

1 ***Cloudberry cultivation in cutover peatland:***
2 ***improved growth on less decomposed peat***

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14 **Abbreviations:** **Fib_Fem**, female clones grown in fibric peat; **Fib_Mal**, male clones grown in
15 fibric peat; **Mes_Fem**, female clones grown in mesic peat; **Mes_Mal**, male clones grown in
16 mesic peat;

18 **Summary (200 words)**

19 Cloudberry cultivation is being seriously considered as a rehabilitation option for
20 industrial peatlands after horticultural peat extraction has ceased. Besides increasing the
21 ecological and economic values of these sites, cloudberry cultivation could improve fruit
22 yield and facilitate fruit harvesting compared to picking in natural peatlands. Previous
23 studies reported slow establishment that was tentatively associated with substrate
24 characteristics. Field and greenhouse experiments were thus conducted to better
25 characterize the impact of different peat substrates in combination with restoration
26 techniques on the growth of male and female clones. Cloudberry grew much better in less
27 decomposed fibric peat (H1-H3) than in more decomposed mesic peat. Restoring the
28 moss layer of the former peat field would thus need to precede cloudberry planting by a
29 few years, in order to plant the rhizomes in a newly formed fibric peat layer. Male clones
30 produced larger leaves and more ramets per rhizome than female clones under common
31 greenhouse conditions, which indicated that differences between sexes are most likely
32 genetic rather than environmental. Furthermore, we found cloudberry clones may be very
33 sensitive to aluminium toxicity. In conclusion, the degree of peat decomposition appears
34 to be one of the key factors determining the success of cloudberry plantations.

35 **Key words (up to 6):** Cutover peatland, substrate, peatland restoration, berry
36 production, *Rubus chamaemorus*, dioecious species.

37

38 **Short title (55 characters):** Cloudberry cultivation in cutover peatland

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40 **Résumé**

41 La culture de la chicouté est sérieusement évaluée comme une option de
42 réhabilitation des tourbières après récolte de la tourbe à des fins horticoles. Outre le gain
43 en termes de valeur écologique et économique de ces sites , la culture de la chicouté
44 pourrait augmenter le rendement en fruits et faciliter la récolte des fruits par rapport à la
45 récolte en tourbières naturelles. Des études antérieures ont montré une croissance initiale
46 lente qui a été provisoirement attribuée aux caractéristiques du substrat. Des expériences
47 sur le terrain et en serres ont donc été mises en place pour mieux caractériser l'effet de
48 différents substrats combinée aux techniques de restauration, sur la croissance des clones
49 mâles et femelles. La chicouté a présenté une meilleure croissance en tourbe fibrique
50 moins décomposée (H1-H3) qu'en tourbe mésique plus décomposée. La restauration
51 devrait donc précéder la mise en culture de la chicouté de quelques années, afin de
52 planter les rhizomes dans la couche de tourbe fibrique nouvellement accumulée. Les
53 clones mâles produisent des feuilles plus grandes et plus de ramets par rhizome que les
54 clones femelles en conditions communes de croissance. Les différences observées entre
55 les sexes sont donc d'ordre génétique plutôt qu'environnemental. De plus, nous avons
56 observé que les clones semblent particulièrement sensibles à la présence d'aluminium. En
57 conclusion, le niveau de décomposition de la tourbe apparaît comme un des facteurs
58 déterminant le succès de plantations de chicouté.

59

60 **Mots clés:** tourbière exploitée, substrat, restauration, production de petits fruits,
61 *Rubus chamaemorus*, espèce dioïque.

INTRODUCTION

62

63 Cloudberry (*Rubus chamaemorus* L.) is a circumboreal berry species, from the Rosaceae
64 family, for which there is a relatively good market in Scandinavia (Saastamoinen et al.
65 2000). An emerging market is developing in North America (Boxall et al. 2003; Centre
66 d'expertise sur les produits agroforestier 2008), the most popular products being jam,
67 jellies and liqueur. The fruit also contains secondary metabolites that can provide health
68 benefits (Thiem 2003). This species is clonal and produces extensive rhizome systems
69 that support few short shoots or ramets. Cloudberry has low fruit productivity
70 (Kortesharju 1988) and berries commercialized fresh, frozen or processed are picked in
71 natural open Sphagnum-dominated ombrotrophic peatlands (bogs). However, there are
72 fewer and fewer pickers due in part to the hostility of the habitat and the low fruit yield
73 productivity, despite being a highly priced berry (Saastamoinen et al. 2000). Growing
74 dense cloudberry cover in cutover peatland could be an interesting alternative to increase
75 fruit yield and meet market needs. It is also a sustainable rehabilitation option for
76 peatlands after horticultural peat harvesting.

