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Differences in C, N, δ^{13}C and δ^{15}N among plant functional types after a wildfire in a black spruce forest, interior Alaska

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17   **Abstract:** We measured differences in %C, %N, δ^{13}C and δ^{15}N of plant functional types

18   (PFTs) between burned and unburned ground surfaces soon after a wildfire on a

19   north-facing slope in interior Alaska.   The C and N were measured for 16 species and

20   *Sphagnum* litter. δ^{13}C differed among the PFTs and was low for trees and shrubs,

21   suggesting that woody stems slowed C dynamics or showed low water use efficiency.

22   δ^{15}N concentrations suggested that the herbaceous plants depended less on the

23   mycorrhizal associations that became weak on the burned surfaces.   The shrub leaves

24   showed the lowest δ^{15}N of PFTs and showed higher δ^{15}N on the burned surface,

25   showing that N transfer from the soils to the leaves in the shrubs was slowed by the

26   wildfire.   Mosses showed the highest C/N ratio.   *Sphagnum* litter decomposed faster

27   on the burned surface, and %N and δ^{15}N in the litter increased from the second to third

28   year on both burned and unburned surfaces, while %C changed little.   In conclusion,

29   the responses to the wildfire differed among the PFTs as characterized by their C and N

30   dynamics.

31

32   *Key words:* Burned and unburned ground surface, carbon (C) and nitrogen (N), Alaskan

33   taiga, plant functional type, stable isotope

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Introduction

Wildfire changes vegetation structure and function, particularly in *Picea mariana* (P. Mill.) B.S.P. forests in Alaska, USA, and Canada, because the forest regeneration is dependent on wildfire (Chapin et al. 2006; Coogan et al. 2021). Wildfires are becoming more intense due to global warming and will lead to changes in carbon and nitrogen dynamics (Mann et al. 2012). Such intense wildfire in boreal forests changes the succession of forest floor vegetation, consisting of various plant functional types (PFTs), i.e., trees, shrubs, forbs, monocots and mosses (Tsuyuzaki et al. 2013).

Carbon (C) and nitrogen (N) status in plants differ among the PFTs, because each PFT has the distinctive strategies for nutrient acquisition (Lambers *et al.* 2008). Mosses represented by *Sphagnum* spp. and *Hylocomium splendens* (Hedw.) Schimp. are often dominant and determine the dynamics of C and N in a peat layer on *Picea mariana* forests (Heijmans et al. 2004). Therefore, characterizing C and N status in plants and *Sphagnum* litter is important to evaluate the effects of wildfire on regeneration through changing nutrient dynamics. The dynamics of C and N are affected by temperature and moisture that change microbial activities in and on peat (Moore et al. 2007).

Stable isotope analysis has various advantages in the quantification of C and N cycles, represented by $\delta^{15}N$ and $\delta^{13}C$. Specifically, $\delta^{13}C$ concentrations in leaves increases with increasing leaf-level intrinsic water use efficiency (WUE) in C_3 plants (Dawson et al. 2002) and $\delta^{15}N$ concentrations are related to mycorrhizal associations and their related N dynamics (Kwon and Tsuyuzaki 2016). These patterns imply that measuring $\delta^{13}C$ and $\delta^{15}N$ could detect interactions between WUE, which is reduced by changes in moisture and temperature in the underground after intensive wildfire (Volik et al., 2021), and mycorrhizal associations in boreal forests (Hobbie and Hogberg 2012). We also measured the relationships of decomposition rates of *Sphagnum* litter with peat moisture and temperature, because this litter is a key source of C and N supply to the

63 peat. When wildfires affect litter decomposition rates, C and N dynamics in PFTs

64 might be different between the burned and unburned surfaces. In addition, in the

65 substances low in N, represented by *Sphagnum* mosses (Bengtsson et al. 2018), the C/N

66 concentration ratio is one of the predictors of litter decomposition rate (Taylor et al.

67 1989). Therefore, the litter quality was evaluated for its effect on litter decomposition

68 rate and for its effect on C and N concentrations in plants.

69   The influence of C and N dynamics on vegetation in *Picea mariana* forests from

70 increasing boreal forest wildfires is of interest. This was investigated through analysis

71 of C and N concentrations and isotopic ratios of plants from burned and unburned

72 surfaces. Peat temperature, moisture and decomposition were monitored to support

73 nutrient data. The major objectives of this study were, therefore, the characterization

74 of N, C δ^{15}N and δ^{13}C in species- and PFT-based plant groups and in moss litter with its

75 decomposition rates. We examined mainly three hypotheses in relation to C and N in

76 plants and litter: 1) there are different responses of C and N properties among PFTs after

77 wildfire. 2) Mycorrhizal associations and WUE, investigated by δ^{15}N and δ^{13}C,

78 respectively, in plants were altered after wildfire. 3) burned microsites were expected to

79 undergo faster *Sphagnum* litter decomposition, because of the alteration of temperature

80 and moisture.

81

82 **Materials and methods**

83 **Study sites and field measurements**

84   The study site is Poker Flat Research Range, approximately 50 km north of

85 Fairbanks in interior Alaska (Tsuyuzaki et al. 2014). The mean annual temperature

86 was -2.0°C with the maximum and minimum monthly temperatures of 17.2°C in July

87 and -22.4°C in January during 1991 and 2020 (Japan Meteorological Agency 2021).

88 The annual precipitation was 293 mm. A huge wildfire (Boundary Fire) occurred in

89 this region from mid-June to late August 2004. Before the wildfire, the trees were

dominated by *Picea mariana* with *Sphagnum*-dominated forest floor.   The two types of vegetation developed several years after the wildfire, i.e., burned surfaces that are occupied by non-*Sphagnums* mosses, shrubs and deciduous trees, and unburned surfaces are dominated by *Sphagnum* mosses (Tsuyuzaki et al. 2013).

A time domain reflectometer (TDR) was installed in each of the burned and unburned habitats to measure moisture content (v/v) in peat.   The two TDR units (Decagon CDC-EC5, Onset, MA, USA) were close to the center of study area. Substrate moisture was measured at a 15 cm depth below living and dead mosses during May 2006 and May 2007.   Temperature profiles at the locations of the moisture measurements were obtained by thermistor sensors (104ET-2, Semitech, Tokyo, Japan) installed at 4, 8 and 15 cm in depths by thermistor sensors.

Sample collection and litterbag establishment

In total, 16 species were randomly sampled from any of 16 10 m × 10 m plots established within a 200 m × 400 m study area on the north slope of Poker Flat Research Range at 260-290 m elevations in summers of 2012 to 2014 (Fig. 1).   Six 1 m × 1 m subplots were randomly established in each plot.   The burned area was visually estimated in each plot and each subplot at 5% intervals.   Burned ground surfaces comprised a mean of 66% of these 96 subplots, ranging from 0% to 100% area burned (Tsuyuzaki et al. 2014).   In this study, a "burned" surface means that most or all of organic layer was removed by the wildfire.   Subsequent sampling was conducted in 0% burned or 100% burned subplots or in equivalent areas adjacent to such subplots. Although the dominant species and floristic composition did not change greatly during the surveyed periods (Tsuyuzaki et al. 2013), inter-annual variations in relative abundance were noted.

