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1 **Changes in litter decomposition across succession in a post-mined**
2 **peatland, northern Japan**

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16

17 **Abstract**

18 Litter decomposition along successional sere after peat mining was monitored for 3 years on
19 post-mined peatland in Sarobetsu Mire, northern Japan. The litter of two dominant species
20 (*Rhynchospora alba* and *Moliniopsis japonica*) was examined in 3 different successional
21 habitats. We used the litterbag method to measure litter decomposition. We also measured
22 changes in litter decomposition properties (carbon, nitrogen, and phospholipid fatty acids)
23 with the environments (solar radiation and peat moisture). Bareground without litter showed
24 the highest fluctuation of solar radiation and temperature, but the litter decomposition was
25 slowest. Therefore, photodegradation and physical abrasion had affected weakly on litter
26 decomposition. The concentrations of carbon, nitrogen, and phosphorus were not different
27 between the two litter species showing that the litter decomposition pathways of these two
28 species were specific in each habitat because of stable isotopes. Phospholipid fatty acids
29 indicated that fungi contributed more to litter decomposition than bacteria in vegetated habitats
30 with time. These results suggested that biodegradation, particularly by fungi, was the
31 determinant in litter decomposition. The fungal activity was determined by vegetation cover
32 suggesting that restoration to the original vegetation was desirable soon after peat mining to
33 return the *in-situ* litter decomposition.

34

35 **Keywords:** litterbag experiment, litter decomposition, stable isotope, phospholipid fatty acids
36 (PLFAs), post-mined peatland

37

38

39 **Introduction**

40 Litter decomposition processes determine the accumulation of litter and the subsequent
41 organic matter and function as a carbon sink in boreal wetlands (Parton et al. 2007). Global
42 climate change alters the functions of wetlands via changing litter decomposition processes
43 (Loisel and Gallego-Sala 2022). In addition, numerous boreal wetlands in Japan have been
44 mined for commercial use and land use change (Tsuyuzaki and Zhang 2020). The restoration
45 of post-mined peatlands is desired to return the original ecosystem function although passive
46 restoration promotes the revegetation slowly or stagnant (Chimner et al. 2017).

47 Litter decomposition is advanced by 3 mechanisms, microbial degradation
48 (biodegradation), physical fragmentation (abrasion), and photodegradation (Henry et al. 2008).
49 These decomposition mechanisms are operated by temperature, light quantity, soil moisture,
50 nutrient availability, etc. Because fungi and bacteria have a dominant role in biodegradation,
51 the activities of fungi and bacteria are measured by fungal and bacterial phospholipid fatty
52 acids (PLFAs) (Otaki et al. 2016). Photodegradation in arid ecosystems such as deserts
53 contributes to litter decomposition because high solar radiation dissolves litter (Cybulski et al.
54 2000). Ground-surface movements promote the physical fragmentation of litter (Austin and
55 Vivanco 2006).

56 Litter decomposition is slower on post-mined peatlands than on natural, un-mined ones
57 (Fennessy et al. 2008), suggesting that the mechanisms of litter decomposition on peat-mined
58 wetlands should be clarified to obtain a key factor for ecological restoration. In addition, a
59 thick litter layer promotes the development of diverse seedbanks across succession on
60 Sarobetsu Mire (Egawa et al. 2009). Vegetation controls litter decomposition via direct and
61 indirect pathways. Species composition and abundance in the standing vegetation directly
62 determine the litter composition, supply, and decomposition (Rejmánková and Houdková
63 2006; Porre et al. 2020). Of the direct effects, home-field advantage (HFA), faster litter
64 decomposition on vegetation dominated by the litter species, has been hypothesized (Hunt et
65 al. 1988, Ayres et al. 2009). HFA is detected in deciduous temperate forests in Hokkaido, Japan,
66 along a xeric succession (Otaki and Tsuyuzaki 2019), while the effects of HFA have been

67 unclear in wetlands. Furthermore, vegetation indirectly influences litter decomposition by
68 manipulating the environments, represented by light, temperatures, and water (van der Krift
69 et al. 2002, Egawa and Tsuyuzaki 2015). Therefore, the mutual effects determine litter
70 decomposition rates across succession. However, litter decomposition process has not been
71 examined well across succession in post-mined peatlands.

72 Peat on Sarobetsu Mire in northern Japan was mined after 1970 or 33 years (Nishimura
73 et al. 2009). The environments for litter decomposition are changed across the succession from
74 baregrounds to grasslands, e.g., an increase in the shade (Egawa and Tsuyuzaki 2015), a
75 decrease in temperature and solar radiation (Koyama and Tsuyuzaki 2010) and a change in
76 peat nutrient (Nishimura and Tsuyuzaki 2014). Using this successional sere, we examined the
77 effects of vegetation and its related factors on litter decomposition. Litter quality is evaluated
78 by carbon, nitrogen, and phosphorus (Manzoni et al. 2010; Tsuyuzaki et al. 2022). Since
79 species richness and evenness change across succession, the patterns of litter decomposition
80 should be different among vegetation types (Asif et al. 2021).

81 The objectives of this study were: to clarify successional and seasonal changes in litter
82 decomposition rates with changes in carbon, nitrogen, and phosphorus and to detect
83 determinants of litter decomposition. We developed 3 hypotheses: (1) the contribution of 3
84 litter decomposition mechanisms will change across succession because of the alteration of
85 environments, and particularly biodegradation will contribute more to vegetated habitats. (2)
86 HFA will not appear clearly because the litter quality of examined species is comparable. (3)
87 the biodegradation will perform by fungi more than by bacteria because the litter quality of
88 monocotyledons will be favored by fungi rather than by bacteria in mesic habitats (Zhan et al.
89 2021).

90

91 **Methods**

92 **Study Sites**

93 The study site was a post-mined peatland in Sarobetsu Mire (45°06'N, 141°42'E, 8 m
94 elevation). The mean annual temperature was 6.3 °C, 6.2 °C, and 6.8 °C in 2008, 2009 and

95 2010 with the daily minimum at -8.0 °C in January, -5.7 °C in February and -6.0 °C in February
96 and with the daily maximum at 18.4 °C in July and August, 18.7 °C in August and 21.9 °C in
97 August at Toyotomi Town, 6 km east from the study site (Japan Meteorological Agency 2021).
98 The annual precipitation recorded 640.5 mm in 2008, 907.5 mm in 2009, and 1167.5 mm in
99 2010. Differences in summer rainfall during June and September were the causes of the
100 interannual variations. Snow cover usually remains from November to April, including in 2008
101 and 2009, and frost occurs even in mid-June, due to radiation cooling (Yamada and Takahashi
102 2004).

103 In Sarobetsu Mire, *Sphagnum* peat was mined every year between 1970 and 2003 at 3-22
104 ha and 3-6 m deep. After extracting high-quality peat for commercial use, the residues returned
105 to their original locations. The post-mined area was covered with open-water soon, and the
106 residues floated and accumulated. The accumulation of floated residues formed a stable
107 ground surface, and then plant colonization was processed. The successional sere after peat
108 mining is bareground to *Rhynchospora alba* sedgeland and then *R. alba* sedgeland to
109 *Moliniopsis japonica* grassland (Nishimura et al., 2009). We selected these two species for
110 examining litter decomposition. We selected 3 habitats based on the vegetation types:
111 bareground (hereafter, i.e., BG) mined in 1982, *R. alba* grassland (RA) mined in 1982, and *M.*
112 *japonica* grassland (MJ) mined in 1977. We established a total of 20 m² experimental plots.