77 Peatlands are now being restored in North America after peat production activities
78 have ceased (Graf et al. 2012). In areas such as the North Shore of the Saint-Lawrence
79 River (Quebec, Canada), where cloudberry is abundant and where economic context is
80 not always easy, commercially grown cloudberry seems a very sustainable rehabilitation
81 option, with or without restoration. Cutover peatland fields present a good potential for
82 cloudberry production because 1) they have few weeds (Salonen 1987), 2) they have not
83 received any pesticides or chemical fertilizers and are thus suitable for organic production

84 and, 3) they have flat fields and roads in place facilitating management and access to site
85 for pickers. All of these characteristics contribute to reducing the cost of production.

86 Cloudberry cultivation in cutover peatland has been studied in Scandinavia and North
87 America for more than ten years. Despite the knowledge gained through different
88 projects including how, when and what propagation units to plant (Bellemare et al.
89 2009a; Th roux-Rancourt et al. 2009), poor results are still observed on some cutover
90 sites. Degree of peat decomposition was suspected as a potential cause of this poor
91 productivity. Fibric peat is the recommended type of peat for cloudberry propagation in
92 beds (Rapp 2004), but the peat left after peat harvesting is usually more decomposed
93 (Graf et al. 2012). An initial greenhouse experiment was conducted over one growing
94 season, testing different degrees of peat decomposition on cloudberry growth (Th roux-
95 Rancourt 2007) and results were not conclusive. Cloudberry was also planted in cutover
96 peatlands in combination with restoration techniques i.e. spreading of sphagnum
97 diaspores covered with straw mulch (Graf et al. 2012). Th roux-Rancourt and
98 collaborators (2009) suggested that rhizomes should be planted two or three years after
99 field restoration as cloudberry growth appears to be affected by the presence of mulch
100 needed during the restoration process.

101 Cloudberry is dioecious and ramets from male and female clones have slightly
102 different morphology ( gren 1988b; Korpelainen 1994). They both carry a single flower
103 per ramet and female produces a polydrupe (Jean and Lapointe 2001). In natural
104 peatlands, the male to female ratios are generally high ( gren 1988b; Dumas and
105 Maillette 1987; Korpelainen 1994) and male ramets have larger leaves and greater
106 rhizome mass than female ramets ( gren 1988b). Repeated higher cost of reproduction

107 for female plants could, over time, induce differential mortality leading to the common
108 spatial segregation reported for perennial dioecious species, where male plants are more
109 abundant in more stressful environments than female plants (Barrett and Hough 2013).
110 However, spatial segregation between sexes has not been conclusively shown in
111 cloudberry (Dumas and Maillette 1987). Although fruit and seed production are generally
112 costly, explaining the high cost of sexual reproduction in females (Obeso 2002), pollen
113 cost may often be significant (Rameau and Gouyon 1991), reducing the difference in cost
114 production between male and female plants.

115 To better understand how the degree of peat decomposition impacts on cloudberry
116 production, we conducted two substrate experiments: 1) a field experiment in cutover
117 peatland, testing a gradient of peat decomposition and the presence of restoration prior to
118 cloudberry planting on cloudberry establishment, and 2) a greenhouse experiment testing
119 the impact of two peat substrates on the growth of male and female cloudberry clones
120 over three seasons. Based on previous results, we posit that cloudberry will perform
121 better on sites restored a few years earlier, and that less decomposed fibric peat will
122 favour growth of both male and female cloudberry compared to more decomposed mesic
123 peat. The greenhouse experiment also allowed us to determine if differences reported
124 previously between male and female clones in field experiments are still present under
125 common growth conditions.

126 **MATERIALS AND METHODS**

127 **Field Experiment**

128 The experiment was set up in 2009 in a coastal cutover ombrotrophic peatland (a former
129 raised bog) in Pointe-Lebel near Baie-Comeau (49°8'N, 68°14'W), Côte Nord region,
130 QC, Canada. Peatland was vacuum- harvested and the operations ceased some years prior
131 to planting or restoration; nevertheless the surface was still devoid of vegetation. Twenty
132 3.5 × 6 m plots were established to cover a range of increasing peat decomposition from
133 H2 to H5 on a Von Post scale (Payette and Rochefort 2001)) either restored 5 to 6 years
134 prior to planting or unrestored. Restoration was performed following the moss layer
135 transfer technique (Graf et al. 2012), spreading *Sphagnum* diaspores and straw mulch, but
136 without blocking drainage ditches. Therefore, the peat surface was not water logged
137 during the growing season.