All species were C_3 plants (Coplen et al. 2002), as well-known in Alaska (Woodward et al. 2004), for which $\delta^{13}C$ in the leaves is expected to be related to

117   leaf-level intrinsic WUE (Dawson et al. 2002).   Shrub samples were collected on
118   August 12 2014 and tree, forb, monocot and moss samples were collected on August 18
119   2012.   Three samples were collected for each species from each of the burned and
120   from unburned surfaces.   All sampled species were common and frequent (Tsuyuzaki
121   et al. 2013), though with 7 species collected from burned habitats, 4 from unburned
122   habitats and 5 species from both habitats, depending on the recovery and establishment
123   patterns.   The sampling locations were separated by more than 5 m.   For seedlings of
124   tree species less than 25 cm in height, the whole plant, including roots, was collected by
125   digging.   For the other species, leaves were cut from the stems with scissors and
126   collected.

127   The species were classified into five PFTs: tree, shrub, monocots (grass, sedge and
128   cottongrass), forb and moss.   Of the four shrubs, two species, *Betula nana* L. and
129   *Vaccinium uliginosum* L., are deciduous and two species, *Rhododendron groenlandicum*
130   (Oeder) Kron et Judd and *Vaccinium vitis-idaea* L., are evergreen.   *Picea mariana*, one
131   of the examined tree species, is an evergreen needle-leaved species and the other two
132   species, *Populus tremuloides* Michaux and *Betula papyrifera* Marsh., are deciduous
133   broad-leaved species.   The properties of C and N were measured for the leaves of all
134   species.   In addition, the three parts (roots, stems and leaves) of trees were separately
135   measured to investigate differences in allocation and transport of C and N between
136   burned and unburned habitats (Dawson et al. 2002).

137   *Sphagnum* litter was cut with scissors in a plot where thick litter was accumulated
138   and collected on May 17, 2008.   A total of 20 litterbags (10 cm × 15 cm) were made of
139   a polyethylene net with 1-mm pore and were filled with pre-weighted 5-6 g of moss
140   litter after the samples were freeze-dried over seven days.   Ten bags were established
141   in a completely-burned plot and 10 bags were established in an unburned plot on August
142   11 2008.   These two plots were close to each other to reduce topographical effects.
143   The bags were set up at about 2 m intervals at and around the western edge of the study

144 area.   The bags were recovered on August 8 2009 and August 12 2010.   At every

145 sampling, five bags were recovered from each of the two habitats.   Then, the

146 remaining biomass was weighed soon after sampling and were freeze-dried over seven

147 days until the measurements of %C, %N, δ^{13}C and δ^{15}N were conducted.

148

149 **Measurements of plant properties**

150 The freeze-dried samples of plants and litter for measuring stable isotope were

151 dried at 80°C in an oven for three days and weighed.   Then, the samples of plants and

152 litter were finely ground in a mill with a metal blade.   %C, %N, δ^{13}C and δ^{15}N were

153 measured with a stable isotope mass spectrometer (Finnigan MAT252, Thermo Fisher

154 Scientific, Yokohama) in GSEES, Hokkaido University (Otaki and Tsuyuzaki 2019).

155 Carbon-to- nitrogen ratio (C/N) was calculated to estimate N use efficiency and

156 degradability of plants (Luo *et al.* 2017).   Tree biomass was measured in three parts,

157 leaf, stem and roots, because allocation among these three parts interacts with the N and

158 C transport (Epron et al. 2012).

159

160 **Statistical analysis**

161 Generalized linear models (GLMs) were used to compare response variables,

162 i.e., %C, %N, δ^{13}C, δ^{15}N and C/N, among PFTs, among the three plant parts of trees and

163 between the burned and unburned habitats of litter.   The PFTs, parts and habitats are

164 used as explanatory categorical variables.   Because tree regeneration was a key

165 indicator of vegetation recovery and resilience, differences were compared between

166 trees and the other PFTs and between leaf and the other parts and between burned and

167 unburned habitats.   A Gaussian distribution with identity link function was assumed

168 for δ^{13}C, δ^{15}N and C/N and a negative binomial distribution with logit link function was

169 used for %C and %N.   Differences in substrate temperatures in the unburned and

170 burned peat and by peat depth were examined by generalized linear mixed-effects

171 model (GLMM) with the assumption of a Gaussian distribution with identity link

172 function and date as the random effect.   Peat moisture was compared between the

173 living and dead peat deposits by GLMM as well as the temperatures except for the

174 comparison of depths.   These statistical models and results are described in

175 supplementary materials (Tables S1 to S6). All statistical analyses were performed by

176 software R (ver. 3.6.1) (R Core Team 2019).

177

Results

The litter environments and decomposition of *Sphagnum* mosses

180 The maximum and minimum temperatures were 21.2°C and -12.6 °C, both of which

181 were recorded at 4 cm deep in the dead moss (Fig. 2).   Temperature decreased with

182 increasing peat depths (GLMM, $p < 0.01$).   The dead moss showed a higher

183 temperature than the living moss ($p < 0.01$).   In total, therefore, the temperature was

184 higher at shallow layers in burned habitats.   An interaction between the depths and

185 habitats was detected ($p < 0.01$).   Peat moisture ranged from 8.8% to 19.6% and was

186 lower, i.e., drier, in the dead moss ($p < 0.01$).

187   *Sphagnum* litter showed 78.8% and 78.3% mass remaining after one and two years

188 on average.   The amount remaining was greater on the unburned surface (GLM, $p <$

189 0.01) (Fig. 3), showing that the litter decomposition was faster on the burned

190 surface.   %C in litter samples did not change over the two years ($p > 0.05$) and %N

191 increased from 1.5% to 3.5% ($p < 0.01$). δ^{13}C was higher on the burned surface than

192 on the unburned surface, independent of the years ($p < 0.01$), showing that C release

193 from litter was greater on the burned surfaces, as indicated by fast litter decomposition

194 on the burned surfaces. δ^{15}N increased with time ($p < 0.01$) and did not differ between

195 the habitats ($p > 0.05$), showing that the wildfire did not affect N dynamics in the litter.

196 C/N ratios decreased slightly from 58.0 to 56.0 and 51.0 on the burned and unburned

197 surfaces, respectively, over one year.   C/N decreased greatly to 12.9 and 12.5 on

198   burned and unburned habitats, respectively, after two years.   C/N differed between the

199   years of 2009 and 2010, while C/N was not significantly different between the habitats

200   overall ($p < 0.01$).

201

202   **Plant functional types**

203   Of the three tree species, the evergreen, needle-leaved tree, *Picea mariana*,

204   established on both habitats and the other two deciduous, broad-leaved trees, *Populus*

205   *tremuloides* and *Betula neoalaskana*, established only on the burned surface (Fig. 4).

206   All the four shrub species established in both habitats.   The examined two forbs,

207   *Polygonum alaskanum* W. Wight ex Hultén and *Epilobium angustifolium* L., established

208   well on the burned surface while the two monocot species, *Carex bigelowii* Torr. ex

209   Schwein. and *Eriophorum vaginatum* L., established mostly on the unburned surface.

210   The four mosses were classified into two types based on the habitat preferences; viz.

211   *Sphagnum fuscum* (Schimp.) H. Klinggr. and *Hylocomium splendens* (Hedw.) Schimp.

212   were found only on the unburned surface while *Ceratodon purpureus* (Hedw.) Brid. and

213   *Polytrichum commune* Hedw. established only on the burned surface.

214   The %C, %N, $\delta^{13}C$ and $\delta^{15}N$ in leaves varied among the five PFTs (Fig. 4), while

215   these did not differ between burned and unburned surfaces.   As compared to the trees,

216   the monocots and mosses showed lower %C and shrub had higher %C (GLM, $p < 0.01$).