113

114 **Litter Sampling and Quantification**

115 We conducted vegetation surveys and biomass harvests to evaluate habitat structure and litter
116 supply. The cover percentage and height were measured on each species within 20 1 m × 1 m
117 plots in each habitat in mid-summer 2008. Litter thickness was measured at 20 points in each
118 habitat by a ruler. Senescent litter produced in the current year was harvested at 40 15 cm ×
119 15 cm plots on 28 to 29 October, 5 November, 2008, and 30 to 31 October, 2009 to estimate
120 the litter production. The harvested samples were kept in paper bags and dried at 80°C in an
121 incubator for 6-8 days. Then, the litter was separated into *R. alba* and others in RA and *M.*
122 *japonica* and the others in MJ.

123 The litter of two examined species were harvested adjacent to RA and MJ on April 17 and
124 October 22, 2008. Litter produced in the current and last years were selected in October and
125 April, respectively. The harvested litter was brought to our lab and dried at 45 °C for 4-6 days.
126 Morphological differences between these two species are (Kitamura et al. 1984; Koyama and
127 Tsuyuzaki 2010): *R. alba* (Cyperaceae) develops filate leaves with 0.5-1.5 mm in width and
128 does not develop rhizomes well while *M. japonica* (Poaceae) forms linear leaves with 5-10
129 mm wide.

130 We investigated the seasonal changes of litter chemistry during snow-accumulated
131 periods by the litter sampled in April and October. We collected 5 litter samples randomly
132 selected for each of the two species, *R. alba* and *M. japonica*. The recovered litter was dried at
133 80 °C for 5-7 days immediately after returning to the lab. The litter samples were homogenized
134 by an electric grinder. The contents of nitrogen (%N) and carbon (%C) were measured by an
135 isotope mass spectrometer (MAT252, Finningan Mat Ltd., Bremen). Carbon-to-nitrogen ratio
136 (C/N) in litter was calculated on each sample based on %C and %N. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were also
137 measured concurrently with %C and %N. Phosphorus (P, $\mu\text{g/g}$) was measured by a
138 spectrophotometer (UV-1200, Shimadzu Corporation, Kyoto) at 880 nm after digestion by 7-
139 ml acid solution ($\text{HNO}_3:\text{H}_2\text{SO}_4:\text{HClO}_4 = 5:1:1$, volume) with 300-mg litter.

140 The litterbags were made of a polyethylene net with 1-mm mesh, and were filled with 5
141 g of either *R. alba* or *M. japonica* litter. The litterbags were deployed on May 8, 2008 for litter
142 collected in April and on November 12, 2008 for litter collected in October. The litterbags
143 were placed on peat surfaces in BG because of no litter and on peat and litter surfaces in RA
144 and MJ. In total, 3 litterbags were recovered 8 times from each layer in each habitat during
145 snow-free periods for the 3 years, except BG in 2010 because of no bags. The recovered
146 samples were kept in a cool box with refrigerant until drying. For measuring PLFAs, the
147 samples were freeze-dried over 7 days immediately after returning to lab and were weighed
148 (Otaki and Tsuyuzaki 2019). When PLFAs were not measured, the litterbags were dried at
149 80°C for 5-7 days.

150 PLFAs in each litter were identified and quantified by phospholipids extracted from the

151 litter under a gas chromatography (G-3000 Gas Chromatograph, Hitachi, Tokyo) and a gas
152 chromatograph-mass spectrometer (Varian Saturn 2200, Agilent Technologies, Santa Clara) to
153 estimate the bacterial and fungal biomass. The phospholipids were separated from the total
154 lipids by thin-layer chromatography with a silica gel under a developer (91:30:8 = acetone:
155 benzene: water). The phospholipids were subjected to mild alkaline methanolysis. Then, the
156 fatty acid methyl esters were detected with the gas chromatography with a flame ionization
157 detector using a 30-m 5% phenyl silicone capillary column (HP-5) exposed to helium as a
158 carrier gas. The temperatures of the injector and detector were adjusted to 270 °C. The
159 temperature in the oven was kept at 160 °C for 5 min and then rose at 1 °C/min up to 180 °C
160 and at 10 °C/min up to 240 °C. PLFAs were identified and quantified in each sample by
161 comparison with the internal standard, nonadecanoate fatty acid (19:0). Fatty acid methyl
162 esters were identified by the standards and previous literature with a gas chromatograph-mass
163 spectrometer (JMS-DX303HF, JEOL, Tokyo). In this study, the PLFAs of 16:1, 17:0, 18:1 and
164 18:2 were focused because the 16:1 and 17:0 were produced only by bacteria and 18:1 and
165 18:2 were by fungi (Šnajdr et al. 2011).

166

167 **Measurements of Environmental Factors**

168 The temperature (°C) and light intensity (lux) were measured at the center of each site by
169 automatic loggers (HOBO UA-002-XX, Onset Computer Corporation, Pocasset) at 1-hour
170 intervals from 8 May, 2008 to 8 November, 2009. The logger was established on litter, when
171 available, or peat surfaces. Ultraviolet radiation (UV, 280-400 nm) was measured by a UV
172 meter (ST-513, Sentry, Taipei). We measured photosynthetic active radiation (PAR) and UV
173 at the ground surface and 1 m above as control in each plot at every census. At each plot, PAR
174 or UV were measured 4 times at the same moment. We calculated daily mean, maximum and
175 minimum temperatures in each day, and the moving average for 5 days on the mean, maximum,
176 and minimum temperatures. The relative intensities of PAR and UV were calculated by
177 (ground surface/above surface × 100) and averaged.

178 We measured peat moisture (% v/v) by time domain reflectometry (TDR) (EC-5,

179 Decagon Devices Inc., Pullman), of which probe length was 5 cm, at 1 and 5 cm in depths
180 from June to November in 2009 and from May to October in 2010. The loggers recorded the
181 data at 1-hour intervals by data loggers (Em5b, Decagon Devices Inc., Pullman). Volumetric
182 water content (% v/v) was measured at every census by TDR (HydroSense, Campbell
183 Scientific Australia Pty. Ltd., Wien) with a 12-cm probe inserted vertically into the ground 20
184 times in each habitat during 2008 and 2009.

185

186 **Statistical Analysis**

187 The amount of litter supply was compared between RA and MJ and between the years by
188 generalized linear models (GLMs) under the assumption of gaussian distribution with the link
189 function of identity. Interaction between the year and vegetation type (RA and MJ) was
190 considered in the models.

