Figure S2) was broken during shipment and thus
 963 excluded in the analysis. The carbon dioxide emissions correlate with the surface soil
 964 temperature (measured at 5 cm soil depth at palsa and bog), measured by Integrated Carbon
 965 Observation System (ICOS) Sweden Abisko – Stordalen¹.

966

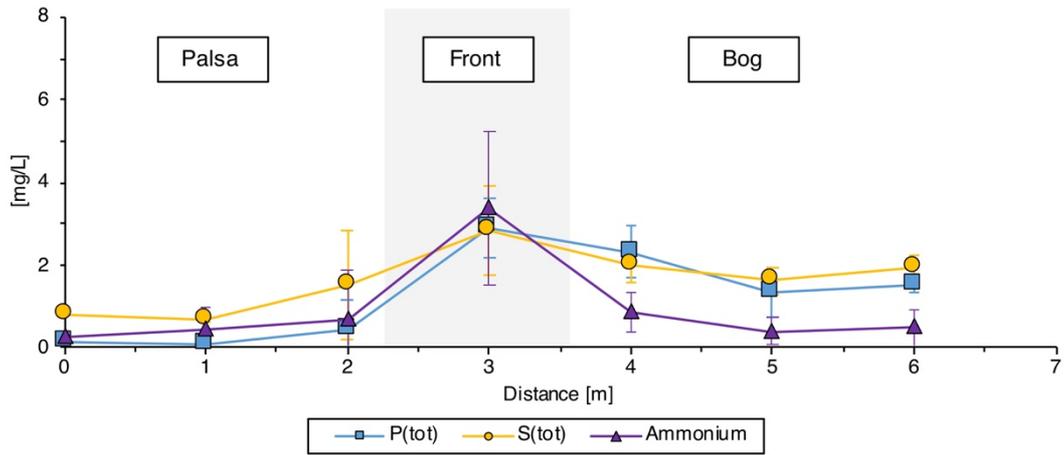


967

968

969 **Figure S4. Porewater sampling along palsa hillslopes.** Along eight palsa hillslopes,
 970 porewater was extracted with lysimeters at six defined sampling points in July 2019. In
 971 transect 1 (shown here, see also Figure S1), lysimeters were installed for 3-4 hours along the
 972 palsa hillslope (68°21'18.70"N, 19° 2'38.00"E) at a distance of 1 m in between each other from
 973 palsa to bog. The porewater with dark brown, reddish color at the collapsing front represents
 974 the sample with the highest aqueous iron and dissolved organic carbon along the palsa hillslope
 975 into bog.

976

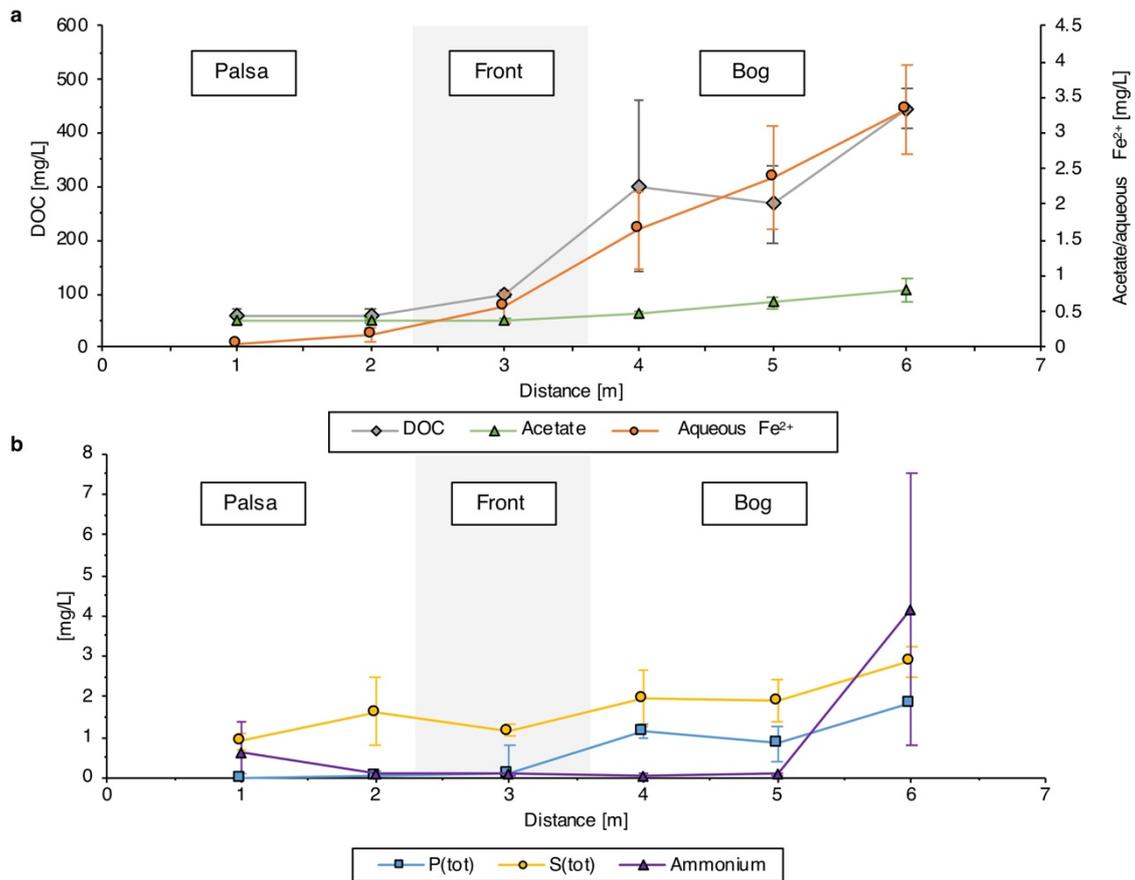


977

978

979 **Figure S5. Aqueous total phosphorous (P(tot)), aqueous total sulfur (S(tot)) and**
 980 **ammonium concentrations in the porewater along the palsa hillslope (30 cm depth,**
 981 **transition zone).** Aqueous concentrations are reported in mg/L from palsa (0-2.7 m) to bog
 982 (2.7-7 m). Reported values represent the average of six sampling spots for eight palsa hillslopes
 983 (0-2.3 m) to collapsing front (2.3-3.6 m) to bog (3.6-7 m), sampled in June/July (see also SI,
 984 Figures S1 and Figure S4). Error bars represent the standard deviation of eight palsa hillslopes
 985 (0-2.3 m) to collapsing front (2.3-3.6 m) to bog (3.6-7 m), sampled in June/July (see also SI,
 986 Figure S1).

987



988

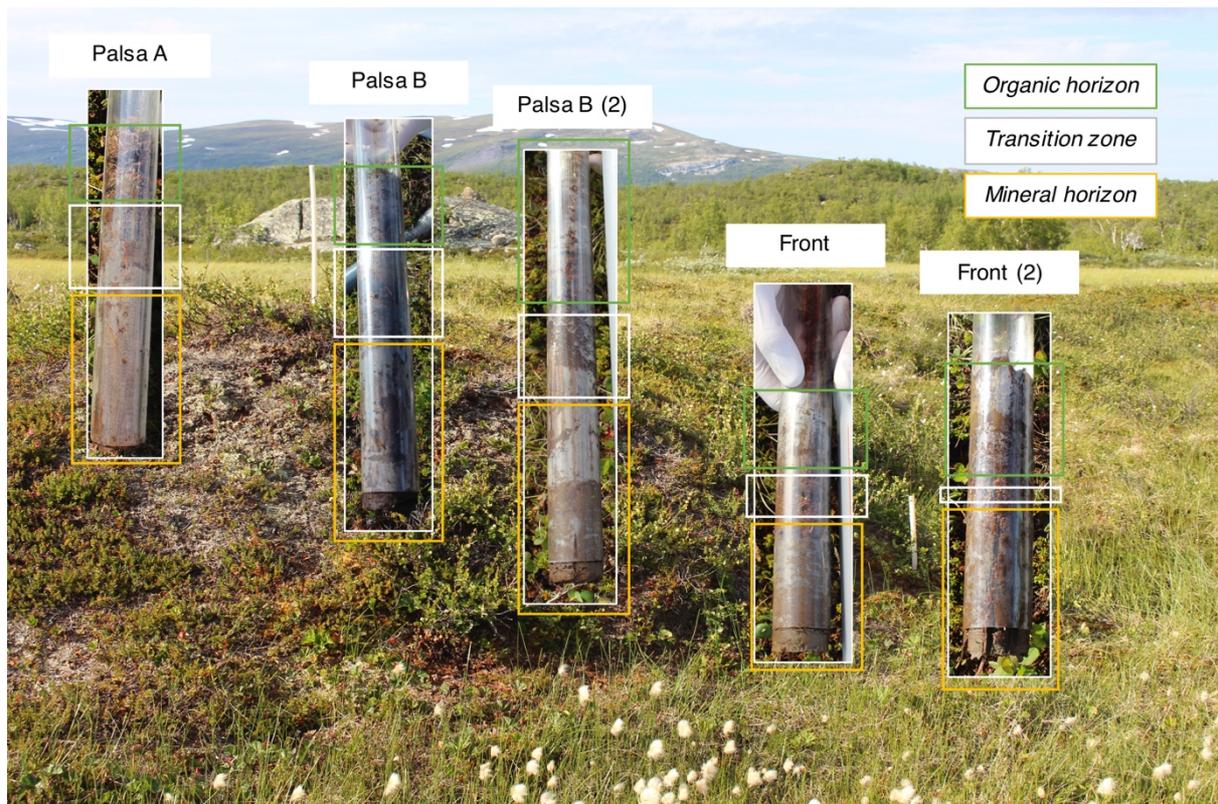
989

990 **Figure S6. Porewater analysis along the palsa hillslope (60 cm, mineral horizon).** a,
 991 Dissolved organic carbon (DOC), acetate and aqueous Fe²⁺ in mg/L and b, aqueous total
 992 phosphorous (P(tot)), aqueous total sulfur (S(tot)) and ammonium concentrations along the
 993 collapsing palsa hillslope into bog. Reported values represent the average of six sampling spots
 994 for eight palsa hillslopes (0-2.3 m) to collapsing front (2.3-3.6 m) to bog (3.6-7 m), sampled in
 995 June/July (see also SI, Figure S1). Error bars represent the standard deviation of eight palsa
 996 hillslopes (0-2.3 m) to collapsing front (2.3-3.6 m) to bog (3.6-7 m), sampled in June/July (see
 997 also SI, Figure S1).