217   The mosses showed lower %N than the trees ($p < 0.01$) and therefore, showed higher

218   C/N than the trees with C/N of four mosses higher than 40, reflecting low values of %N.

219   C/N ratios ranged from 20 to 60 for all the examined vascular plants except for

220   *Epilobium angustifolium*, of which C/N ratio was 90. $\delta^{13}C$ ranged from -33.5‰ to

221   -25.3‰ for all the examined species.   The trees showed the lowest $\delta^{13}C$ of the five

222   PFTs and the monocots showed the highest value ($p < 0.01$). $\delta^{15}N$ showed remarkable

223   differences among the examined PFTs, i.e., the shrubs showed the lowest $\delta^{15}N$

224   (-4.894‰ in average) and herbs (monocots and forbs) showed the highest $\delta^{15}N$ (5.987‰

225 and 3.950‰, respectively) ($p > 0.05$).

226

C and N in shrubs grown in burned and unburned substrates

228 %C was higher in *Rhododendron groenlandicum* and *Vaccinium vitis-idaea* leaves

229 than in *Betula nana*, while it was lower in *Vaccinium uliginosum* than in *Betula nana*

230 (GLM, $p < 0.01$) (Fig. 5). %N was lower in *Rhododendron groenlandicum* and

231 *Vaccinium vitis-idaea* than in *Betula nana* ($p < 0.01$). Except for high δ^{13}C in

232 *Rhododendron groenlandicum*, δ^{13}C did not differ among the other three shrub species

233 ($p > 0.05$). δ^{15}N was higher in *Vaccinium uliginosum* than in *Betula nana* ($p < 0.05$).

234 δ^{15}N and δ^{13}C in the shrub leaves was higher on the burned surface than on the

235 unburned surface (GLM, $p = 0.01$ and 0.04, respectively), while the %C, %N, C/N did

236 not differ between the two habitats (Fig. 5).

237

Dominant trees

239 %C in all the three examined tree species did not differ among the three parts, leaf,

240 stem and root (GLM, $p > 0.05$) (Table 1). %N also did not differ among the three tree

241 species ($p > 0.05$). However, %N was higher in leaves than in stems or roots for *Picea*

242 *mariana* on the unburned surface and *Betula papyrifera* on the burned surface ($p < 0.01$).

243 δ^{13}C in *Picea mariana* was higher in the roots than in the leaves ($p < 0.01$).

244 Furthermore, it was higher on the unburned surface than on the burned surface ($p <$

245 0.01). *Populus tremuloides* showed the highest δ^{13}C of the tree species on the burned

246 surface ($p < 0.01$). δ^{15}N did not differ among tree species and among the three parts (p

247 > 0.05). C/N was higher in stems on *Picea mariana* and *Betula papyrifera* on the

248 burned surface ($p < 0.01$).

249

Discussion

Plant functional types

252   The mosses with high C/N tended to show lower %C and %N than the vascular

253   plants (Fig. 1), indicating that the mosses stored N less so than the vascular plants.

254   Because %N did not differ among the vascular species, differences in C/N among them

255   were determined mostly by %C. N concentration averages 2.1% and 1.0% for 49

256   boreal vascular plant species on burned and unburned surfaces, respectively, and 1.8%

257   and 0.7% for mosses, soon after a wildfire (Mack 2007). Because mosses, including

258   four species examined in this study (Fig. 4), generally have low N concentrations with a

259   narrow range (Zechmeister et al. 2008), as compared with vascular plants, the difference

260   between the vascular plants and mosses became more than among the vascular

261   plants. %C, %N, δ^{13}C, δ^{15}N and C/N ratio did not differ between the burned and

262   unburned habitats, probably because all the PFTs except for shrubs were restricted to

263   one habitat. On the shrub species, all of which were sampled from burned and

264   unburned habitats, δ^{13}C and δ^{15}N were different between the two habitats. These

265   suggested that C and N dynamics were altered after wildfire when they survived on

266   burned surfaces.

267   The δ^{13}C of examined plants, all of which were C$_3$ plants, showed a wide range

268   among the species, depending on PFTs (Fig. 4). These results suggested that WUE

269   differed greatly among the PFTs, also found in arid regions (Dugas et al. 1996; Peng et

270   al. 2007) and in tropical forests (Wright et al. 2021). δ^{13}C was lower in the woody

271   plants (trees and shrubs) than in the herbaceous plants (monocots and forbs). δ^{13}C in

272   *Picea mariana*, of the trees, was reduced by the wildfire (Table 1), suggesting that *Picea*

273   *mariana* reduced WUE after wildfire in boreal forests. Soil scarification increases

274   WUE for *Picea mariana* in boreal forests (Wotherspoon et al. 2020), while

275   paludification does not alter WUE for *Picea mariana* in boreal peatlands while

276   decreasing the growth rate (Beaulne et al. 2021). These results showed that the

277   responses of WUE on *Picea mariana* vary with the characteristics of disturbances or

278   habitats. However, the estimation of WUE by δ^{13}C must be made with caution

279 because the highest WUE occurs during the driest years and does not always hold true

280 (Golluscio and Oesterheld 2007; Medlyn et al. 2017).

281 Three monocot species showed the highest $\delta^{15}N$ (Fig. 4). Of the monocots, *Carex*

282 *bigelowii* and *Eriophorum vaginatum*, are non-mycorrhizal plants (Hobbie et al. 2009,

283 Ruotsalainen and Aikio 2011). When plants are non-mycorrhizal, $\delta^{15}N$ becomes high

284 and stable because there is no fractionation of N isotopes (Hobbie and Agerer 2010).

285 Therefore, $\delta^{15}N$ of *Carex bigelowii* and *Eriophorum vaginatum* can be used as the

286 benchmark to investigate the dependence of plants on mycorrhizal fungi. Another

287 monocot, *Calamagrostis canadensis*, showed high $\delta^{15}N$, as well. The development of

288 mycorrhizal fungi in *Calamagrosits canadensis* roots might be expected to be low after

289 disturbance by wildfire. Although the mycorrhizal association of *Polygonum*

290 *alaskanum*, a forb, is unknown, a phylogenetically and morphologically similar species,

291 *Polygonum sachalinense*, is non- or less- arbuscular mycorrhizal (Obase et al. 2008).

292 Low $\delta^{15}N$ in the leaves also suggested that mycorrhizal associations are low for the

293 monocots and forbs. $\delta^{15}N$ of shrubs was higher on the burned surfaces. $\delta^{15}N$ varies

294 among species and PFTs, corresponding with mycorrhizal association in the plant roots

295 (Aerts et al. 2009). Three of the four shrubs, showing low $\delta^{15}N$, develop ericoid

296 mycorrhizae (Hbobie et al. 2009). Furthermore, all of these shrubs tended to show

297 higher $\delta^{15}N$ on the burned habitat (Fig. 5), suggesting that the mycorrhizal associations

298 were weakened by the wildfire. Short-term environmental factors may interact with

299 species- or PFT-specific plant traits related to water and N use strategies and override

300 $\delta^{13}C$ and $\delta^{15}N$ (Gatica et al. 2017).