191 GLMs detected the determinants of litter decomposition with the assumption of gaussian
192 distribution. We developed 2 models: model I used chemical properties, and model II did not
193 use them because we did not always measure the chemical properties. The response variable
194 of models I and II was litter mass remaining. The explanatory variables of model I were habitat,
195 litter species, layer, and date. Model II additionally used %C, %N, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, C/N, and P with
196 the explanatory variables of model I. Akaike's information criterion (AIC) selected the
197 meaningful explanatory variables in the best models. The differences in 6 chemical properties
198 in the initial litter were compared between the 2 litter species and 2 seasons (April and
199 October) by GLMs with the same assumptions with the litter decomposition. We investigated
200 the chemical properties of decomposed litter in the same way. These analyses did not consider
201 interactions between the explanatory variables. GLMs examined the temporal changes in
202 PLFAs with the explanatory variables of date, habitat, litter species, and layer.

203 GLMs compared the relative PAR and UV between the seasons and between the habitats
204 with their interactions as well as the litter supply. The daily mean, maximum and minimum
205 temperatures were compared between litter species, among habitats, and among date by GLMs.
206 Peat moisture was compared between habitat, layer, and date. All statistical analyses were

207 performed by R (ver. 4.1.1) (R Development Core Team 2021).

208

209 **Results**

210 **Habitat and Litter Supply**

211 In total, 21 vascular plant species were recorded from the 40 plots of 1 m × 1 m. The total
212 cover averaged 5% in BG, 74% in RA to 99% in MJ. As well as the cover, plant height
213 increased from BG to MJ. These results showed that shade became stronger with progress the
214 succession. On non-vascular plant species, a few *Sphagnum* mosses, most of which were *S.*
215 *papillosum*, were recorded from MJ. Only 3 species grew on BG (Table 1). *Rhynchospora*
216 *alba* showed the highest frequency (8) on BG, although the cover averaged 3% only. *R. alba*,
217 of which cover averaged 69%, was predominant on RA. The other species showed less than
218 8% in cover on RA. *Moliniopsis japonica* (76% in cover) and *Carex middendorffii* (52%) were
219 dominant in MJ.

220 BG produced the least litter for the 2 years, i.e., litter thickness was nearly zero. The mean
221 of litter thickness was 3 times higher in MJ than in RA, 2.4 ± 1.3 cm (mean \pm standard
222 deviation) in RA and 8.7 ± 1.6 cm in MJ. *R. alba* on RA produced 191 ± 90 g/m² of litter in
223 2008 and 147 ± 67 g/m² in 2009. The other species on RA did not produce less litter in 2008
224 and did less than 67 g/m² and averaged 3 ± 11 g/m² in 2009. The litter supply on RA was 192
225 ± 91 g/m² and 150 ± 67 g/m² in 2008 and 2009, respectively. Therefore, litter on RA was
226 supplied mostly by *R. alba*. *M. japonica* on JM produced 546 ± 412 g/m² in 2008 and $523 \pm$
227 366 g/m² in 2009. The other species supplied 213 ± 175 g/m² and 161 ± 122 g/m² on the
228 grassland in 2008 and 2009, respectively. The total amount of litter was 759 ± 450 g/m² in
229 2008 and 685 ± 343 g/m² in 2009. The litter production of *M. japonica* on MJ was 4 to 5 times
230 more than that of *R. alba* on RA. The total amount of litter supply was significantly different
231 between RA and MJ (GLM, $p < 0.01$) and was not different between the years ($P = 0.252$).

232

233 **Litter Decomposition**

234 The initial litter of *R. alba* contained $46.6\% \pm 0.8$ of %C, $0.93\% \pm 0.96$ of %N and 104.2 µg/g

235 ± 5.4 of P in October, while it did $46.3\% \pm 0.7$ of %C, 0.90 ± 0.06 of %N and $115.0 \mu\text{g/g} \pm 7.1$
 236 of P in April. The C/N of *R. alba* averaged 50.2 ± 3.6 in October and 51.6 ± 2.3 in April. *M.*
 237 *japonica* litter contained $49.3\% \pm 0.9$ of %C, $0.81\% \pm 0.98$ of %N, $48.8 \mu\text{g/g} \pm 5.8$ of P, and
 238 61.1 ± 5.7 of C/N in October. These in *M. japonica* litter changed to $46.1\% \pm 0.5$, $0.79\% \pm$
 239 0.05 , $121.4 \mu\text{g/g} \pm 5.7$ and 61.1 ± 3.5 in April. These 4 chemical properties did not differ
 240 between the seasons (GLMs, $P > 0.01$), and between the 2 species (GLM, $P > 0.01$), showing
 241 that the litter did not change the chemical composition greatly during the snowfall period for
 242 both *R. alba* and *M. japonica*.

243 $\delta^{13}\text{C}$ in the initial litter of *R. alba* was $-29.2\% \pm 0.9$ in October and $-27.3\% \pm 0.3$ in April,
 244 while $\delta^{13}\text{C}$ in *M. japonica* was $-27.1\% \pm 0.1$ in October and $-26.2\% \pm 0.3$ in April. $\delta^{13}\text{C}$ was
 245 higher in April than in October (GLM, $p < 0.001$) and was higher in *M. japonica* litter than in
 246 *R. alba* litter ($p < 0.01$). The interaction between the litter species and season was not
 247 significant on $\delta^{13}\text{C}$. $\delta^{15}\text{N}$ in *R. alba* litter was $1.69\% \pm 0.20$ in October and $0.20\% \pm 0.63$ in
 248 April. $\delta^{15}\text{N}$ in *M. japonica* litter changed from $-2.72\% \pm 0.29$ in October to $-3.15\% \pm 0.18$ in
 249 April. $\delta^{15}\text{N}$ was higher in *M. japonica* litter than in *R. alba* litter ($p < 0.001$) with the interaction
 250 between litter species and season ($p < 0.01$). Although the chemical composition, %C, %N,
 251 and P, did not differ between the litter species, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ were different between the litter
 252 species. These suggested that the transportation of carbon and nitrogen differed between the 2
 253 litter species and occurred even in winter.

254 Litter mass remaining decreased with time, particularly in vegetated habitats, independent
 255 of the litter species (Fig. 1). Model I, which did not use chemical properties, discarded litter
 256 species (Table 2) and used habitat. The slopes of GLMs were steeper in RA and MJ. These
 257 results meant that the litter decomposition was faster in vegetated habitats, RA and MJ. Model
 258 II, including chemical properties, discarded %N and $\delta^{15}\text{N}$ in the final model, showing that N
 259 was not a strong determinant of litter decomposition for these 2 species. The litter
 260 decomposition did not correlate to the other examined variables, except $\delta^{13}\text{C}$, which remained
 261 in the model but was insignificant. When chemical properties were not considered (Model I),
 262 litter species did not remain in the model. However, the species remained in the model when

263 chemical properties were considered (Model II). In total, excluding N parameters, all the
264 parameters remained in Model II.

265 %C, %N, C/N, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and P were different among the dates and litter species (GLMs,
266 $p < 0.01$) (Fig. 2). On the litter species, $\delta^{13}\text{C}$ was higher in *M. japonica* litter than in *R. alba*
267 litter, while $\delta^{15}\text{N}$ was higher in *R. alba* litter than in *M. japonica* litter. %C and $\delta^{13}\text{C}$ did not
268 differ between the habitats. %N was highest in MJ and $\delta^{15}\text{N}$ was also highest in MJ. C/N was
269 higher in BG and RA. %C did not differ among the habitats, so the difference came from %N.
270 P was highest in MJ, as well as %N and $\delta^{15}\text{N}$.