998

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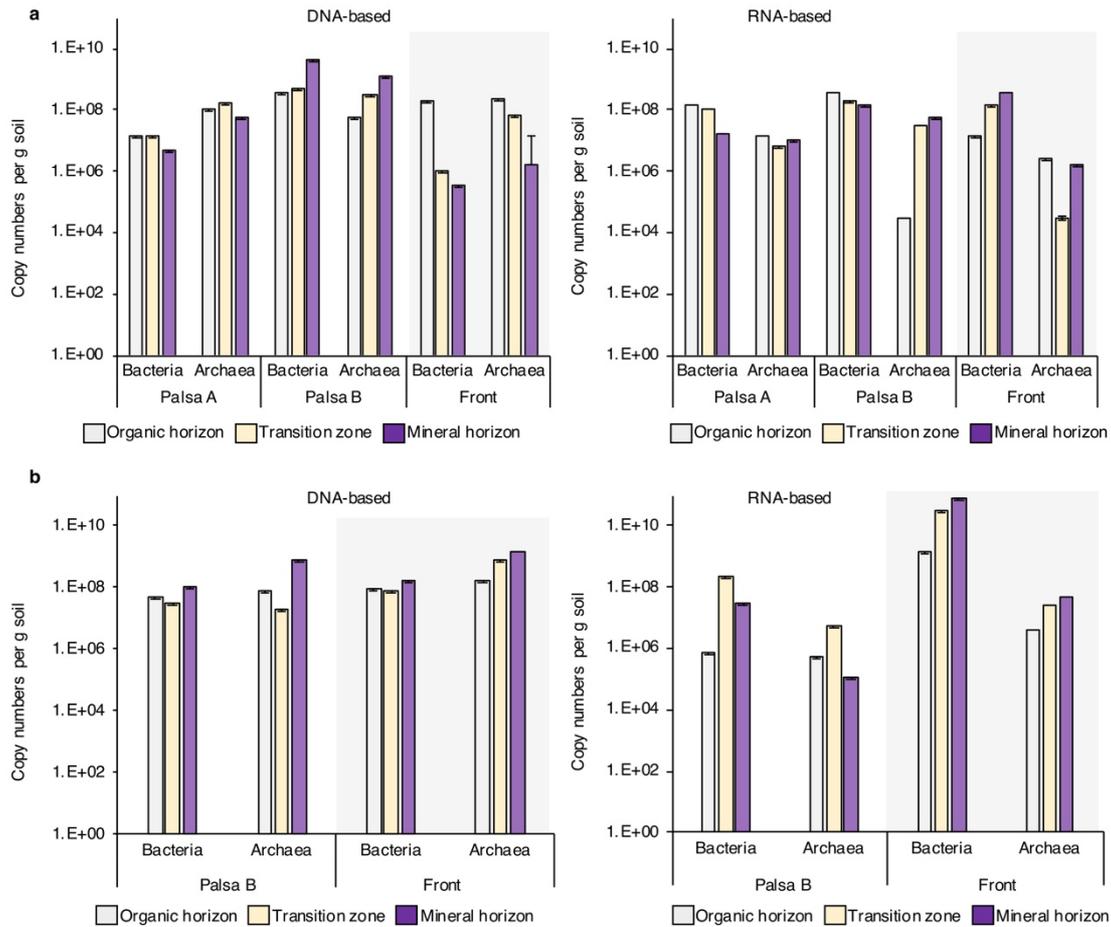


1001

1002

1003 **Figure S7. Coring along palsa hillslopes.** Ten soil cores were taken along different palsa
1004 hillslopes to capture spatial heterogeneity of iron-carbon associations along the peatland mire.
1005 In transect 3 (shown here, see also Figure S1), five cores were taken along a palsa hillslope
1006 towards the collapsing front into bog (68°21'27.33"N, 19° 3'1.91"E), immediately frozen in
1007 liquid nitrogen and stored at -80°C until analysis of iron-carbon associations and of present and
1008 active microbial community.

1009

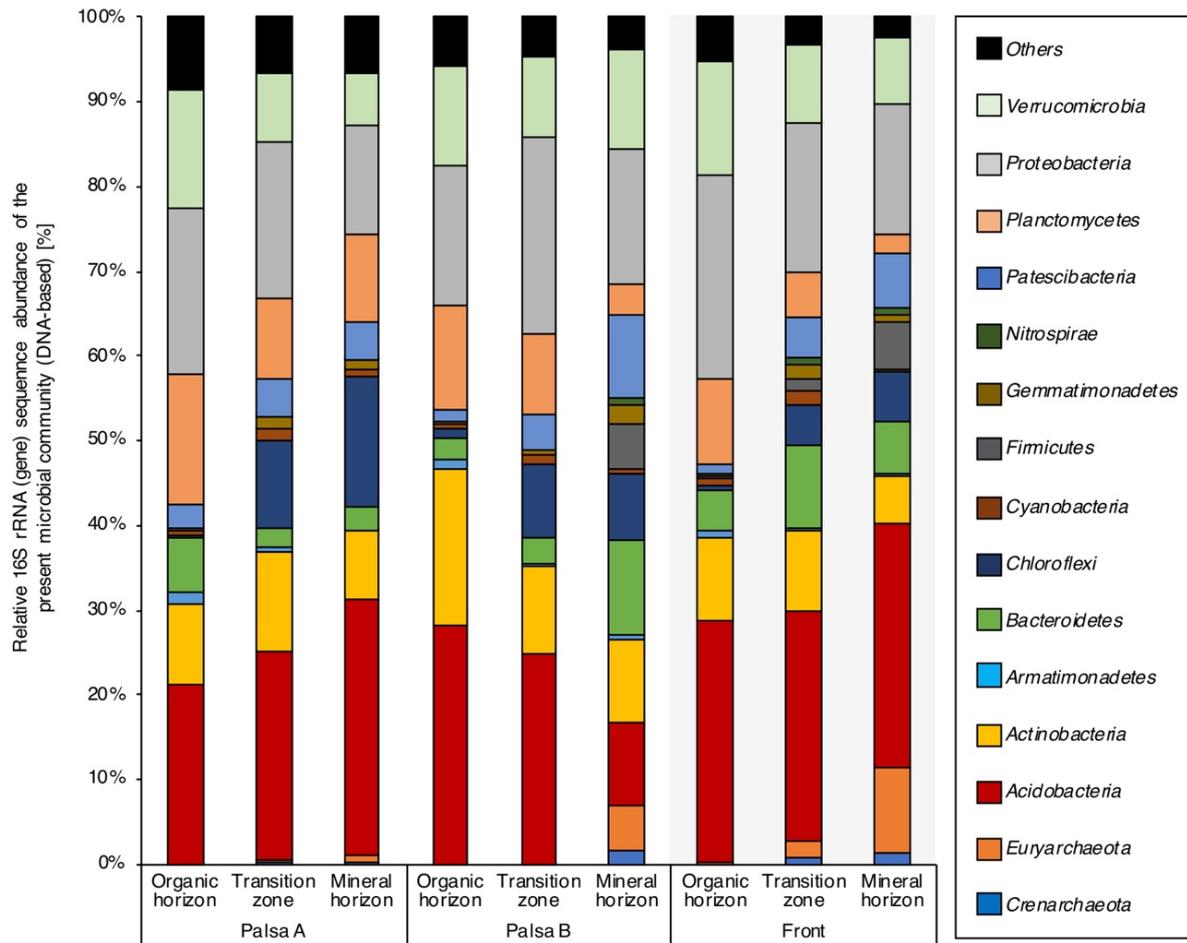


1010

1011

1012 **Figure S8. Abundance of bacteria and archaea** (copy numbers based on qPCR analysis
 1013 specific for 16S rRNA genes; DNA-based on the left, RNA-based on the right). a, and b, show
 1014 replicate analysis for Palsa A, Palsa B and Front (a), and Palsa A and Front (b).