301

302 ***Sphagnum* litter**

303 The *Sphagnum* litter decomposed faster on the burned surface (Fig. 3). The

304 albedo is higher on dead or burned *Sphagnum* mosses than on the unburned, living

305 mosses (Chambers et al. 2005, Tsuyuzaki et al. 2009). The peat moisture decreased

306 with increasing temperature on the burned surface, probably due to evaporation. In

307 addition, high evaporation of living mosses induces cooling effects on the temperature

308 in peat in a boreal black spruce forest (Heijmans et al. 2004). Litter decomposition

309 increases with increasing temperature and rainfall due to microbial activities in various

310 wetlands (Bell et al. 2018). These relationships suggest that intensive wildfires change

311 nutrient dynamics in the ground by accelerating litter decomposition, due probably to

312 high temperature and low water content in the burned ground (Fig. 2). Low water

313 content in peat results in great aeration and opportunities for aerobic decomposition

314 (Granath et al. 2016).

315 N loss was influenced less by the wildfire, as shown by no differences of %N and

316 δ^{15}N between burned and unburned habitats. %N increased drastically from 2009 to

317 2010, probably because N immobilization in *Sphagnum* litter was absent during the first

318 year and then occurs (Bragazza et al. 2001). C/N decreased drastically in the second

319 year, due mostly to an increase in %N that was 4-6 times higher in 2010 than in

320 2009. %C did not change with time. These results suggested that the wildfire

321 enhanced the loss of N-unbounded components more in the second year. C-use

322 efficiency in litter increases when the litter is N-poor, as shown in the initial stage of

323 litter decomposition in this study because the decomposers are initially N-limited

324 (Manzoni 2017). The decomposition of *Sphagnum* litter, which is made mostly of

325 cellulose, is limited in lignin or phenolic compounds as well as the N availability of the

326 microbes (Hajek 2009).

327 It should also be noted that C/N ratios are diverse among *Sphagnum* species. The

328 initial liter of *Sphagnum angustifolium* showed C/N ratios of 25-30 two years after

329 installing litterbags in a peat bog in northeastern Hungary (Peli et al. 2016). The initial

330 C/N of *S. balticum* C/N was 66 in an Alaskan tundra habitat (Hobbie 1996).

331 *Sphagnum fuscum* litter had C/N values of more than 100 in a peatland-forest complex,

332 British Columbia, Canada, even two in two years after setting up litterbags (Asada et al.

333 2005). The decomposition of *Sphagnum* litter may be more species-specific than

334 previously thought because differences in litter decomposition rates are related to

335 carbon quality more than to nitrogen concentration (Hobbie 1996). Of the four moss

336 species, *Polytrichum commune* showed the highest $\delta^{13}C$ and $\delta^{15}N$ (Fig. 4). $\delta^{13}C$ and

337 $\delta^{15}N$ values as measured for *Polytrichum juniperinum* on unburned surfaces in an

338 interior Alaska were -28.8‰ and 3.0‰, respectively (Mack, 2007). These results may

339 reflect the fact that *Polytrichum* mosses develop water-conducting tissue (Glime 2017),

340 which probably accounts for the stable isotope signatures.

341

342 **Behaviors of shrubs and trees**

343 The shrubs established in both burned and unburned habitats and did not show

344 significant differences in the properties of C and N among them, except for $\delta^{15}N$ (Fig.

345 5). Six shrub species, including *Vaccinium vitis-idaea* and *Rhododendron*

346 *groenlandicum*, had been previously shown to have higher %N on unburned surfaces

347 (Mack 2007), as found in this study. These shrubs regenerated mostly by vegetative

348 reproduction on the two habitats (Tsuyuzaki et al. 2013). Vegetative reproduction

349 often alleviates resource limitation through below-ground nutrient transport under

350 stressful conditions or in disturbed habitats (Lovett Doust and Lovett Doust 1990).

351 Their capabilities to adapt to various habitats, particularly on the burned surface via

352 vegetative reproduction, should facilitate their establishment and growth and

353 consequently did not show the significant differences of chemical properties between

354 the two habitats due to belowground nutrient transport.

355 On the burned surfaces, *Picea mariana* allocated to the aboveground biomass more

356 than *Betula papyrifera* and to the height growth more than *Populus tremuloides* (Fig. 6).

357 These two broad-leaved tree species, which are pioneer trees after wildfire (Chapin et al.

358 2006), do not establish on the unburned surfaces while they establish well on the burned

359 surfaces (Tsuyuzaki et al. 2014). However, *Picea mariana* grows in height much more

360 slowly than do the broad-leaved trees (Tsuyuzaki et al. 2014).

361 In conclusion, the Boundary Wildfire, which altered the temperature and water

362 content in peat, greatly affected C dynamics, particularly through the *Sphagnum* litter

363 and *Picea mariana* shoots. The N dynamics were characterized well by the

364 combination of PFTs (in particular, differences among woody, herbaceous and moss

365 species), burned vs. unburned habitats and mycorrhizal associations.

366

373

374 **References**

375 Aerts, R., Callaghan, T.V., Dorrepaal, E., van Logtestijn, P., and Cornelissen J.H.C.

376 2009. Seasonal climate manipulations result in species-specific changes in leaf

377 nutrient levels and isotopic composition in a sub-arctic bog. Funct. Ecol. **23**:

378 680-688.

379 Asada, T., Warneer, B., and Aravena, R. 2005. Effects of the early stage of

380 decomposition on change in carbon and nitrogen isotopes in *Sphagnum* litter. J.

381 Plant Ecol. **1**: 229-237.

382 Bell, M.C., Ritson, J.P., Verhoef, A., Brazier, R.E., Templeton, M.R., Graham, N.J.D.,

383 Freeman, C., and Clark, J.M. 2018. Sensitivity of peatland litter decomposition to

384 changes in temperature and rainfall. Geoderma **331**: 29-37.

385 Bengtsson, F., Rydin, H., and Hajek T. 2018. Biochemical determinants of litter quality

386 in 15 species of *Sphagnum*. Plant and Soil **425**: 161-176.

387 Bragazza, L., Buttler, A., Siegenhaler, A., and Mitchell, E.A.D. 2001. Plant litter
388   decomposition and nutrient release in peatlands. Geophys. Monogr. Ser. **184**:
389   99-110.

390 Chambers, S.D., Beringer, J., Randerson, J.T., and Chapin,F.S. III. 2005. Fire effects on
391   net radiation and energy partitioning: contrasting responses of tundra and boreal
392   forest ecosystems. J. Geophys. Res. Atmos. **110**: D09106.doi:10.1029/2004JD
393   005299.

394 Chapin III, F.S., Moilanen, L., and Kielland, K. 1993. Preferential use of organic
395   nitrogen for growth by a non-mycorrhizal arctic sedge. Nature **361**:150–153.

396 Chapin III, F.S., Oswood, M.W., van Cleve K., Viereck, L.A., and Verbyla, D.L. 2006.
397   *Alaska's changing boreal forest*. Oxford University Press, Oxford.

398 Coogan, S.C.P., Daniels, L.D., Boychuk, D., Burton, P.J., Flannigan, M.D., Gauthieer, S.,
399   Kafka, V., Park, J.S., and Wotton, B.M. 2021. Fifty years of wildland fire science in
400   Canada. Can. J. For. Res. **51**: 283-302.

401 Coplen, T.B., Böhleke, J.K., De Bié P., Ding T., Holden N.E., Hopple J.A., Krouse K.J.,
402   Lamberty, A., Peiser, H.S., Révész, K., Rieder, S.E., Rosman, K.J.R., Roth, E.,
403   Taylor, P.D.P., Vocke, Jr., R.D., and Xiao, Y.K. 2002. Isotope-abundance variation
404   of selected elements. Pure Appl. Chem. **74**: 1987-2017.