271 PLFAs indicated that bacteria and fungi were less abundant in the first year (Fig. 3). The
272 amounts of PLFAs produced by bacteria did not change for the 3 years in any habitats, while
273 those by fungi increased with time. In addition, fungal PLFAs were more in MJ with high
274 vegetation cover. Litter species affected 18:1 PLFA concentration but did not do the 18:2 PLFA.
275 The amount of PLFAs did not differ between the burial layers, i.e., on peat and litter.

276

277 **Solar Radiation and Temperature**

278 Light intensity was highest on BG (Fig. 4). With increasing vegetation cover across the
279 succession, the light intensity decreased throughout the surveyed period. The light intensity
280 was lower on the peat than on the litter because of shade. As well as the light intensity, relative
281 PAR and UV decreased with increasing vegetation cover. The PAR and UV were higher in
282 early spring because of no shade by shoots, although the date was not significant for
283 determining PAR and UV.

284 The temperatures, expressed by mean, maximum, and minimum, differed among the
285 habitats with above and below litter in 2008 and 2009 (Fig. 5). With increasing vegetation
286 cover, the temperatures decreased. The temperature fluctuations, expressed by standard
287 deviation, were higher above than below the litter, showing that the litter accumulation
288 reduced the effects of heat derived from direct sunshine.

289 Peat moisture was lower at 1 cm in depth than 5 cm in all habitats for the 2 years from
290 2009 to 2010 (Fig. 6). The moisture was highest in RA and lowest in BG. The peat moisture

291 fluctuated in response to precipitation, particularly above the litter in BG. The peat moisture
292 on the shallow peat layer was affected directly by precipitation. In contrast, peat moisture at 5
293 cm deep in RA was stable, probably because the water table was constantly higher than 5 cm
294 deep in RA. The water content measured by a 12 cm probe inserted from the litter surface
295 showed that MJ was driest. The content was higher in RA than in BG, although the difference
296 was small. Since the locations for measuring water content in MJ contained the litter layer, the
297 thick litter decreased the water content.

298

299 **Discussion**

300 **Litter Decomposition and Litter Supply**

301 The litter decomposition was fast in the vegetated habitat, MJ, independent of the litter species,
302 showing that the characteristics of habitats determined the litter decomposition more than litter
303 species. The 2 litter species showed equivalent %C, %N, and P suggesting that the litter quality
304 did not differ greatly between them. $\delta^{13}\text{C}$ ranging from -34‰ to -24‰ in the initial litter for
305 the 2 examined species indicated that these species are C_3 plants (Coplen et al. 2002).
306 Therefore, HFA on litter decomposition was weak because of the non-significant difference in
307 litter quality between the 2 species. There is little evidence of HFA on litter decomposition in
308 an Italian mountain forest because the chemical composition in litter affects decomposition
309 more in the early stages (Pastorelli et al. 2021). The litter species was not adopted in model I
310 not including litter chemistry and was adopted in model II including litter chemistry. These
311 also implied that the habitat differences were more important than the litter chemistry for the
312 litter decomposition.

313 PAR, UV and temperature fluctuation decreased with increasing vegetation cover.
314 Biodegradation does not favor high PAR and UV because microbial activities are inhibited by
315 strong solar radiation, particularly by UV (Wei et al. 2022). The orders of water content and
316 peat moisture were not synchronized with the successional sere, i.e., water content lowered as
317 $\text{RA} < \text{BG} < \text{MJ}$. These results suggested that the litter decomposition rates were determined
318 by temperature and solar radiation more than by water in peat. The inundation regime does not

319 affect the decomposition of refractory stem litter, while prolonged and stable inundation
320 stimulates the degradation of labile leaf litter (Xie et al. 2019a). However, the litter was not
321 inundated on the peat surface, as shown by seasonal changes in peat moisture at 1 cm and 5
322 cm in depths except during melting snow. Furthermore, the peat moisture was unstable in BG.
323 Therefore, fast litter decomposition was not expected in BG because of less inundation and
324 strong UV.

325 Although the litter decomposition rate on MJ was faster, litter accumulation was 2.5 times
326 higher on MJ than on RA. Therefore, litter accumulation on MJ was due mostly to the litter
327 input. *Rhynchospora alba* is an early colonizer on BG and *Moliniopsis japonica* is a late
328 colonizer (Nishimura and Tsuyuzaki 2014), and PLFAs showed that fungal activities were
329 higher in MJ. The quality and quantity of litter input affect litter decomposition processes more
330 than the temperature on permafrost in northern Greenland because the litter input determines
331 the composition and abundance of microbiome (Adamczyk et al. 2020). These results suggest
332 that the carbon sink enlarges with the succession from *R. alba* to *M. japonica*.

333

334 **Biological Decomposition**

335 Biological decomposition processes had a dominant role in litter decomposition and differed
336 between *R. alba* and *M. japonica* litter. However, the resultant litter decomposition rates did
337 not differ between the 2 species. BG showed the least PLFAs produced by bacteria and fungi,
338 suggesting that biodegradation in BG was less throughout the surveyed period.

339 Fungi showed a faster increase in PLFAs in MJ, while bacteria did not increase the PLFAs
340 in most samples. Peat moisture was highest in RA, but the litter decomposition did not differ
341 between RA and MJ. Higher water levels facilitate litter decomposition and organic carbon
342 release from *Carex brevicuspis* leaf litter into the soil via water leaching and microbial activity
343 (Zhu et al. 2021). However, this pattern was not observed in the litter of *R. alba* and *M.*
344 *japonica*, probably because the temperature effects precluded the moisture effects on fungi.
345 The activities of litter-decomposed fungi are often determined more by temperature than by
346 moisture because moisture was higher than the threshold for litter decomposition in wetlands

347 (Xie et al. 2019b).

348

349 **Photodegradation and Physical Abrasion**

350 BG receiving high UV and PAR showed the slowest litter decomposition. The litter
351 decomposition did not differ between the 2 burial layers, not adopted in the models I and II.
352 These results indicated that photodegradation did not act strongly. Photodegradation is a
353 determinant of litter decomposition in (semi-)arid ecosystems that receive high direct sunlight,
354 although bacterial decomposition is weak due to dryness (Cybulski et al. 2000; Pancotto et al.
355 2005; Austin and Vivanco 2006). The photodegradation did not work highly in mesic habitats,
356 i.e., post-mined peatlands in Sarobetsu Mire, with low temperatures. These suggest that peat
357 moisture is related to the intensity of photodegradation.

358 As well as photodegradation, physical abrasion in the litterbags was weak in the post-
359 mined peatland, as shown on BG. Leaves are more fragile than stems because of less
360 recalcitrant components, such as cellulose and lignin (Fonseca et al. 2013). Although the 2
361 litter species did not produce their stems except the flowering stalks, physical abrasion was
362 less. These also suggested the leaf morphology, i.e., filate and linear leaves, affected less the
363 litter decomposition. In conclusion, the 3 proposed hypotheses were supported by the present
364 results as: (1) the litter decomposition was performed weakly by photodegradation and
365 physical abrasion even on bareground and was done mostly by biodegradation on the vegetated
366 habitats. Therefore, the litter decomposition patterns and mechanisms changed across the
367 succession. (2) HFA was not detected clearly in the 2 litter species. (3) Of the biodegradation,
368 the activities of fungi, of which establishment was determined by vegetation cover, were
369 critical for the litter decomposition. Ecosystem restoration should be conducted promptly after
370 peat mining to return the original litter decomposition patterns before starting the distortion of
371 ecosystems (Nishimura and Tsuyuzaki 2015).