1015



1016

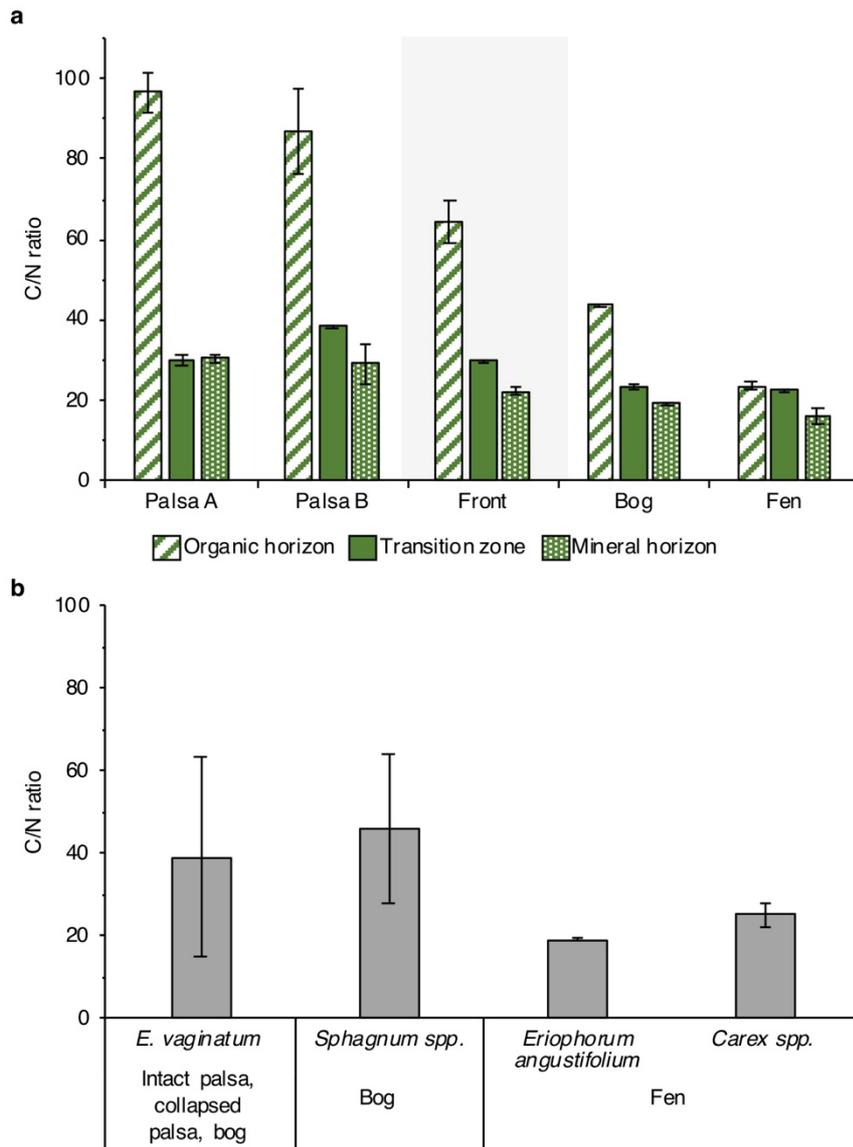
1017

1018 **Figure S9 Taxonomic identification of the microbial communities along the palsa hillslope**

1019 based on 16S rRNA gene amplicon analysis (DNA-based). Data were averaged among triplicate

1020 analysis of each soil horizon (organic horizon, transition zone, mineral horizon).

1021



Values obtained from Hodgkins *et al.* (2014)

1022

1023

1024 **Figure S10. C/N weight ratios of a, soil samples of distinguished soil horizon along palsa**

1025 **hillslope and thaw gradient, in comparison to b, living plant samples of dominant**

1026 **Stordalen species (modified and adapted from Hodgkins *et al.*²). Reported values represent**

1027 the average of triplicate analysis of cores Palsa A, Palsa B, Front (transect 1, Figure S1) and

1028 Bog C and Fen E, which were previously published³. Error bars represent the standard deviation

1029 of triplicate analysis of cores Palsa A, Palsa B, Front (transect 1, Figure S1), one bog (Bog C)

1030 and one fen core (Fen E) (see also Patzner *et al.*³).

1031

1032 **Table S1. Overview of iron(II)-oxidizing microorganisms that were cross-checked in the**
1033 **16S rRNA amplicon gene sequencing results (DNA- and RNA-based) in this study (adapted**
1034 **from Otte *et al.*⁴ and Weber *et al.*⁵, see also Dinh *et al.*⁶ and Berg *et al.*⁷).**

1035

Iron(II)-oxidizing microorganisms (species or strains)

Acidianus brierleyi
Acidiplasma aeolicum; *A. cupricumulans*
Acidimicrobium ferrooxidans sp. strain DSM 10331
Acidithiobacillus ferrooxidans sp. strain ATCC 23270
Acidovorax sp. strains 2AN, BoFeN1, BrG1; *A. delafieldii*; *A. ebreus* strain TPSY
Alicyclobacillus disulidooxidans; *A. tolerans*
Aquabacterium sp. strains BrG2, HidR2
Azoarcus sp. strain ToN1
Azospira sp. strain TR1; *A. oryzae*
Bradyrhizobium japonicum sp. strains 22, in8p8, wss14
Candidatus Brocadia sinica
Candidatus Scalindua sp.
Chlorobium luteolum DSM273; *C. ferrooxidans* sp. strain KoFox
Chromobacterium violaceum sp. strain 2002
Citrobacter freundii sp. strain PXL1
Comamonas sp. strain MPI12
Crenothrix sp. #
Cupriavidus necator sp. strains A5-1, ss1-6-6
Dechlorobacter hydrogenophilus sp. strain LT-1
Dechloromonas sp.; *D. agitata* sp. strains CKB, is5; *D. aromatica* sp. strains RCB, UWNR4; *D. suillum* sp. strain PS
Dechlorospirillum sp. strain M1
Denitromonas indolicum
Desulfitobacterium frappieri sp. strain G2
Desulfobacterium corrodens (Dinh *et al.*, 2004)
Escherichia coli sp. strain E4
Ferrimicrobium acidiphilum
Ferriphaselus amnicola (*Siderooxydans*)
Ferrithrix thermotolerans
Ferritrophicum raditicola
Ferroglobus placidus sp. strain AEDII12DO
Ferroplasma acidarmanus sp. strain fer1
Ferrovibrio denitrificans
Gallionella capsiferriformans; *G. ferruginea*; *G.* strain ES-2
Geobacter metallireducens sp. strain GS-15
Geothrix spp.
Hoeflea marina; *H. siderophila* sp. strain Hf1
Hyphomicrobium sp.

1036

(Table continues on next page)

1037

Hyphomonas sp.
Klebsiella-like sp. strain FW33AN
Leptospirillum ferrooxidans; *L. ferriphilum*
Leptothrix cholodnii; *L. discophora*
Magnetococcus sp. (Berg *et al.*, 2016)
Magnetospirillum bellicus sp. strain VDY
Marinobacter aquaeolei sp. strain VT8
Mariprofundus ferrooxidans sp. strains PV-1, RL-1, JV-1, GSBS
Metallosphaera sedula sp. strain J1
Nocardioides sp. strain In31
Paracoccus sp.; *P. denitrificans*; *P. ferrooxydans* sp. strain BDN-1; *P. pantotrophus*; *P. versutus*
Parvibaculum sp. strain MBN-A2
Pedomicrobium spp.
Propionivibrio militaris sp. strain MP
Pseudogulbenkiania ferrooxidans sp. strain 2002
Pseudomonas sp. strains LP-1, SZF15; *P. stutzeri*
Ralstonia solanacearum sp. strain in4ss52
Rhodanobacter sp. strain MPN-A3
Rhodobacter sp. strain SW2; *R. ferrooxydans*
Rhodomicrobium vannielii
Rhodopseudomonas palustris strain TIE-1
Rhodovulum sp.; *R. iodosum*; *R. robiginosum*
Rubrivivax group sp. strains BrG4, BrG5
Siderocapsa sp.
Sideroxydans paludicola; *S. lithotrophicus* sp. strain ES-1
Sphaerotilus natans sp. strain DSM 6575
Sulfobacillus spp.
Sulfobus acidocaldarius
Thauera aromatica sp. strain AR-1
Thermomonas sp. strain BrG3
Thiobacillus denitrificans
Thiodictyon sp.
Thiomicrospira denitrificans
Zixibacteria sp. strain RBG-1

1038

Crenothrix is most often associated with methanotrophy but there are also hints for Fe(II) oxidation.

1039

1040 **Table S2. Overview of iron(III)-reducing microorganisms that were cross-checked in the**
1041 **16S rRNA amplicon gene sequencing results in this study** (adapted from Otte *et al.*⁴ and
1042 Weber *et al.*⁵, see also Berg *et al.*⁷, Li *et al.*⁸, Holmes *et al.*⁹, Finneran *et al.*¹⁰).

1043

Iron(III)-reducing microorganisms (species or strains)

Acidithiobacillus ferrooxidans
Aeromonas hydrophila
Albidoferax ferrireducens
Alteromonas hydrophila
Anaeromyxobacter sp.
Bacillus infernus
Clostridium sp.
Deferribacter thermophilus
Desulfobacter propionicus
Desulfobacterium sp.
Desulfobulbus spp.
Desulfococcus spp.
Desulfotalea spp.
Desulfotomaculum sp. (Berg *et al.*, 2016)
Desulfovibrio sp. (Li *et al.*, 2016)
Desulfuromonas spp.
Desulfuromusa spp.
Ferribacterium limneticum
Ferrimonas balearica
Ferroglobus placidus
Geobacter spp.
Geoglobus sp.
Geothrix fermentans
Geovibrio ferrireducens
Malonomonas sp. (Holmes *et al.*, 2004)
Myxococcales sp.
Pantoea agglomerans sp. strain Sp1
Pelobacter sp.
Pseudomonas sp.
Pseudorhodoferax (Berg *et al.*, 2016)
Pyrobaculum sp.
Rhodobacter sp.
Rhodoferax sp. (Finneran *et al.*, 2003)
Shewanella colwelliana
Sinorhodobacter sp.
Sulfurospirillum barnesii
Thermoterrabacterium ferrireducens
Thermotoga maritima
Thermus sp. strain SA01
Thiobacillus ferrooxidans

1044

1045

1046 **Table S3. Overview of methanogenic microorganisms that were cross-checked in the 16S**
1047 **rRNA amplicon gene sequencing results in this study (see also Kim&Whitman¹¹ and**
1048 **Mondav *et al.*¹²).**

1049

Methanogenic microorganisms (genera, species or strains)

Bathyarchaeia
Methanobacterium spp.
Methanobrevibacter spp.
Methanocaldococcus spp.
Methanocella spp.
Methanococcoides spp.
Methanococcus spp.
Methanocorpusculum spp.
Methanoculleus spp.
Methanoflorens spp., *M. stordalenmirensis* (Mondav *et al.*, 2014)
Methanofollis spp.
Methanogenium spp.
Methanohalobium spp.
Methanohalophilus spp.
Methanoignis spp.
Methanolacinia spp.
Methanolinea spp.
Methanolobus spp.
Methanomassillicoccaceae spp.
Methanomethylovorans spp.
Methanomicrobium spp.
Methanoplanus spp.
Methanopyrus spp.
Methanoregula spp.
Methanosaeta spp.
Methanosalsum spp.
Methanosarcina spp.
Methanosphaera spp.
Methanosphaerula spp.
Methanospirillum spp.
Methanothermobacter spp.
Methanothermococcus spp.
Methanothermus spp.
Methanotherix spp.
Methanotorris spp.
Methermicoccus spp.