405 Dawson, T.E., Mambelli, S., Plamboeck, A.H., Templer, P.H., and Tu, K.P. 2002. Stable
406   isotopes in plant ecology. Ann. Rev. Ecol. Syst. **33**: 507-559.

407 Dugas, W.A., Hicks, R.A., and Gibbens, R.P. 1996. Structure and function of C_3 and C_4
408   Chihuahuan Desert plant communities. Energy balance components. J. Arid
409   Environ. **34**: 63-79.

410 Epron, D., Bahn, M., Derrien, D., Lattanzi, F.A., Pumpanen, J., Gessler, A., Hogberg, P.,
411   Maillard, P., Dannnoura, M., Gerant, D., and Buchmann, N. 2012. Pulse-labelling
412   trees to study carbon allocation dynamics: a review of methods, current knowledge
413   and future prospects. Tree Physiol. **32**: 776-798.

414 Gatica, M.G., Aranibar, J.N., and Pucheta, E. 2017. Environmental and species-specific controls on $\delta^{13}C$ and $\delta^{15}N$ in dominant woody plants from central-western Argentinian drylands. Austral Ecol. **42**: 533-543.

417 Glime, J.M. 2017. Chapter 7-1. Water relations: conducting structures. *In*: Glime, J.M. Bryophyte ecology. Vol. 1. Michigan Technology University, Houghton. 7-1-1 - 7-1-28.

420 Golluscio, R.A., and Oesterheld, M. 2007. Water use efficiency of twenty-five co-existing Patagonian species growing under different soil water availability. Oecologia **154**: 207-217.

423 Granath, G., Moore, P.A., Lukenbach, M.C., and Waddington, J.M. 2016. Mitigating wildfire carbon loss in managed northern peatlands through restoration. Scientific Rep. **6**: 28498, doi: 10.1038/srep28498.

426 Hajek, T. 2009. Habitat and species controls on *Sphagnum* production and decomposition in a mountain raised bog. Boreal Env. Res. **14**: 947-958.

428 Heijmans, M.M.P.D., Arp, W.J., and Chapin III, S. 2004. Controls on moss evaporation in a boreal black spruce forest. Global Biogeochem. Cycles **18**: GB2004, doi: 10.1029/ 2003BG002128.

431 Hobbie, E.A., and Hogberg, P. 2012. Nitrogen isotopes link mycorrhizal fungi and plants to nitrogen dynamics. New Phytol. **196**: 367-382.

433 Hobbie, J.E., Hobbie, E.A., Drossman, H., Conte, M., Weber, J.C., Shamhart, J., and Weinrobe M. 2009. Mycorrhizal fungi supply nitrogen to host plants in Arctic tundra and boreal forests: ^{15}N is the key signal. Can. J. Microbiol. **55**: 84-94.

436 Hobbie, S.E. 1996. Temperature and plant species control over litter decomposition in Alaskan tundra. Ecol. Monogr. **66**: 503-522.

438 Hobbie, S.E., and Agerer, R. 2010. Nitrogen isotopes in ectomycorrhizal sporocarps correspond to belowground exploration types. Plant Soil **327**: 71–83.

440 Japan Meteorological Agency. 2021. ClimatView. Fairbanks, AK. https://www.data.jma.

441   go.jp/gmd/cpd/monitor/climatview/graph_mkhtml.php?n=70261&y=2013&m=8&s

442   =3&r=4&e=3&k=0&d=0

443   Kwon, T., and Tsuyuzaki, S. 2016. Differences in nitrogen redistribution between early

444   and late plant colonizers through ectomycorrhizal fungi on the volcano Mount

445   Koma. Ecol. Res. **31**: 557-567.

446   Lambers, H., Chapin, III F.S., and Pons, T.L. 2008. *Plant physiological ecology (2nd*

447   *edition)*. Springer, Berlin.

448   Lovett Doust, J., and Lovett Doust, L. 1990. *Plant reproductive ecology: patterns and*

449   *strategies*. Oxford University Press, Oxford.

450   Luo, W., Li, M.-H., Sardans, J., Lu, X.-T., Wang, C., Penuelas, J., Wang, Z., Han, X.-G.,

451   and Jiang, Y. 2017. Carbon and nitrogen allocation shifts in plants and soils along

452   aridity and fertility gradients in grasslands of China. Ecol. Evol. **7**: 6927-6934.

453   Mack, M.C. 2007. Biomass %N, %C, natural abundance ^{15}N and ^{13}C isotopic signatures

454   for common and rare under-and overstory plants in long unburned and burned

455   (1999) boreal forest stands, Caribou-Poker Creek and Delta Junction, Bonanza

456   Creek LTER. UAF, Fairbanks. https://www.lter.uaf.edu/data/data-detail/id/522

457   (unpublished data).

458   Mann, D.H., Rupp, T.S., Olson, M.A., and Duffy P.A. 2012. Is Alaska's boreal forest

459   now crossing a major ecological threshold? Arct. Antarct. Alp. Res. **44**: 319-331.

460   Manzoni, S. 2017. Flexible carbon-use efficiency across litter types and during

461   decomposition partly compensates nutrient imbalances-results from analytical

462   stoichiometric models. Front. Microbiol. **26**: Article 661. doi: 10.3389/fmicb.

463   2017.00661.

464   Medlyn, B.E., De Kauwe, M.G., Lin, Y.-S., Knauer, J., Duursma, R.A., Williams, C.A.,

465   Arneth, A., Clement, R., Isaac, P., Limousin, J.-M. 2017. How do leaf and

466   ecosystem measures of water-use efficiency compare? New Phytol. **216**: 758-770.

467   Moore, T.R., Bubier, J.L., and Bledzki, L. 2007. Litter decomposition in temperate

468   peatland ecosystems: the effect of substrate and site. Ecosystems 10: 979-963.

469   Obase, K., Tamai, Y., Yajima, T., and Miyamoto, T. 2008. Mycorrhizal colonization
470   status of plant species established in an exposed area following the 2000 eruption
471   of Mt. Usu, Hokkaido, Japan. Landscape Ecol. Eng. **4**: 57-61.

472   Otaki, M., and Tsuyuzaki, S. 2019. Succession of litter-decomposing microbial
473   organisms in deciduous birch and oak forests, northern Japan. Acta Oecol. **101**,
474   103485.

475   Peli, E.R., Nagy, J., and Cserhalmi, D. 2016. Decomposition rate, and carbon and
476   nitrogen dynamics of Sphagnum litter: lessons from a peat bog. Pol. J. Ecol. **64**:
477   231-240.

478   Peng, Y., Jiang, G.M., Liu, X.H., Niu, S.L., Liu, M.Z., and Biswas, D.K. 2007.
479   Photosynthesis, transpiration and water use efficiency of four plant species with
480   grazing intensities in Hunshandak Sandland, China. J. Arid Environ. **70**: 304-315.

481   R Core Team. 2019. R: *A language and environment for statistical computing*. R
482   Foundation for Statistical Computing, Vienna, Austria.

483   Ruotsalainen, A.L., and Aikio, S. 2011. Mycorrhizal inoculum and performance of
484   nonmycorrhizal *Carex bigelowii* and mycorrhizal *Trientalis europaea*. Canad. J.
485   Bot. **82**: 443-449.