372

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379

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385 **Data Availability** The datasets are available from the corresponding author on reasonable
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387 **Code Availability** Not applicable.

388

389 **Declarations**

390 **Consent for Publication** Everybody entitled so gave their consent for this research being
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392 **Ethics Approval** Not applicable.

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396

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Table 1. Habitat characteristics evaluated by averaged cover (%) \times averaged shoot height (cm) with frequency (n) in the summer of 2008. Species with $F > 20\%$ ($n > 12$) are shown. -: not observed. Species richness and total cover are shown by mean with standard deviation.

Species*	Habitat		
	Bareground	<i>R. alba</i> grassland	<i>M. japonica</i> grassland
<i>Moliniopsis japonica</i> (Hack.) Hayata	0.8 \times 2 (1)	7.9 \times 19 (18)	75.8 \times 61 (20)
<i>Lobelia sessilifolia</i> Lamb.	-	1.6 \times 10 (16)	7.8 \times 37 (18)
<i>Rhynchospora alba</i> (L.) Vahl.	3.2 \times 5 (8)	62.0 \times 19 (20)	-
<i>Phragmites australis</i> (Cav.) Trin. ex Steud.	-	2.0 \times 21 (8)	11.3 \times 72 (17)
<i>Carex middendorffii</i> Fr. Schmidt	1.3 \times 1 (1)	0.5 \times 1 (1)	52.0 \times 49 (18)
<i>Drosera rotundifolia</i> L.	-	0.8 \times 2 (17)	-
<i>Iris laevigata</i> Fisch.	-	0.1 \times 2 (3)	4.3 \times 18 (11)
Total species richness	3	15	13
Plot species richness	0.5 \pm 0.6	5.8 \pm 2.2	6.0 \pm 1.5
Total cover (%) with maximum height	5.2 \pm 9.7 (38)	73.5 \pm 23.2 (65)	98.8 \pm 3.2 (115)

* The other species include: *Sanguisorba tenuifolia* Fisch. ex Link var. *alba* Trautv. et Mey. (10), *Andromeda polifolia* L. (7), *Hosta rectifolia* Nakai (7), *Vaccinium oxycoccos* L. (7), *Rubus chamaemorus* L. (6), *Lycopus uniflorus* Michx. (5), *Calamagrostis epigeios* (L.) Roth (4), *Gentiana triflora* Pall. (4), *Sphagnum* spp, mostly *S. papillosum* Lindb. (4), *Myrica gale* L. var. *tomentosa* C. DC. (3), *Sasa senanensis* (Franch. et Sav.) Rehder (3), *Solidago virgaurea* L. ssp. *leiocarpa* (Benth.) Hultén (3), *Thelypteris palustris* (Salisb.) Schott (2), *Ilex crenata* Thunb. var. *radicans* (Nakai ex H. Hara) Murai (1), and *Platanthera tipuloides* (L. f.) Lindl. ssp. *nipponica* (Makino) Murata (1).

Table 2. Litter mass remaining predicted by GLM with the assumption of a Gaussian distribution and identity link. The values show the intercepts and estimates of the models. AICs are used to select the parameters that led to the best GLMs by a backward method. Differences between models I and II are: explanatory variables in model I are habitat and litter species only and those in model II are habitat, litter species and litter chemistry. **: significant at $p < 0.001$, *: $p < 0.01$, NS: not significant. -: removed by AIC procedure. Blank: not inserted in the model at the first step. See also, Fig. 1.

	Model I (without chemical properties)	Model II (with chemical properties)
Number of samples	587	339
Intercept	-0.048**	-1.109**
Habitat (from bareground)		
<i>Rhynchospora alba</i> grassland	-0.111**	-0.115**
<i>Moliniopsis japonica</i> grassland	-0.145**	-0.079**
Litter (from <i>Rhynchospora alba</i>)		
<i>Moliniopsis japonica</i>	-	+0.034*
Carbon content (%)		+0.008*
Nitrogen content (%)		-
C/N		+0.004**
$\delta^{13}\text{C}$ (‰)		-0.020 ^{NS}
$\delta^{15}\text{N}$ (‰)		-
Phosphorus ($\mu\text{g/g}$)		-0.001**
AIC	-1115.2	-945.19

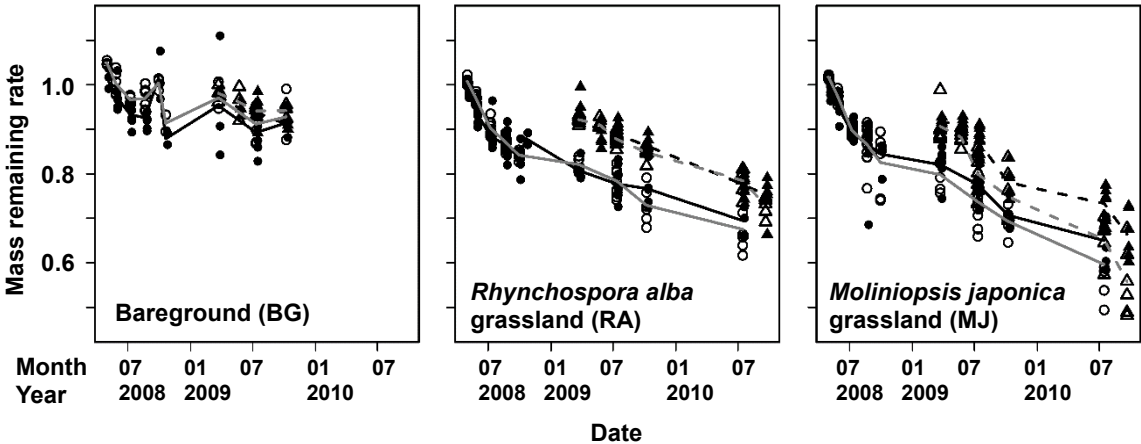


Fig. 1. Litter mass remaining in the 3 habitats for the 3 surveyed years from 2008 to 2010. Closed and open symbols show mass remaining of *Rhynchospora alba* litter and *Moliniopsis japonica* litter, respectively. Circles and triangles show mass remaining of litter established in the fall of 2007 and the spring of 2008, respectively. Solid and interrupted lines show averaged mass remaining of litter established in the fall of 2007 and the spring of 2008, respectively. O the statistical results, see Table 2.