1050

1051

1052 **Table S4. Overview of methanotrophic microorganisms that were cross-checked in the**
1053 **16S rRNA amplicon gene sequencing results (DNA- and RNA-based) in this study (see**
1054 **also Jiang *et al.*¹³ and Singleton *et al.*¹⁴).**

1055

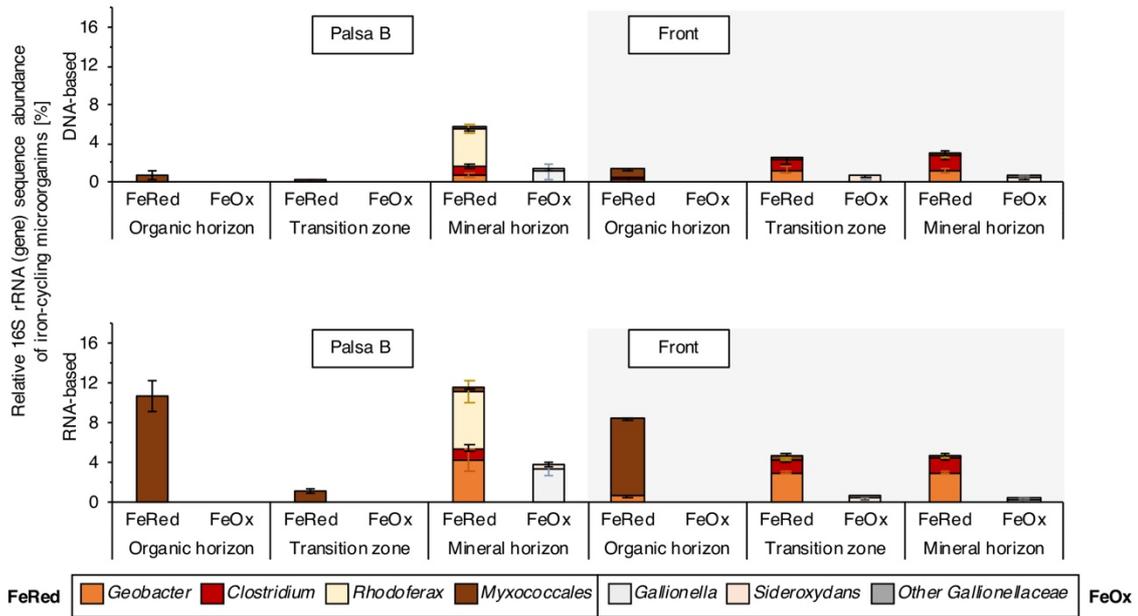
Methanotrophic microorganisms (species or strains)

Acidimethylosilex spp.
Clonothrix spp., *Clonothrix fusca*
Crenothrix spp., *Crenothrix polyspora*
Methylosinus spp.
Methyloacida spp.
Methylobacter spp.
Methylobacterium spp.
Methylocaldum spp.
Methylocapsa spp.
Methylocella spp., *Methylocella silvestris*
Methylococcus spp.
Methylocystis spp.
Methylokorus spp.
Methylomonas spp.
Methylosphaera spp.
Methylothermus spp.
Methylovirgula spp.
Rhodoblastus spp.
Roseiarcus spp.

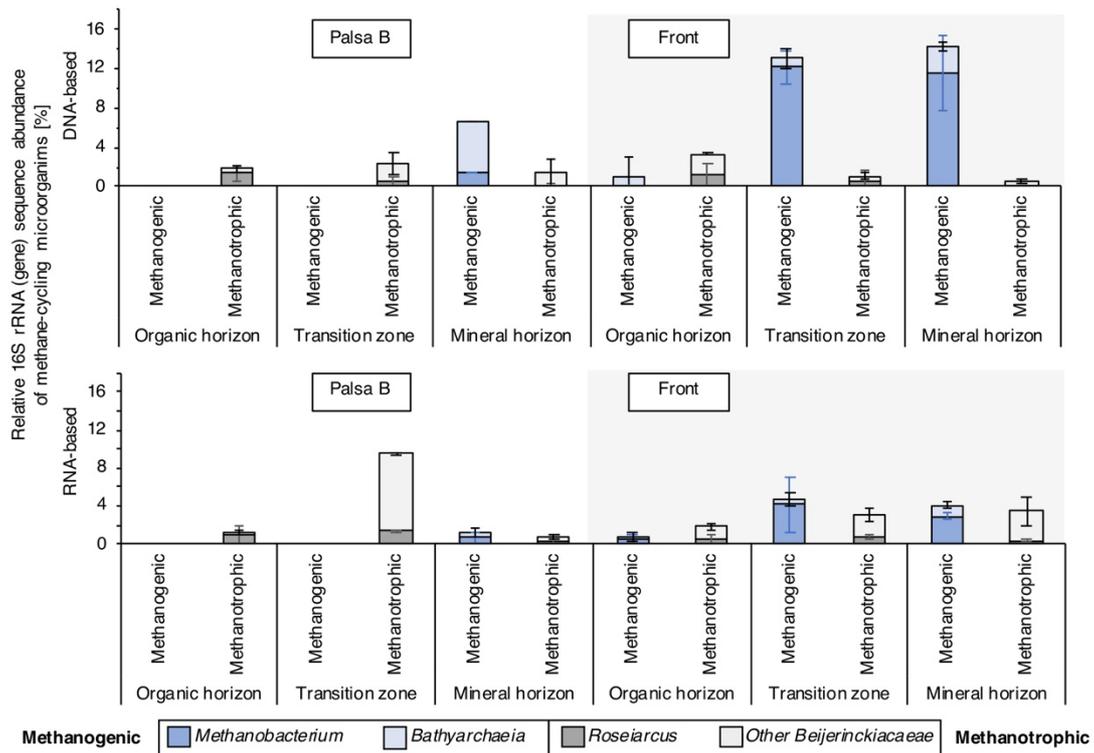
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1057

a Iron-cycling microorganisms along the palsa hillslope



b Methane-cycling microorganisms along the palsa hillslope



1058

1059

1060 **Figure S11. Relative abundance of 16S rRNA (gene) sequence of a, iron- and b, methane**

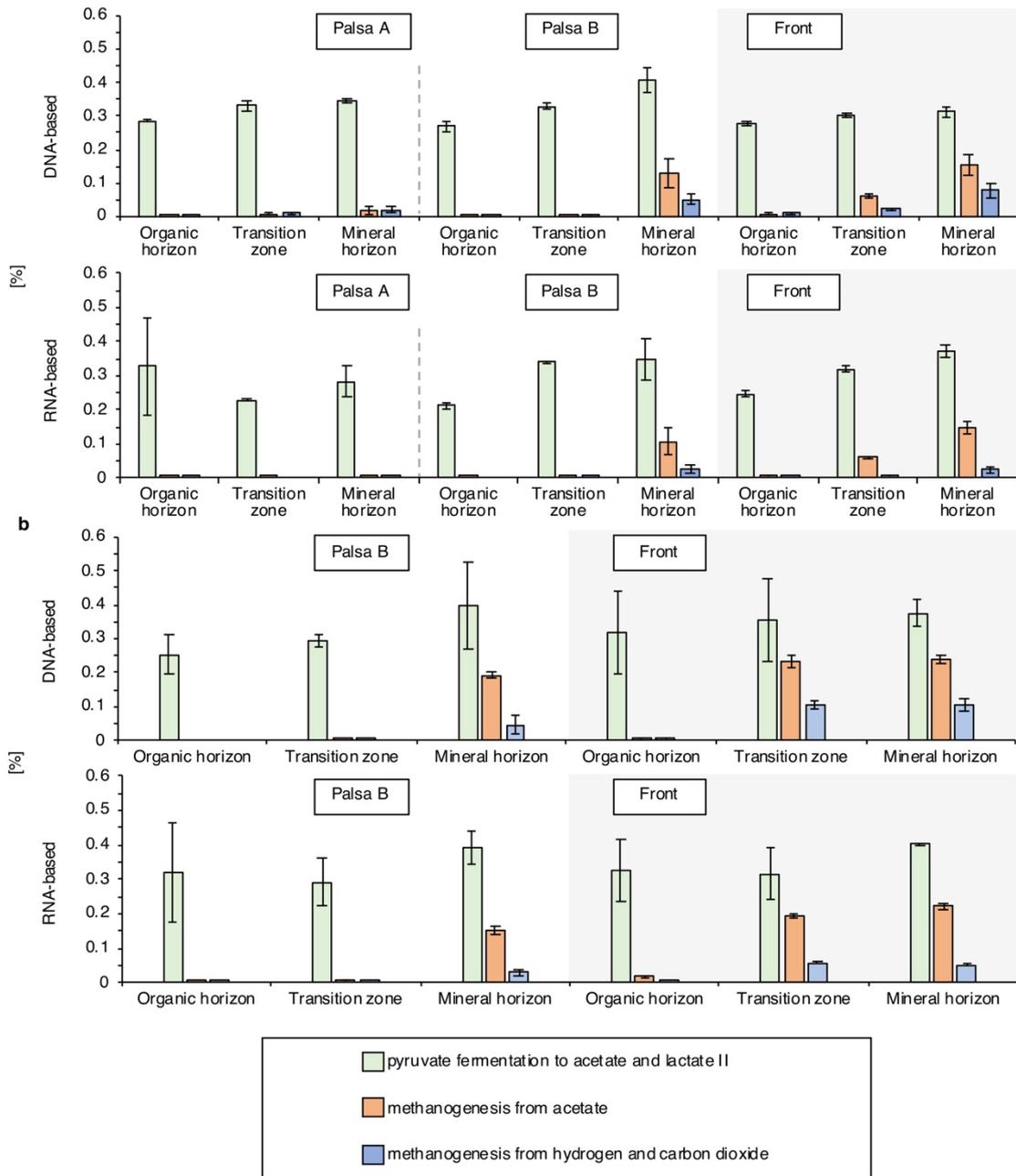
1061 **cycling microorganisms (DNA- and RNA-based) in replicate cores (Palsa B and Front)**