486   Taylor, B.R., Parkinson, D., and Parsons, W.F.J. 1989. Nitrogen and lignin content as
487   predictors of litter decay rates: a microcosm test. Ecology **70**: 97-104.

488   Tsuyuzaki, S., Kushida, K., and Kodama, Y. 2009. Recovery of surface albedo and plant
489   cover after wildfire in a *Picea mariana* forest in interior Alaska. Clim. Change **93**:
490   517-525.

491   Tsuyuzaki, S., Narita, K., Sawada, Y., and Harada K. 2013. Recovery of forest-floor
492   vegetation after a wildfire in a *Picea mariana* forest. Ecol. Res. **28**: 1061-1068.

493   Tsuyuzaki, S., Narita, K., Sawada, Y., and Kushida, K. 2014. The establishment patterns
494   of tree seedlings are determined immediately after wildfire in a black spruce (*Picea*

495   *mariana*) forest. Plant Ecol. **215**: 327-337.

496 Volik, O., Petrone, R., Kessel, E., Green, A., and Price, J. 2021. Understanding the peak

497   growing season ecosystem water-use efficiency at four fens in the Athabasca oil

498   sands region. Hydrol. Proc. **35**: e14323.

499 Woodward, F.I., Lomas, M.R., and Kelly, C.K. 2004. Global climate and the distribution

500   of plant biomes. Proc. Royal Soc. B **359**: 1465-1476.

501 Wotherspoon, A., Thiffault, N., and Bradley, R.L. 2020. Resource availability and

502   physiological response of black spruce to scarification in two climatic regions of

503   Québec (Canada). Silva Fennica **54**: 10375. doi: 10.14214/sf.10375.

504 Wright, C.L., de Lima, A.L.A., de Souza, E.S., West, J.B., and Wilcox, B.P. 2021. Plant

505   functional types broadly describe water use strategies in the Caatinga, a seasonally

506   dry tropical forest in northeast Brazil. Ecol Evol. **11**: 11808-11825.

507 Zechmeister, H.G., richter, A., Smidt, S., Hohenwallner, D.H., Order, I., Maringer, S.,

508   and Wanek, W. 2008. Total nitrogen content and δ^{15}N signatures in moss tissue:

509   indicative value for nitrogen deposition patterns and source allocation on a

510   nationwide scale. Environ. Sci. Technol. **42**: 8661-8667.

511 **Table 1.** Carbon content (%), nitrogen content (%), δ^{13}C (‰), δ^{15}N (‰) and C/N ratio in three tree species established on burned or

512 unburned habitats. Each cell shows mean with standard deviation. For statistical differences, see appendix (Table S5).

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Habitat	Species	Tissue	Carbon (%)	Nitrogen (%)	C/N	d^{13}C (‰)	d^{15}N (‰)
Unburned	*Picea mariana*	Leaf	48.9 ± 1.7	1.57 ± 0.35	32.0 ± 6.6	-31.4 ± 0.2	0.49 ± 2.09
		Stem	52.4 ± 0.9	0.84 ± 0.17	64.5 ± 14.3	-30.3 ± 0.4	-3.22 ± 1.43
		Root	49.7 ± 1.4	1.37 ± 0.05	36.2 ± 2.0	-29.1 ± 0.7	-1.20 ± 0.77
Burned	*Picea mariana*	Leaf	49.5 ± 0.6	1.03 ± 0.12	48.6 ± 5.7	-31.7 ± 0.8	-0.01 ± 2.09
		Stem	49.0 ± 3.6	0.63 ± 0.17	81.4 ± 20.9	-31.5 ± 1.0	-1.74 ± 1.85
		Root	47.9 ± 2.2	1.29 ± 0.29	38.5 ± 8.1	-30.9 ± 0.2	2.25 ± 1.10
	Betula neoalaskana	Leaf	47.4 ± 0.8	1.24 ± 0.16	38.6 ± 5.3	-30.5 ± 0.9	0.83 ± 2.67
		Stem	51.6 ± 1.9	0.74 ± 0.11	70.8 ± 12.1	-30.7 ± 0.8	0.11 ± 2.87
		Root	48.0 ± 0.9	0.94 ± 0.18	59.4 ± 5.9	-30.4 ± 0.3	1.07 ± 1.95
	Populus tremuloides	Leaf	50.5 ± 1.2	1.71 ± 0.35	30.4 ± 5.0	-30.5 ± 0.1.2	0.68 ± 1.98
		Stem	49.4 ± 1.9	0.75 ± 0.24	73.1 ± 28.2	-30.1 ± 1.4	-0.68 ± 1.77
		Root	47.3 ± 1.6	0.94 ± 0.18	52.0 ± 10.4	-29.0 ± 0.4	0.37 ± 1.66

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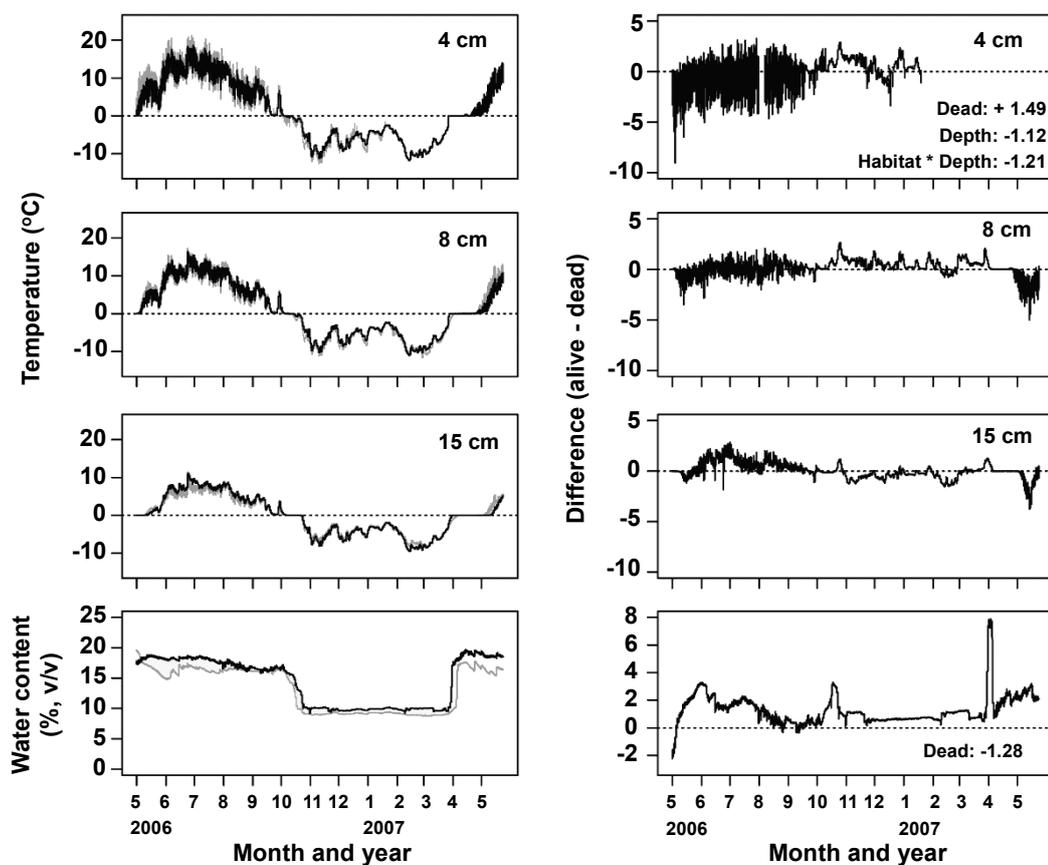