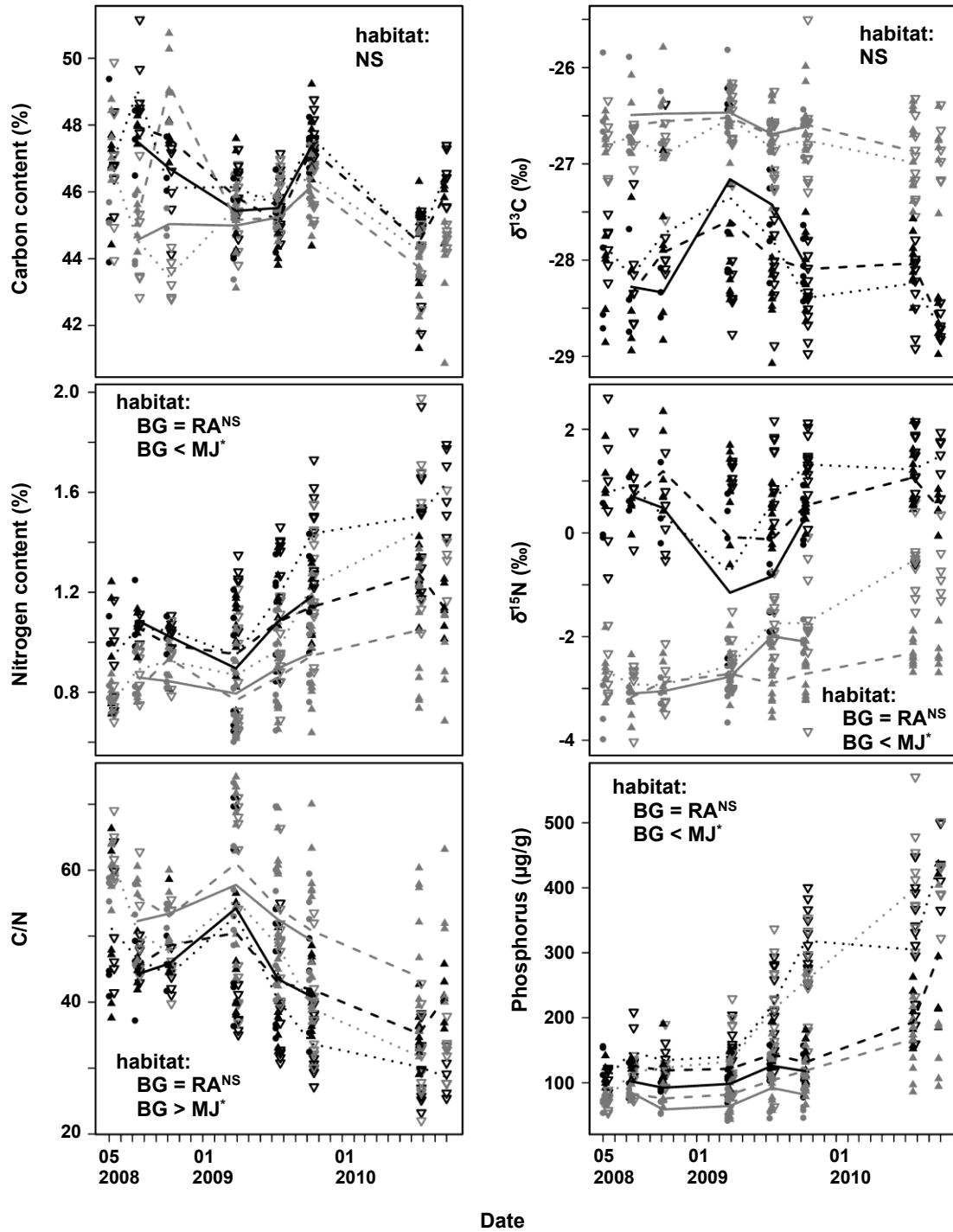


Fig. 2. Temporal changes in litter chemistry from 2008 to 2010. Solid circles, solid triangles and open reverse triangles indicate BG, RA and MJ. Solid, interrupted and dotted lines indicate mean values in BG, RA and MJ. Black and gray colors indicate litter of *R. alba* and *M. japonica*, respectively. The significant differences are indicated in each panel. All the intercepts, dates and litter species in the GLMs are significant at $p < 0.01$. *: significant at $p < 0.01$. NS: not significant. Inequality signs indicate the magnitude relations.

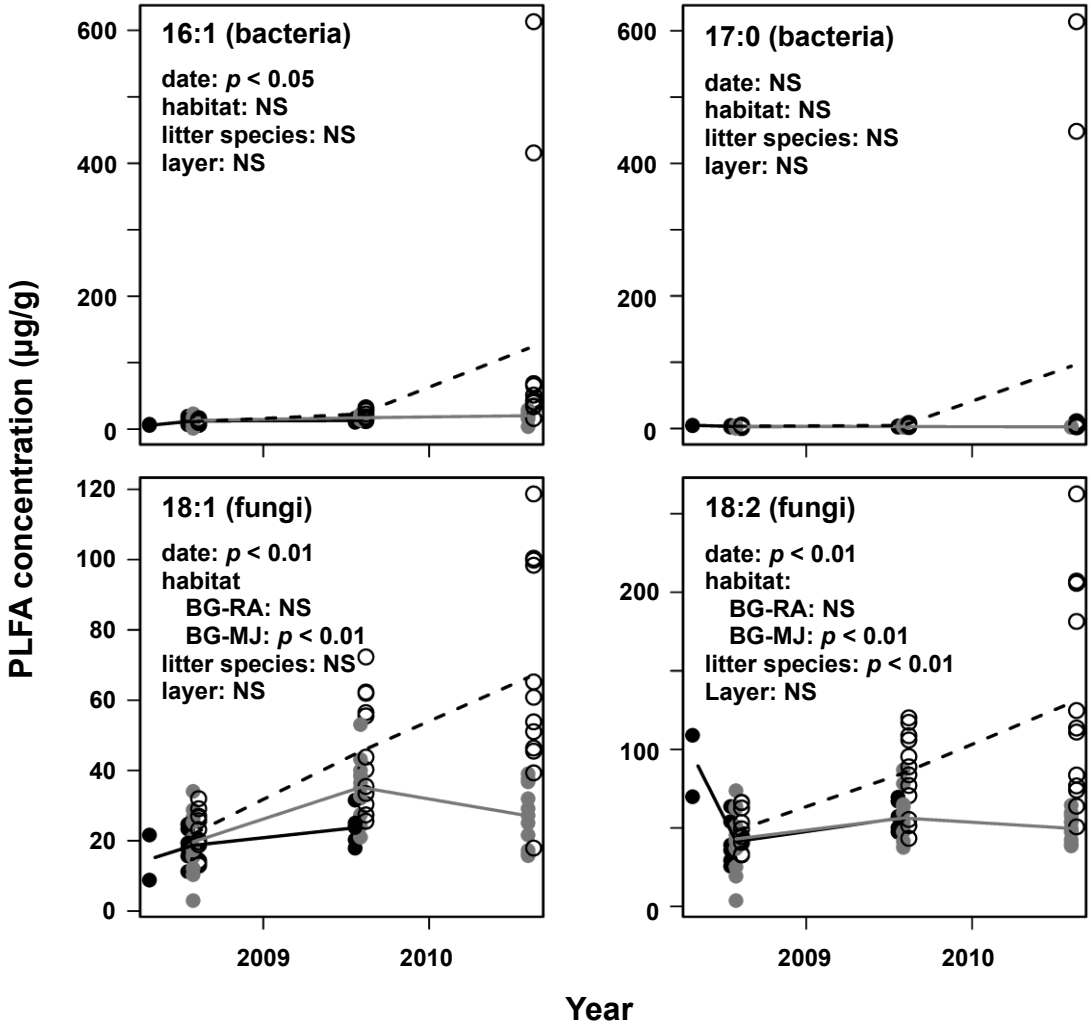


Fig. 3. Yearly fluctuations of 4 taxon-specific PLFAs (16:1 and 17:0 produced by bacteria and 18:1 and 18:2 by fungi) in the 3 habitats, bareground (BG, closed circles), *Rhynchospora alba* grassland (RA, grayed circles) and *Moliniopsis japonica* grassland (MJ, open circles). Solid, grayed and interrupted lines show the averages of PLFAs in bareground, *Rhynchospora alba* grassland and *Moliniopsis japonica* grassland. The significant differences among the habitats are shown under the taxon-specific PLFA.