1062 **along the palsa hillslope (transect 3, Figure 1). All iron- and methane-cycling taxa below**

1063 **0.1% relative 16S rRNA gene sequencing abundance are not illustrated in this figure. For**

1064 absolute abundance of bacteria and archaea based on qPCR analysis, specific for 16S rRNA
1065 gene (based on DNA) and Fe- and methane-cycling microorganisms, analyzed in these study
1066 (adapted from Otte *et al.*⁴ and Weber *et al.*⁵), see also Figure S8. Reported values and error bars
1067 represent the average and standard deviation of triplicate analysis of each soil horizon (organic
1068 horizon, transition zone and mineral horizon) along the palsa hillslope.

1069



1070

1071

1072 **Figure S12. Predicted metabolic pathways of the present (DNA-based) and potentially**
 1073 **active (RNA-based) microbial communities along the palsa hillslope in a, cores presented**
 1074 **in the main text (transect 3, Figure S1) and b, replicate cores. Analyses were performed**
 1075 **with MetaCyc Metabolic Pathway Database. Reported values and error bars represent the**
 1076 **average and standard deviation of triplicate analysis of each soil horizon (organic horizon,**
 1077 **transition zone and mineral horizon) along the palsa hillslope.**

1078

1079 **Table S5. Elemental composition percentage and number of FT-ICR-MS assigned**
 1080 **formula.** Values are derived from total assigned formula tables. Not available (N/A) values for
 1081 CHNOS formula in porewater samples and salt control extractions are due to lack of reliable
 1082 formula series detected in those samples. A comparison of the soil extractions using salt or
 1083 dithionite solutions indicates that the extracts are not identical, but provides little to no evidence
 1084 of CHOS molecular artifacts formed through reactions with dithionite, as reported by Lv *et*
 1085 *al.*¹⁵.

1086

Sample Type	Sample	Extraction	CHO	CHNO	CHOS	CHNOS
			(% abundance / # formula)			
Soil extraction	Palsa A, Transition zone	Dithionite	74.19%/7486	11.21%/2887	13.76%/2018	0.84%/319
		Salt control	58.67%/4952	7.08%/1716	34.25%/6609	N/A
	Front, Transition zone	Dithionite	76.12%/7466	9.25%/2668	14.42%/2881	0.21%/98
		Salt control	76.22%/6536	7.08%/1716	13.79%/2249	N/A
Porewater	Palsa A	N/A	89.79%/9009	7.81%/3374	2.40%/991	N/A
	Front	N/A	87.41%/9072	10.80%/4172	1.79%/984	N/A

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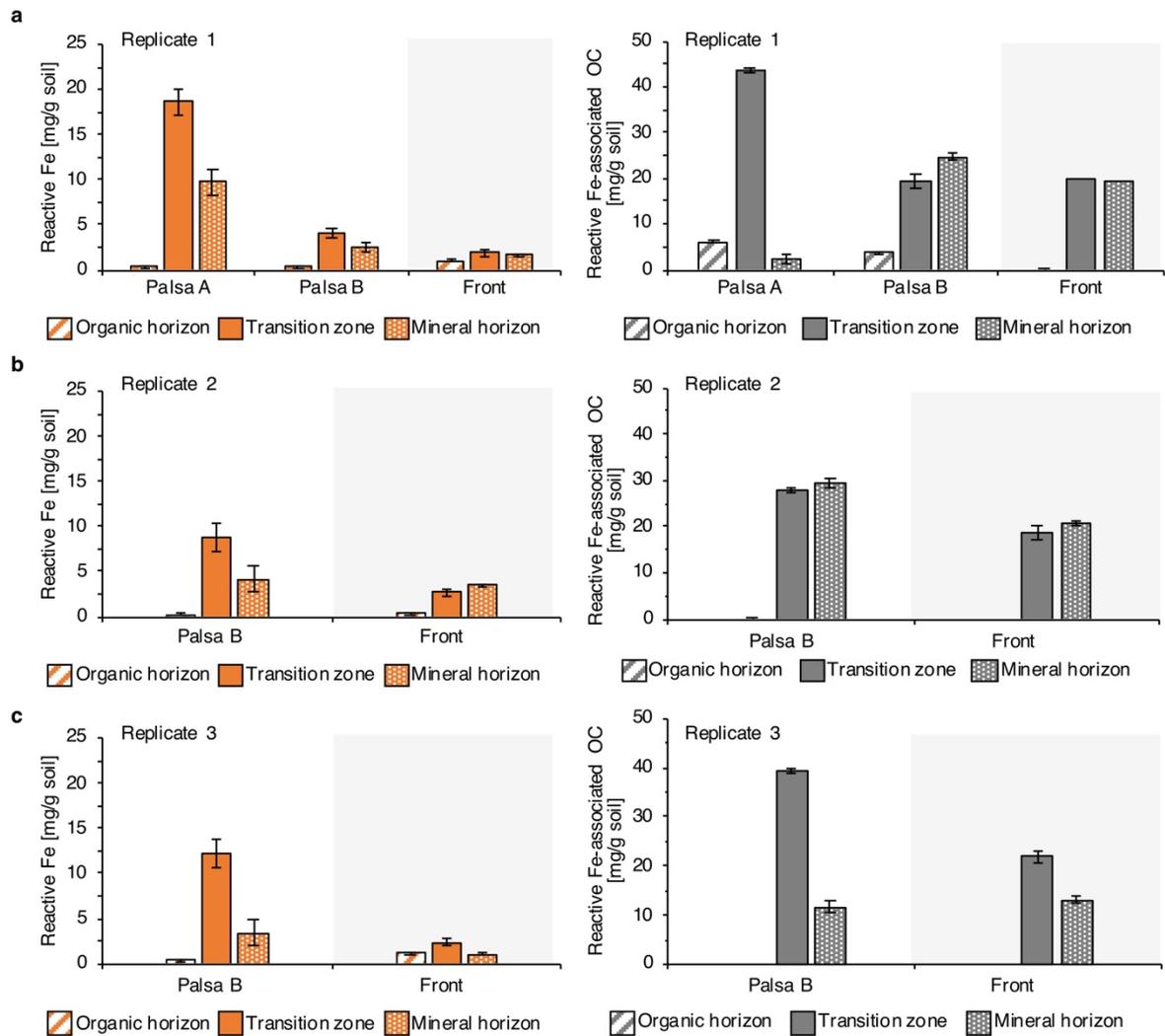
1089 **Table S6. Absolute values of iron and carbon in locations Palsa A, Palsa B and Front of**
 1090 **the cores reported in the main text.** Errors of the dithionite/citrate extractable a, iron (reactive
 1091 Fe, control corrected) and b, carbon (carbon bound to reactive iron, control corrected) represent
 1092 a combined standard deviation of sodium chloride bicarbonate extractable a, iron and b, carbon,
 1093 b, citrate blank and dithionite/citrate extractable a, iron and b, carbon (not control corrected).

	Palsa A				Palsa A			
	Reactive Fe [mg/g soil]	Error bars	Associated OC [mg/g soil]	Error bars	Leachable Fe [mg/g soil]	Error bars	Leachable OC [mg/g soil]	Error bars
Organic horizon	0.20	0.12	1.42	1.42	0.05	0.07	2.26	0.45
Transition zone	10.04	0.07	83.69	10.04	0.09	0.07	4.06	0.00
Mineral horizon	3.76	0.48	47.21	14.30	0.05	0.03	2.00	0.19
	Palsa B				Palsa B			
	Reactive Fe [mg/g soil]	Error bars	Associated OC [mg/g soil]	Error bars	Leachable Fe [mg/g soil]	Error bars	Leachable OC [mg/g soil]	Error bars
Organic horizon	0.67	0.07	4.80	0.52	0.00	0.00	3.05	0.71
Transition zone	4.61	0.78	76.60	16.89	0.00	0.00	2.76	0.00
Mineral horizon	4.22	0.09	25.08	4.22	0.00	0.00	0.85	0.00
	Front				Front			
	Reactive Fe [mg/g soil]	Error bars	Associated OC [mg/g soil]	Error bars	Leachable Fe [mg/g soil]	Error bars	Leachable OC [mg/g soil]	Error bars
Organic horizon	0.59	0.30	0.00	0.00	0.07	0.10	2.01	1.11
Transition zone	3.22	0.06	40.88	24.76	0.31	0.01	3.67	0.25
Mineral horizon	3.46	1.40	11.60	8.54	0.63	0.59	1.53	0.47

*Reactive Fe = dithionite extractable Fe - salt extractable Fe (leachable OC)