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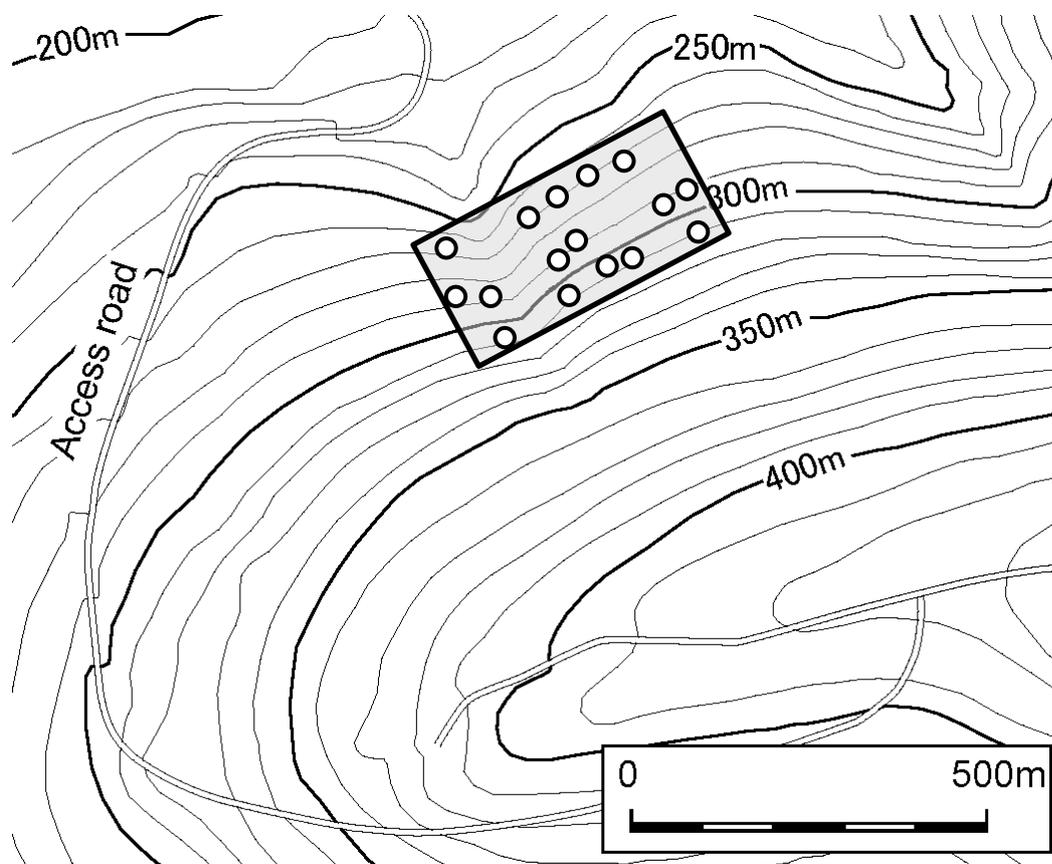
Fig. 1. Study area (+65.12, -147.46), shown by a gray square, in Poker Flat Research Range, interior Alaska.   Open circles indicate the locations of 16 10 m × 10 m plots for surveying revegetation patterns after the 2004 wildfire. North is upward on the map. The coordinates are confirmed by GoogleEarth.

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Fig. 2. Fluctuations of temperature and water content at 4, 8 and 15 cm depths in

living and dead *Sphagnum* mats. Left panels: black and grey lines show living and

dead *Sphagnum* mats, respectively. Right panels: differences between the living and

dead mats (living minus dead mats). The overall differences are confirmed statistically

by GLMM (p < 0.001). Temperature at 4 cm depth in the living moss is absent after

January 2007, because of data logger breakdown.

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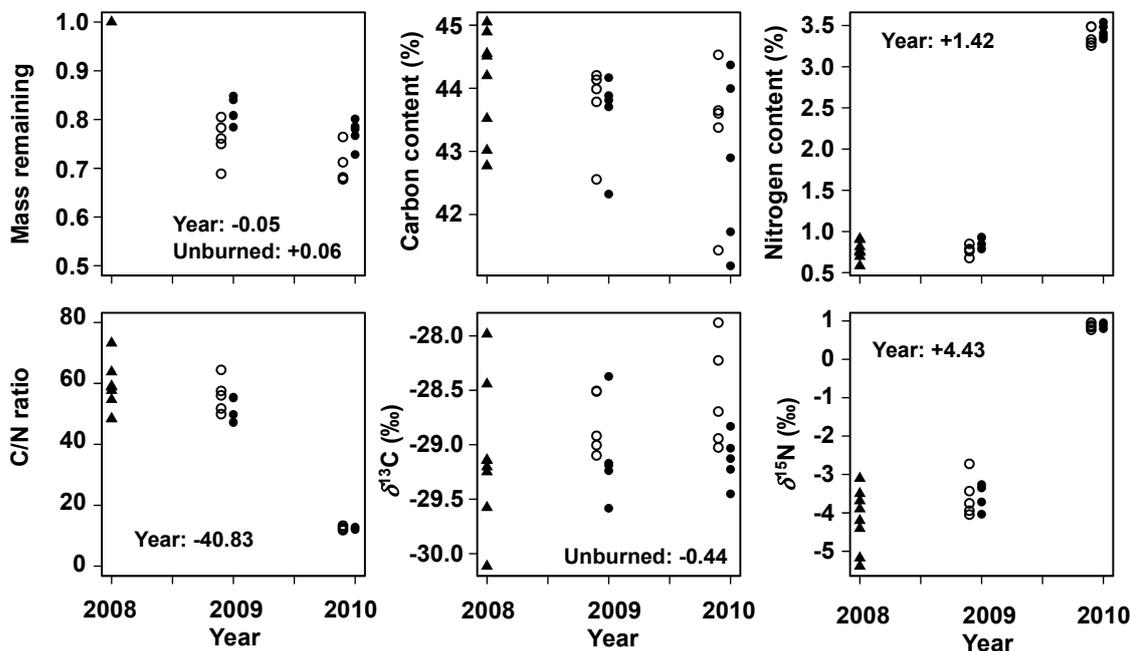
Fig 3. Yearly fluctuations of chemical properties (mass remaining, carbon content, nitrogen content, $\delta^{13}C$ and $\delta^{15}N$) in *Sphagnum* litter on the burned (open circles) and unburned (closed circles) habitats.   Triangles indicate the initial status prior to setting the litterbags up in the field.   Differences among the years and between the habitats are examined by GLM ($p < 0.01$).   The litterbags were set up in the summer of 2008.   When the estimates are significantly different, the difference in estimates is indicated in each panel.