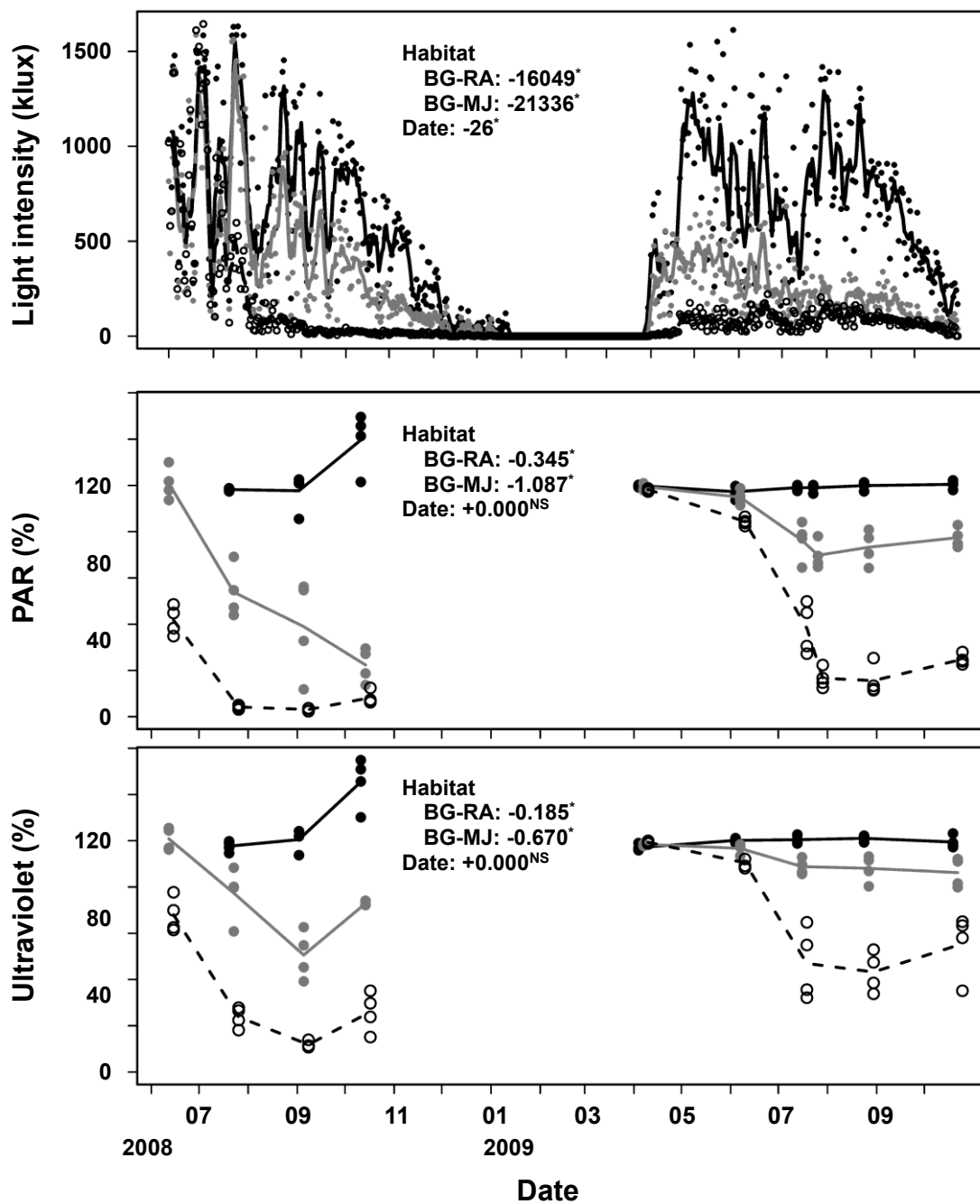


Fig. 4. Fluctuation of daily cumulative light intensity (klux), relative PAR and relative UV from June 2008 to October 2009. Solid, gray and open circles indicate BG, RA and MJ, respectively. Averaged relative intensities of PAR and UV are shown by solid, gray and interrupted lines for BG, RA and MJ, respectively. Solid, gray and open circles indicate bareground, *Rhynchospora alba* grassland and *Moliniopsis japonica* grassland. The light intensity is expressed by a cumulative value in a day. The moving average for 5 days is also shown by broken lines, as solid, gray and interrupted lines for bareground, *R. alba* grassland and *M. japonica* grassland. The results of GLMs are shown in each panel. *: significantly different at $p < 0.001$. NS: not significant at $p > 0.05$.