1094

1095



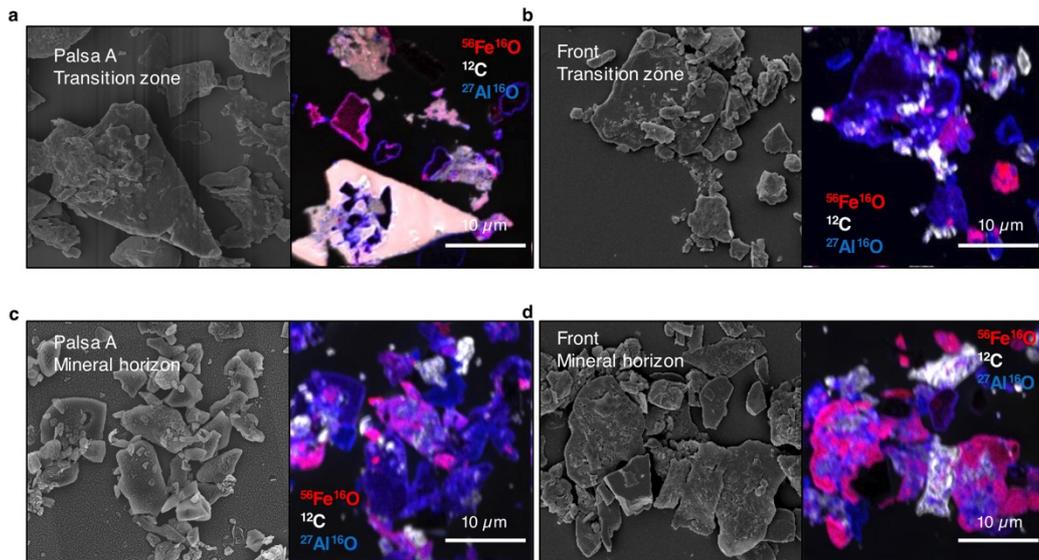
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1098 **Figure S13. Organic carbon (OC, grey) associated with reactive iron (Fe, orange) along**
 1099 **three replicate palsa hillslopes: a, replicate 1 (transect 3), b, replicate 2 (transect 3) and c,**
 1100 **replicate 3 (transect 2).** Reactive Fe and associated organic carbon along the palsa hillslope
 1101 (Palsa A, Palsa B and Front) per each soil horizon (organic horizon, transition zone and mineral
 1102 horizon) [mg/g] decreases towards the collapsing front. Palsa A shows the highest reactive Fe
 1103 and associated organic carbon in intact permafrost soils. Along the palsa hillslope towards the
 1104 collapsing front, reactive Fe and associated OC are lost in the solid phase. Reactive Fe reported
 1105 values are the average of sodium dithionite citrate duplicate extractions of each soil horizon,
 1106 control corrected by a sodium chloride bicarbonate extractable Fe (leachable Fe). Associated
 1107 OC reported values are the average of sodium dithionite citrate extractions of each soil horizon,
 1108 control corrected by the citrate background and the sodium chloride bicarbonate extractable OC
 1109 (leachable OC) (see also Material and Methods). Cores were taken in July 2019 (see Figure

1110 S1). Error bars of the reactive Fe represent a combined standard deviation of sodium chloride
1111 bicarbonate extractable iron and dithionite/citrate extractable Fe. Errors of the associated carbon
1112 represent a combined standard deviation of citrate blank, sodium chloride bicarbonate
1113 extractable OC and dithionite/citrate extractable OC.

1114



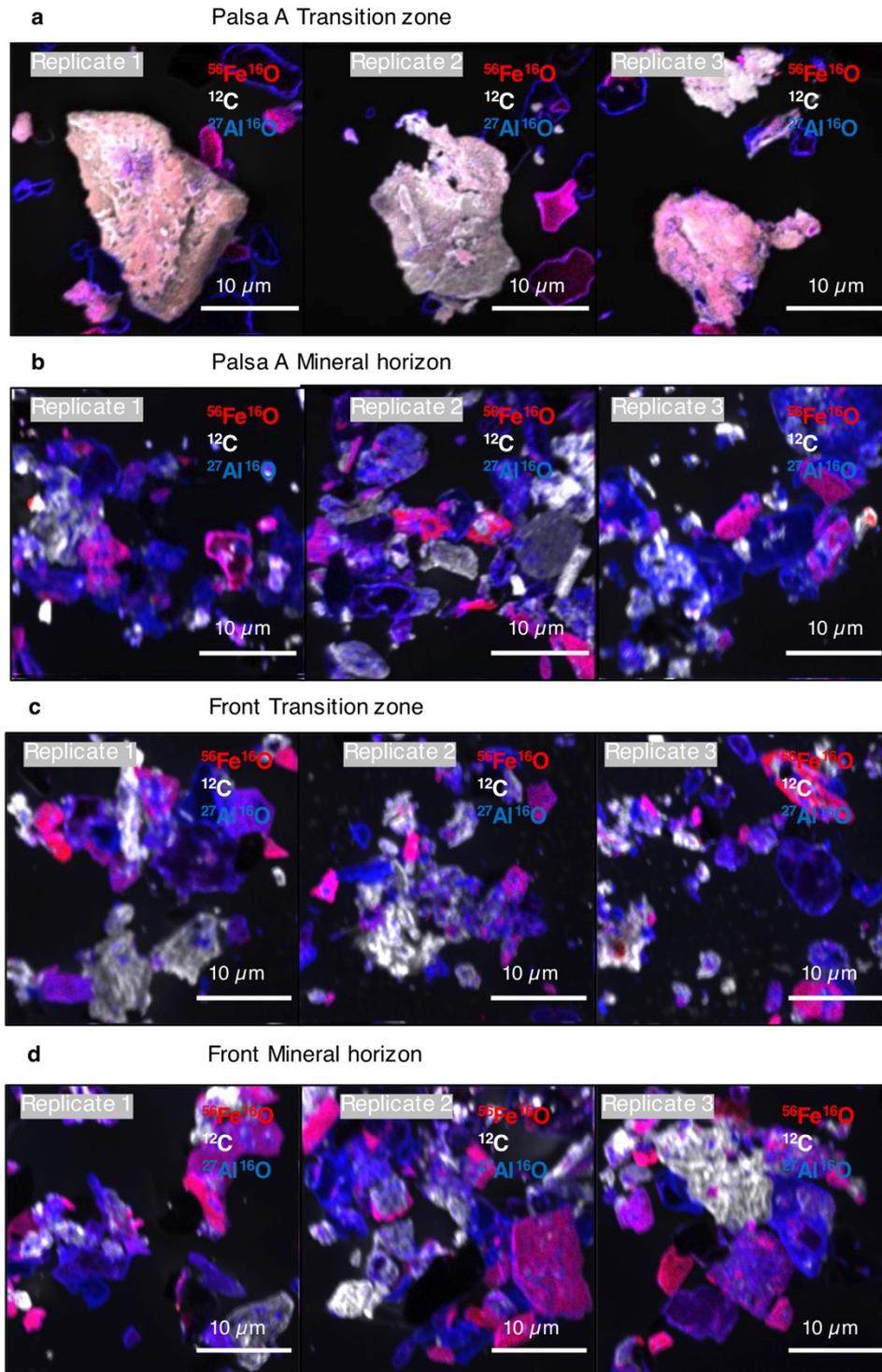
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1117 **Figure S14. Correlative scanning electron microscopy and nanoscale secondary ion mass**
 1118 **spectrometry (nanoSIMS) of fine fraction of palsa soil horizons along the palsa hillslope.**

1119 Fine particle analysis of a, Palsa A transition zone; b, Front transition zone; c, Palsa A mineral
 1120 horizon and d, Front mineral horizon (transect 1, Figure S1). Seven detectors were used during
 1121 nanoSIMS measurements for $^{12}\text{C}^-$, $^{16}\text{O}^-$, $^{12}\text{C}^{14}\text{N}^-$, $^{31}\text{P}^-$, $^{32}\text{S}^-$, $^{27}\text{Al}^{16}\text{O}^-$ and $^{56}\text{Fe}^{16}\text{O}^-$ and $^{27}\text{Al}^{16}\text{O}^-$. Here,
 1122 $^{12}\text{C}^-$ (white), $^{56}\text{Fe}^{16}\text{O}^-$ (red) and $^{27}\text{Al}^{16}\text{O}^-$ (blue) are overlaid in a single composite image.

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1126 **Figure S15. Replicate analysis of nanoscale secondary ion mass spectrometry (nanoSIMS)**