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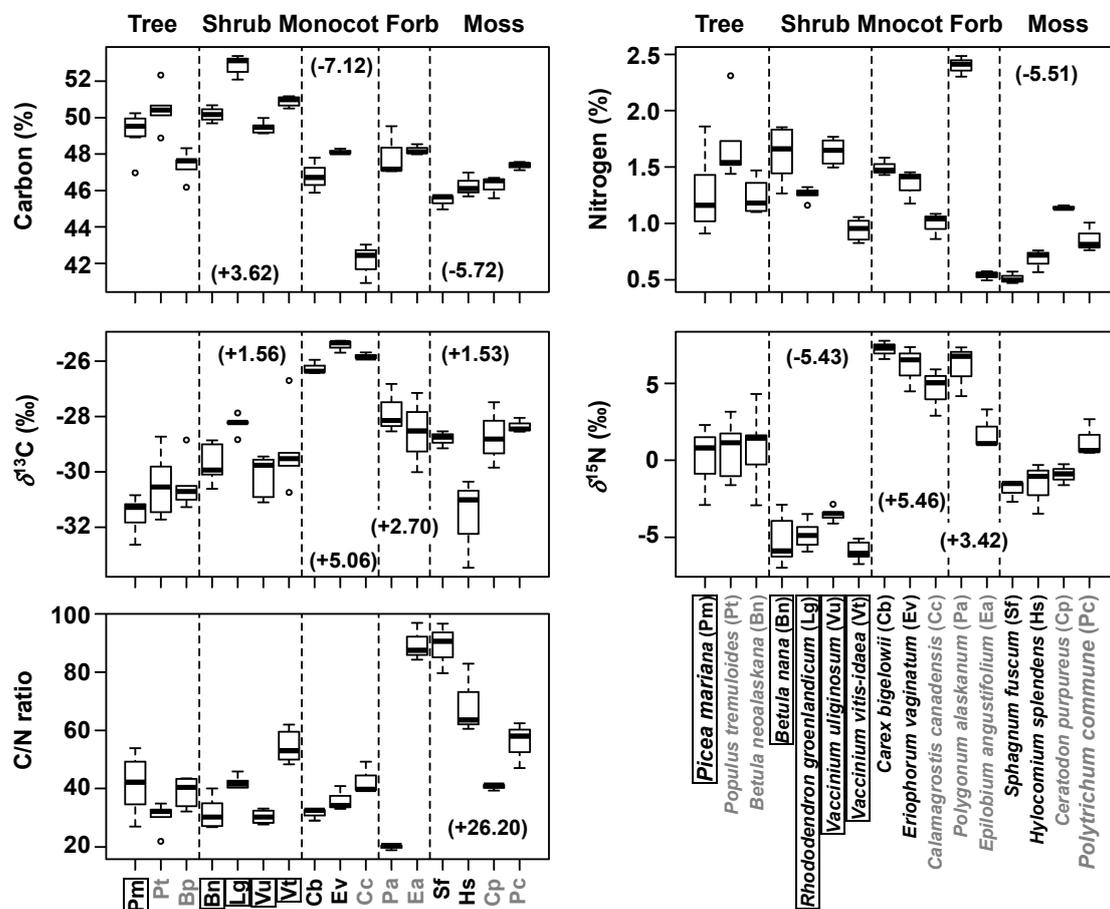
Fig. 4. Carbon content (%), nitrogen content (%), $\delta^{13}C$ (‰), $\delta^{15}N$ (‰) and C/N ratio in the leaves of 16 boreal plant species. The species are classified into five plant functional types (PFTs). Black, grey and enclosed letters indicate the samples collected from the unburned, burned and both habitats. Differences between each PFT and trees and between burned and unburned surfaces were examined by GLM ($p < 0.01$). The differences between the burned and unburned surfaces are not significant at $p > 0.05$ for all the response variables. When the estimates are significantly different, the difference in estimates is shown in the parentheses.
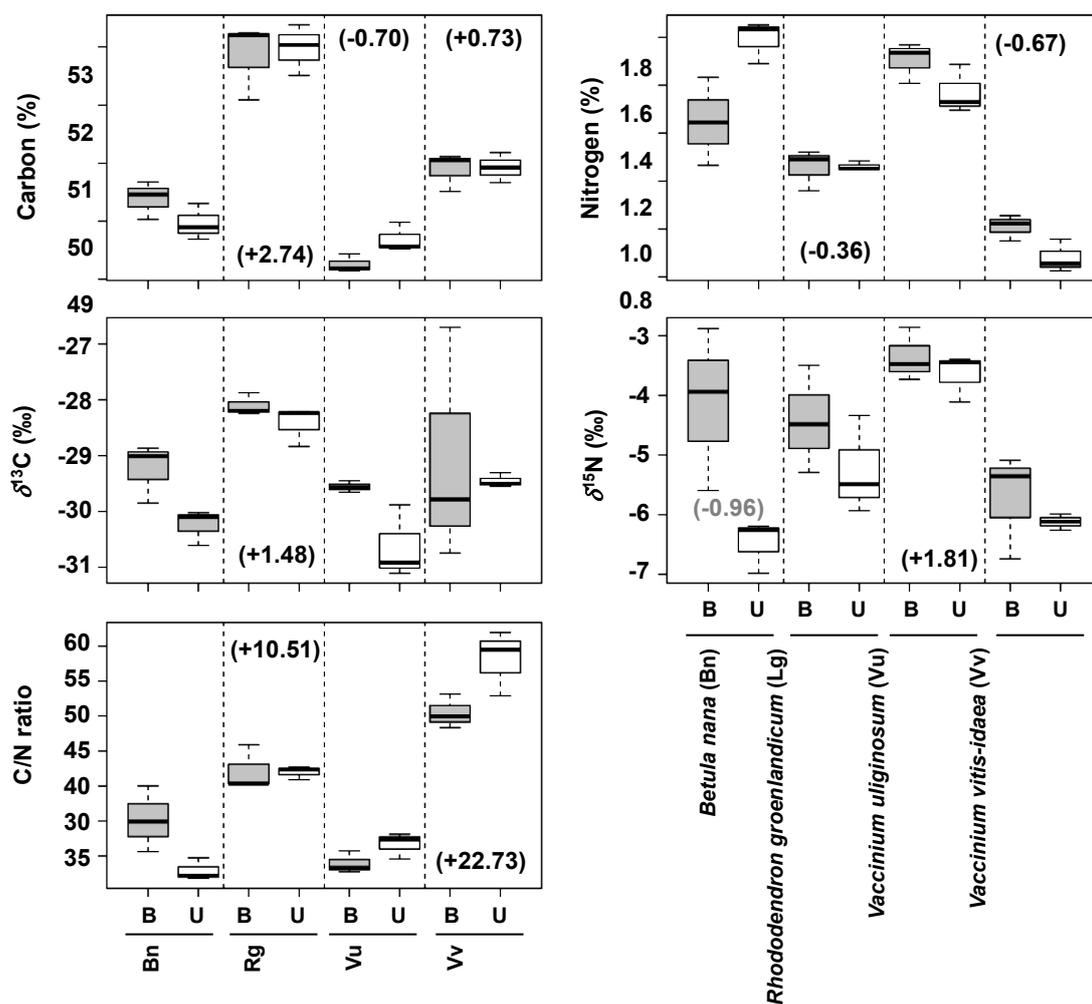
Fig. 5. Chemical properties, carbon content (%), nitrogen content (%), $\delta^{13}C$, $\delta^{15}N$ and C/N ratio in the leaves of shrubs, collected in 2014 from burned (B) and unburned (U) surfaces.　The differences between the two habitats and among species were examined by GLM ($p < 0.01$).　When the estimates are significantly different, the difference in estimates is shown in parentheses.　The comparisons by GLMs were conducted between burned to unburned habitats (shown on each species in black letters) and between *Betula nana* to the other shrub species.　See also, Fig. 2.