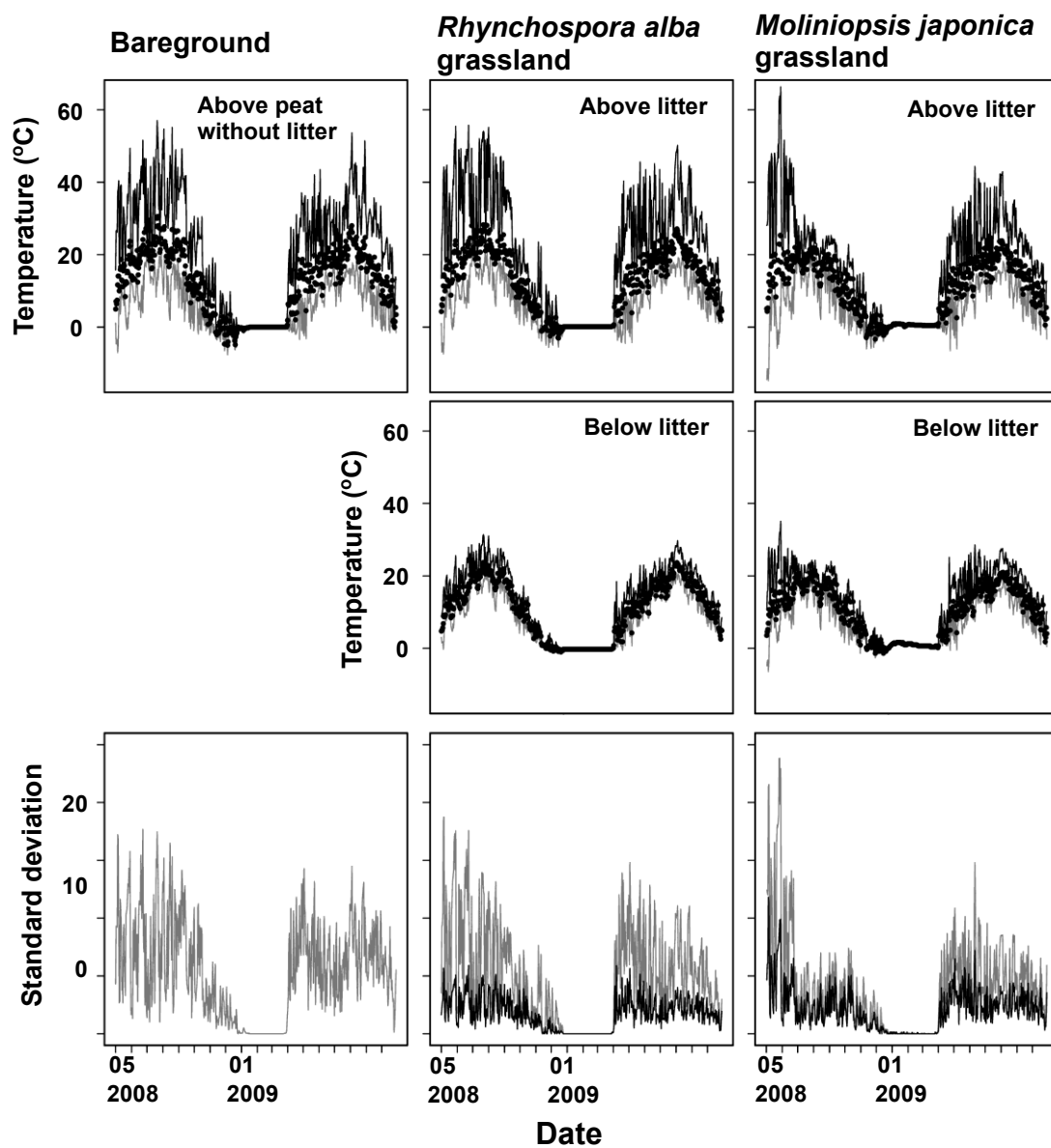


Fig. 5. Temperature fluctuations (mean, maximum, minimum and standard deviation in a day) in 3 habitats, bareground, *Rhynchospora alba* grassland and *Moliniopsis japonica* grassland. Mean (closed circles) is shown with the maximum (black lines) and minimum (gray lines) daily temperature. All the 4 parameters (mean, maximum, minimum and standard deviation) are significantly different among the 3 habitats and between the layers (GLMM, $p < 0.001$).

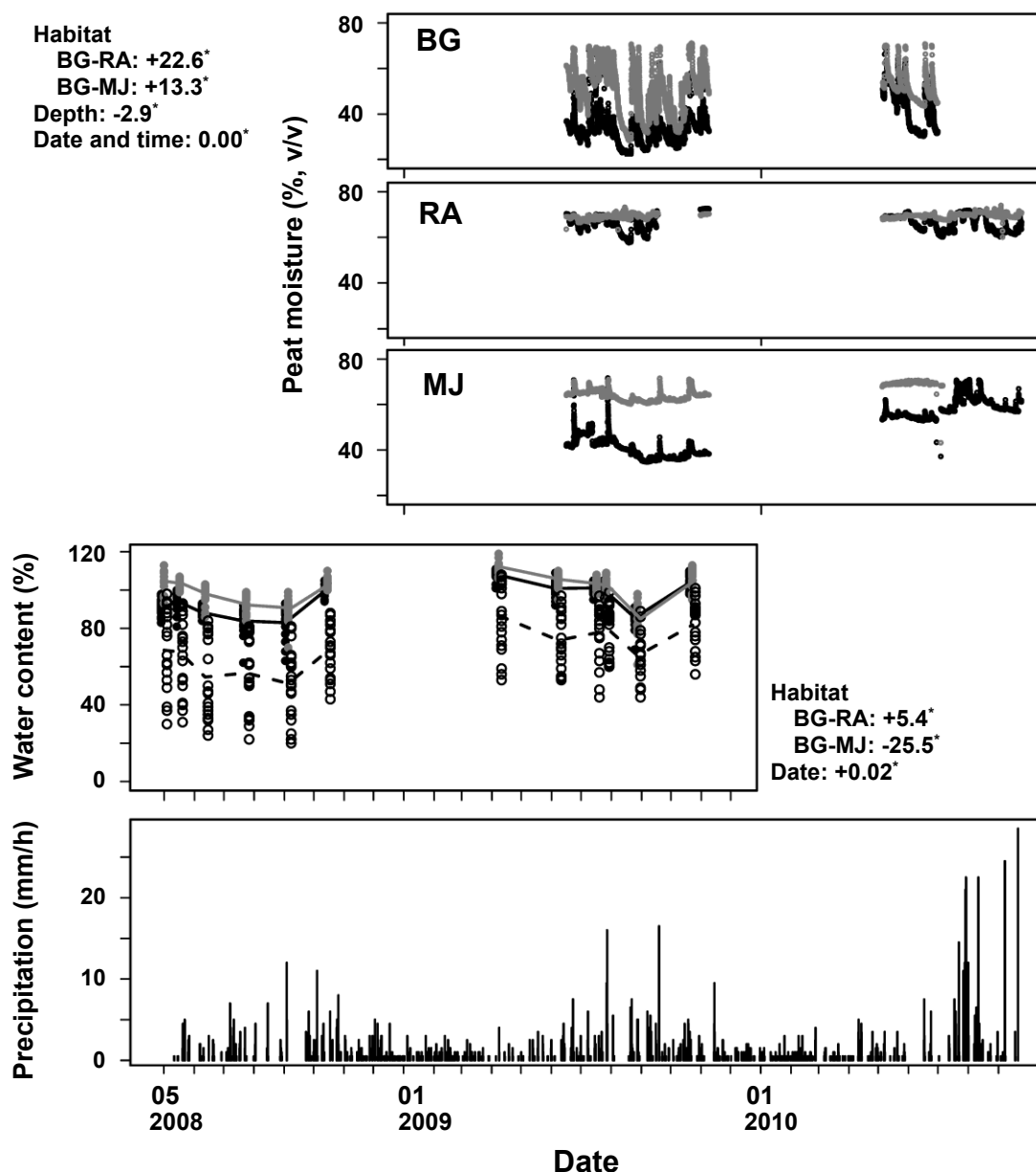


Fig. 6. Seasonal fluctuations of peat moisture (% v/v) during snow-free periods from early spring in 2009 to late fall in 2010 in 3 habitats, bareground (BG), *Rhynchospora alba* grassland (RA) and *Moliniopsis japonica* grassland (MJ) at 1 cm and 5 cm in depths and water content from 2008 to 2009 with daily precipitation (Toyotomi Town, 6 km east from the site). On water content, black, gray and open circles indicate BG, RA and MJ. The averaged peat moisture is shown by solid and black lines for bareground, solid and gray lines for RA and interrupted and black lines for MJ. The results of GLMs are shown in the sides of panels and are significantly different at $p < 0.001$.