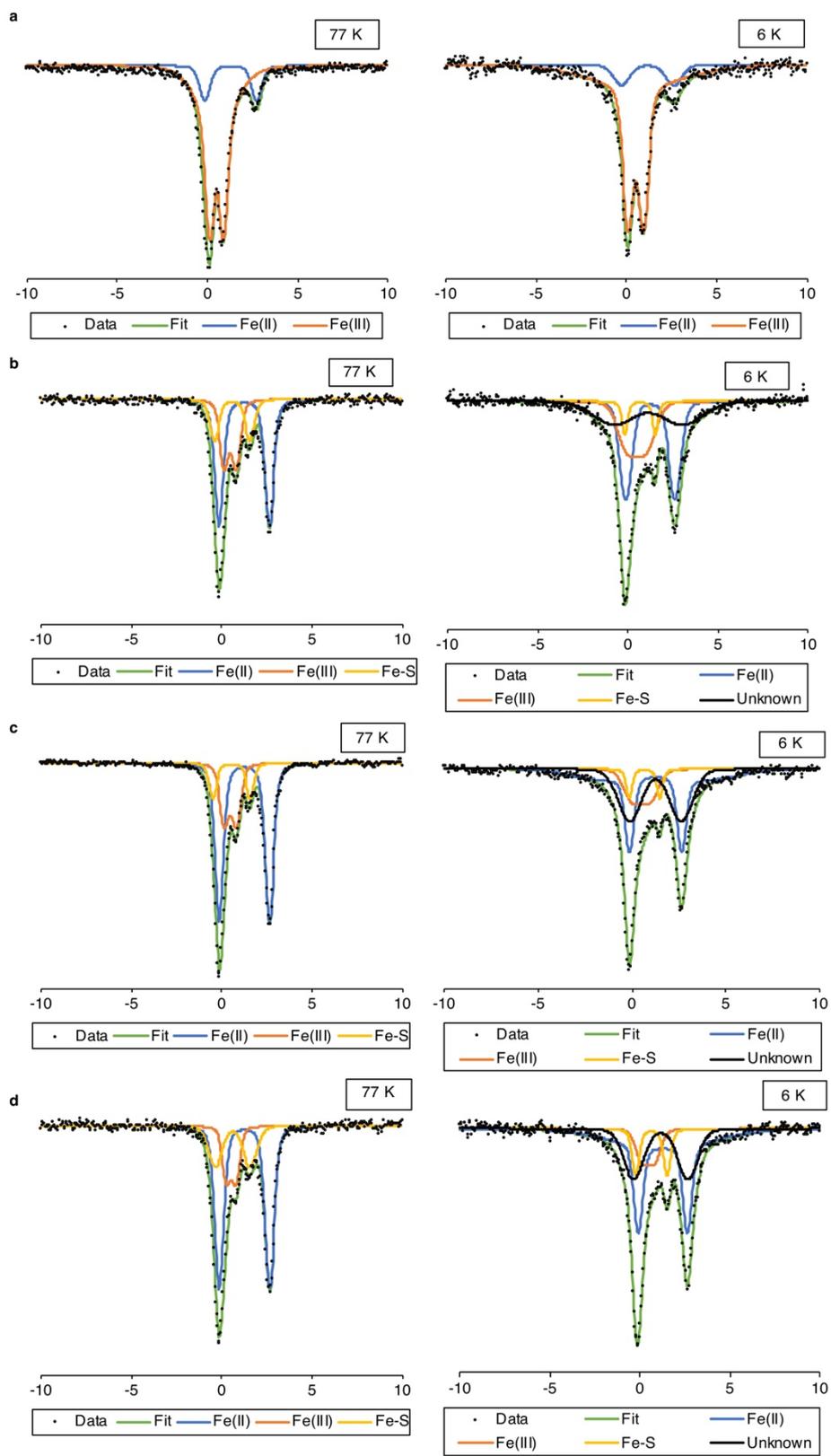
1127 **of fine fraction:** a, Palsa A transition zone; b, Palsa A mineral horizon; c, Front transition zone

1128 and d, Front mineral horizon (transect 1, Figure S1). Seven detectors were used during

1129 nanoSIMS measurements for for $^{12}\text{C}^-$, $^{16}\text{O}^-$, $^{12}\text{C}^{14}\text{N}^-$, $^{31}\text{P}^-$, $^{32}\text{S}^-$, $^{27}\text{Al}^{16}\text{O}^-$ and $^{56}\text{Fe}^{16}\text{O}^-$ and $^{27}\text{Al}^{16}\text{O}$.

1130 Here, $^{12}\text{C}^-$ (white), $^{56}\text{Fe}^{16}\text{O}^-$ (red) and $^{27}\text{Al}^{16}\text{O}^-$ (blue) are overlaid in a single image. In total,
1131 four representative fine particles were analyzed with nanoSIMS.

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1135 **Figure S16. Mössbauer spectroscopy analysis at 77 K and 6 K of the present Fe minerals**
1136 **along the thaw gradient:** a, Palsa A transition zone; b, Palsa A mineral horizon; c, Bog (Bog
1137 C³) mineral horizon; d, Fen (Fen E³) mineral horizon.

1138 **Table S7. Mössbauer spectroscopy parameters (measured at 77 and 6 K)** derived from
 1139 fitting spectra obtained for Palsa A transition zone and mineral horizon, Bog (Bog C³) and Fen
 1140 (Fen E³) mineral horizon.

1141

Sample	Components	CS ^a (mm/s)	ΔE_Q ^b (mm/s)	$\sigma(\Delta)$ ^c (mm/s)	B _{hf} ^d (T)	RA ^e (%)	\pm (%)	χ^2 ^f
77 K								
Palsa A								
Transition zone	Fe(II)	1.28	2.87	0.45	0.00	15.30	0.10	1.77
	Fe(III)	0.50	1.18	1.01	2.06	84.70	0.10	
Palsa A								
Mineral horizon	Fe(II)	1.24	2.79	0.39	0.00	54.46	0.97	0.88
	Fe(III)	0.46	0.67	0.35	0.26	27.45	0.91	
	Fe-S	0.58	1.96	0.40	0.00	18.09	0.93	
Bog (Bog C, see Patzner <i>et al.</i> , 2020)								
Mineral horizon	Fe(II)	1.25	2.78	0.37	0.00	62.87	0.38	3.47
	Fe(III)	0.47	0.68	0.37	0.31	24.73	0.34	
	Fe-S	0.54	2.07	0.30	0.00	12.40	0.34	
Fen (Fen E, see Patzner <i>et al.</i> , 2020)								
Mineral horizon	Fe(II)	1.25	2.79	0.44	0.00	61.30	0.21	0.61
	Fe(III)	0.52	0.54	0.31	0.41	17.30	0.18	
	Fe-S	0.61	1.93	0.67	0.03	21.40	0.22	
6 K								
Palsa A								
Transition zone	Fe(II)	1.17	2.90	0.80	0.00	12.30	0.16	0.85
	Fe(III)	0.52	2.17	2.65	2.26	87.70	0.16	
Palsa A								
Mineral horizon	Fe(II)	1.25	2.73	0.58	0.00	38.10	0.25	0.81
	Fe-S	0.68	1.68	0.19	0.00	6.50	0.16	
	Fe(III)	0.49	1.19	0.79	0.64	26.90	0.27	
	Unknown	1.13	3.87	2.06	0.29	28.50	0.30	
Bog (Bog C, see Patzner <i>et al.</i> , 2020)								
Mineral horizon	Fe(II)	1.26	4.07	2.55	1.50	42.10	0.77	0.75
	Fe-S	0.66	1.64	0.22	0.00	6.00	0.18	
	Fe(III)	0.48	1.07	0.70	0.60	15.90	0.34	
	Unknown	1.26	2.77	1.13	0.08	36.00	0.76	
Fen (Fen E, see Patzner <i>et al.</i> , 2020)								
Mineral horizon	Fe(II)	1.28	3.65	2.62	2.03	49.20	0.54	0.54
	Fe-S	0.64	1.74	0.27	0.00	10.70	0.27	
	Fe(III)	0.47	0.76	0.48	0.55	11.50	0.27	
	Unknown	1.16	3.02	0.98	0.02	28.50	0.58	

a CS = center shift

b ΔE_Q = quadrupole splitting;

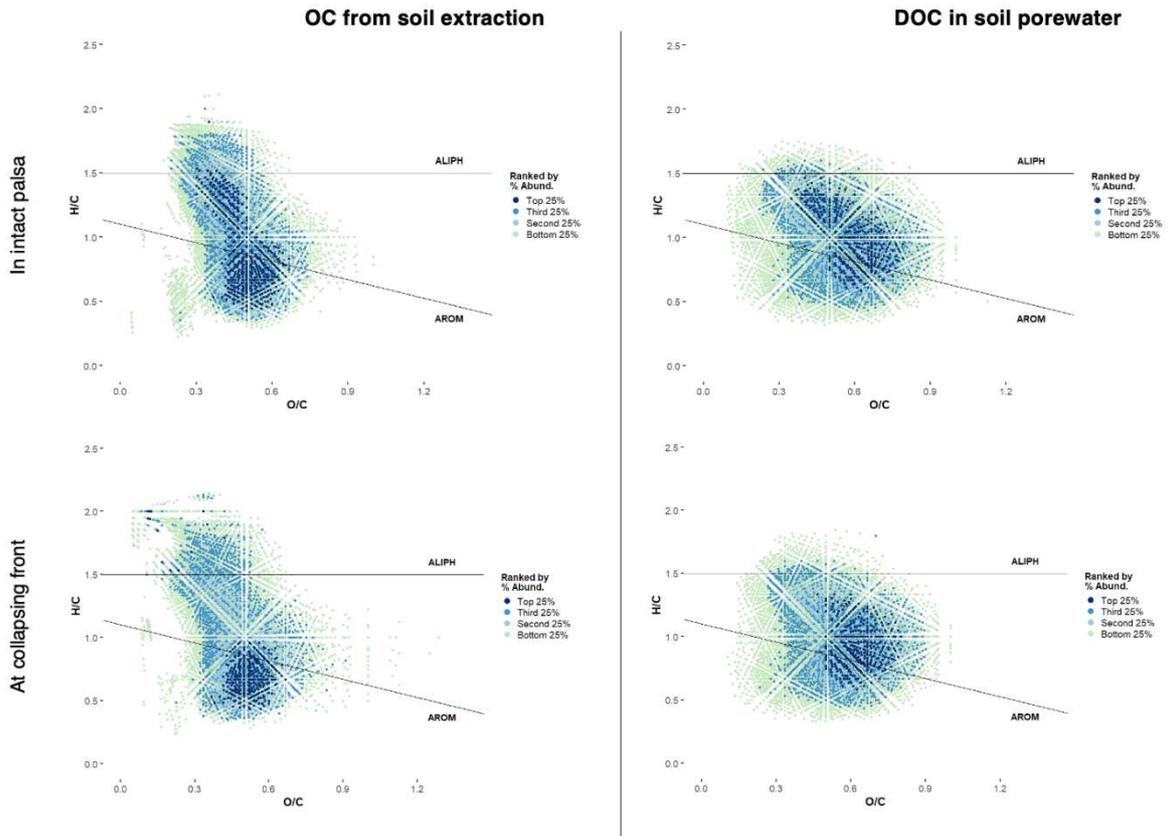
c $\sigma(\Delta)$ = standard deviation of quadrupole splitting component d B_{hf} = hyperfine magnetic field

e RA = Relative abundance

f χ^2 = goodness of fit

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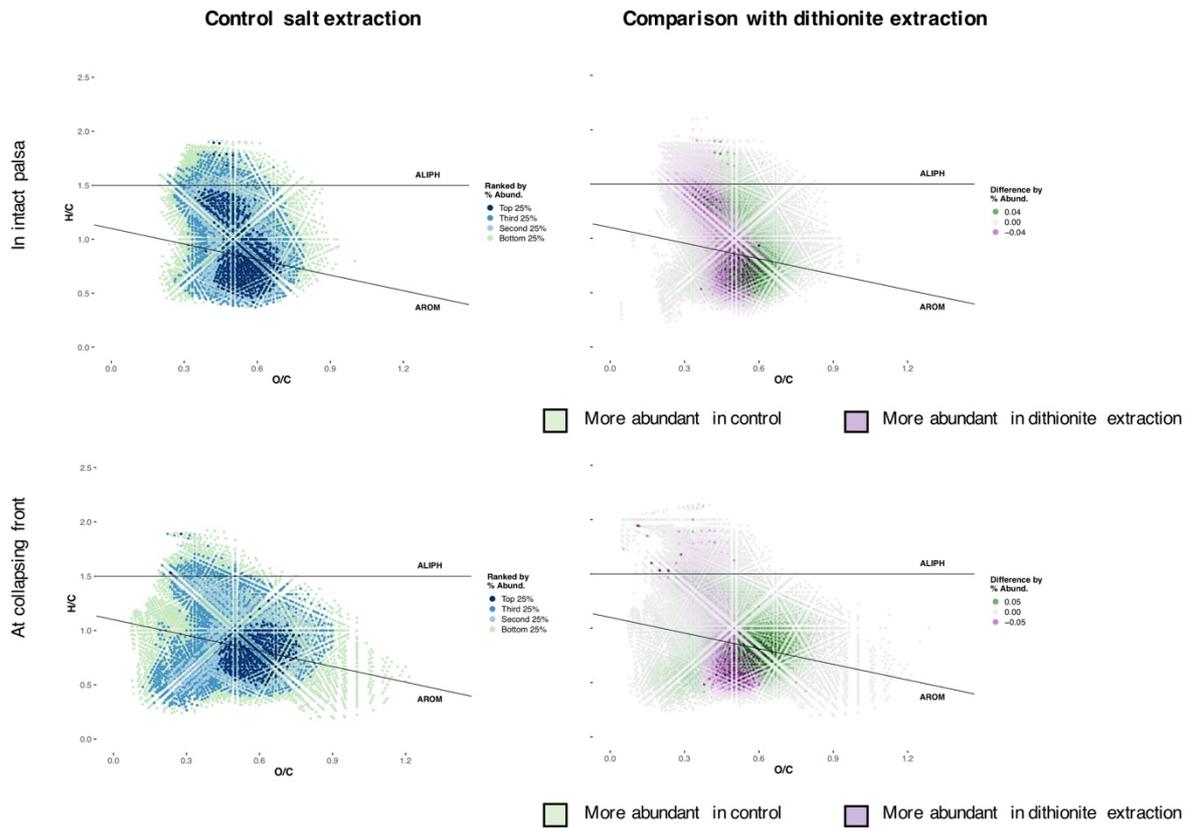


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1146 **Figure S17. Van Krevelen diagrams for individual samples for solid phase extracted OC**
 1147 **(dithionite extractable) from the transition zone of the intact palsa and of the collapsing**
 1148 **front (transect 1, Figure S1) and DOC, extracted from 30 cm depth, in intact palsa and at**
 1149 **the collapsing front.**

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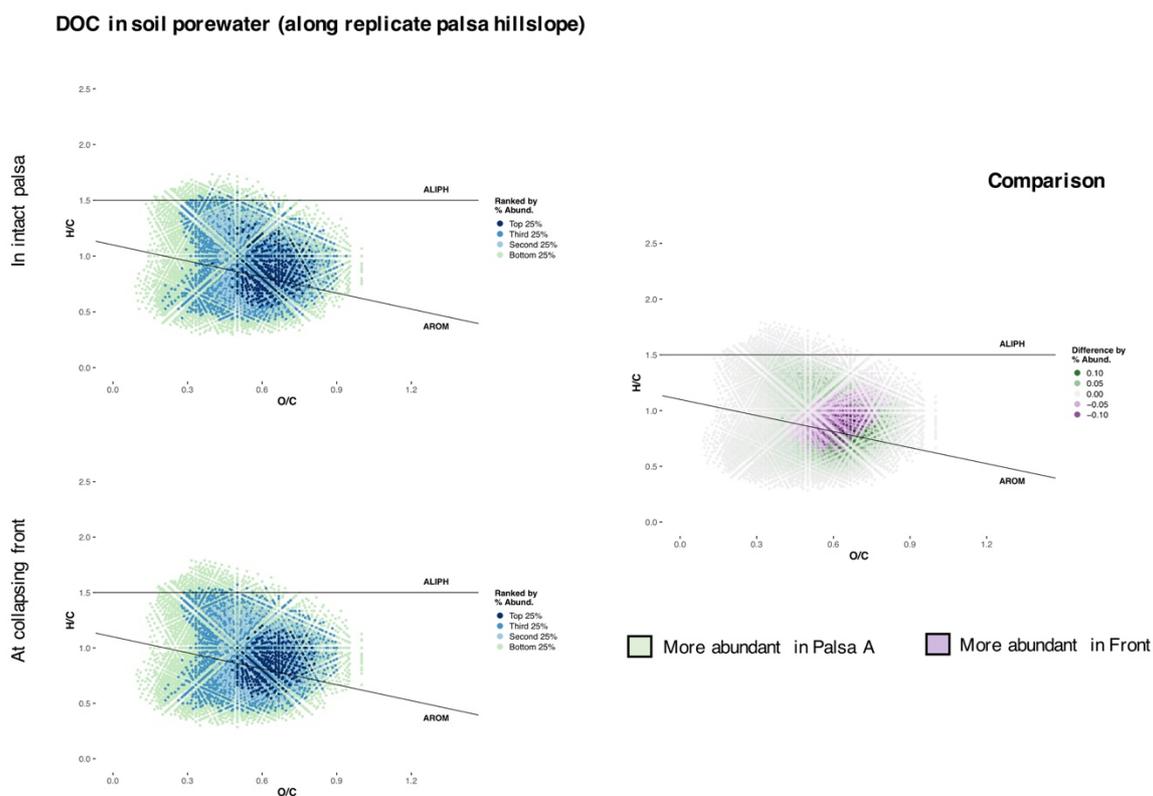
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1153 **Figure S18. Van Krevelen diagrams of control extractions (sodium chloride bicarbonate**
 1154 **with the same ionic strength and pH as the sodium dithionite extraction):** left, each
 1155 individual van Krevelen diagram and right, in direct comparison with the sodium dithionite
 1156 citrate extractable CHO. Organic carbon (OC) which is more abundant in control (green)
 1157 represents OC which is leachable of the soil by the same ionic strength and pH as the sodium
 1158 dithionite extraction. OC which is more abundant in the sodium dithionite extraction (purple)
 1159 is only dissolved by reductive dissolution.

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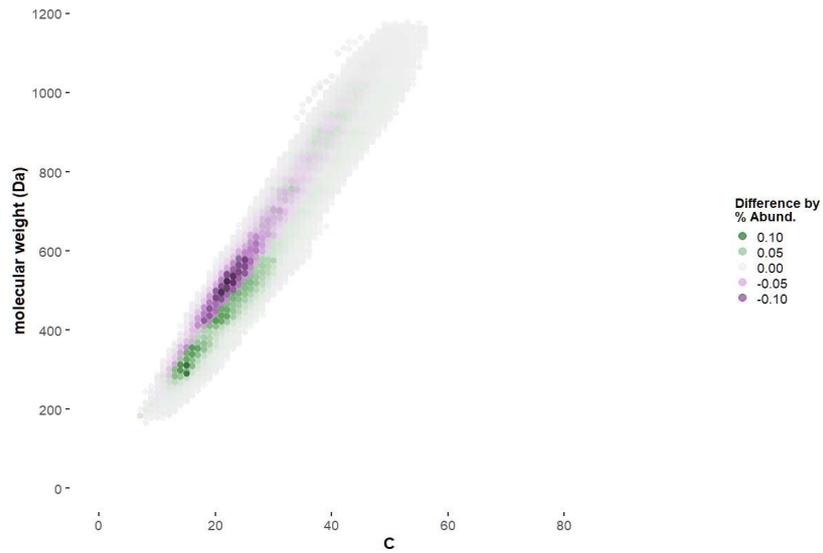


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1164 **Figure S19. Van Krevelen diagrams for replicate porewater samples (30 cm depth) (left)**
1165 **and in direct comparison to each other (right).** Dissolved OC, which is only found in Palsa
1166 A, is enriched in more aliphatic compounds (green). Towards Front and Bog, only more
1167 aromatic molecules (purple) remain.

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1170 **Figure S20. Molecular weight (MW) of dissolved organic carbon compounds in intact**
1171 **palsa versus at the collapsing front.** Lower MW compounds have higher relative abundance
1172 in porewater samples from intact palsa at a lower molecular weight for a given carbon number
1173 (colored in green). Higher MW compounds have higher relative abundance for a given carbon
1174 number at the collapsing front (colored in purple).

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1177

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