138 Cloudberry rhizomes were harvested in October 2008, in the Pointe-Lebel peatland in
139 a sector that contains mostly female clones. Rhizomes were dug, then cut in 20 cm long
140 sections, then put in plastic bags filled with moist sphagnum. They were cold stratified at
141 4°C for three months. They were then planted in 10 × 10 cm pots filled with fibric peat
142 and grown for one season in the greenhouse (3 months), then cold stratified for 10 weeks.
143 Fifty rooted cloudberry plants were planted every 50 cm from 11 to 15 June 2009 (Year
144 1) in each plot. No fertilizer or water was applied throughout the duration of the
145 experiment.

146 Peat was sampled from 23 to 26 June 2009 in order to estimate its initial degree of
147 decomposition on the von Post scale (Payette and Rochefort 2001), bulk density (dry
148 mass of peat after 24h at 105°C per known volume) and nutrient content (see Greenhouse
149 experiment for complete description of nutrient analysis). Ramets were counted in June
150 of Year 2 to 4, while in Year 1, they were counted in July, to take account of mortality

151 following planting in June. Statistical analyses were performed using Statistix 9
152 Analytical Software (Tallahassee, FL). Pearson correlations were performed between the
153 number of ramets per plot and nutrients available in peat along with bulk density. Two-
154 sample *T* tests between restored and unrestored plots and ANOVA with polynomial
155 contrasts testing the degree of decomposition were also performed on the number of
156 ramets per plot.

157 **Greenhouse Experiment**

158 This experiment took place at Laval University from June 2010 to December 2011.
159 Both male and female rhizomes were separately planted in either fibric or mesic peat, for
160 a total of 32 containers (4 treatments, $n = 8$). Each experimental unit was
161 $40W \times 55L \times 30D$ cm deep, in which four rhizomes were planted (either male or female).
162 Peat was manually harvested from a peat pile in the field, after laboratory tests had
163 confirmed the degree of decomposition: fibric (Fib; H1 to H3 on a Von Post scale) or
164 mesic (Mes; H4 to H6). Male (Mal) and female (Fem) rhizomes, each with an apical bud,
165 were collected at flowering time in mid-June 2010 in the field at Pointe-Lebel and cut 20
166 cm long prior to being planted 5 cm deep in containers (Bellemare et al. 2009a). These
167 rhizomes were harvested in a different sector of the peatland from the one where we
168 harvested cloudberry rhizomes for the field experiment in autumn 2008. However, there
169 is no reason to believe that they differ in terms of edaphic and climatic conditions they
170 are adapted to. Containers were then transferred in greenhouses for three growing seasons
171 of 13 to 14 weeks each, at 20/15° C and 60-70 / 70% humidity (day/night), with 12 to 16
172 hours of natural light supplemented when needed with artificial light. Plants were
173 watered two to three times per week, the first season with the nutrient solution proposed

174 by Rudolph et al. (1988), and the other seasons with rain water collected from rainwater
175 harvesting tanks. Excess water was drained from the bottom of the containers, thus
176 avoiding water logging. Between each season, containers were moved to a cold room at
177 4°C for 14 (between season 1 and 2) and 20 weeks (between season 2 and 3).

178 Survival was estimated in the middle of the second growing season. It was impossible
179 to properly estimate survival in the third season because of vegetative propagation.
180 Number of ramets and leaf diagonal (an indicator of leaf size) were measured at full
181 expansion (between week 9 and 12). Percent flowering was estimated at bloom, in third
182 growing season, based on the number of ramets in flower within each container. Since we
183 noticed differences in leaf coloration, experimental units were ranked based on the
184 percentage of the leaves that were red at the end of the third season. The total number of
185 ramets produced during the third growing season was divided by four to be reported per
186 rhizome initially planted. At final harvest, at the end of the third growing season,
187 biological material within each container was sorted as rhizomes, roots, petioles and
188 leaves. Samples were oven dried at 70°C and weighed after constant dry weight has been
189 reached (48 h). Four leaf subsamples per treatment (degree of decomposition × sex) were
190 analysed for nutrients. N was extracted by Kjeldahl digest (QuickChem Method 13-107-
191 06-2-D) then quantified using flow injection analysis (FIA QuikChem4000, Lachat
192 Instruments, Loveland, CO). P, K, Ca, Mg, Cu, Fe, Mn, Zn, Al and Na were extracted
193 according to Parkinson and Allen (1975) and their concentration assessed with an
194 inductively coupled plasma optimal emission spectrometer (Optima 4300 DV ICP,
195 PerkinElmer Instruments, Norwalk, CT). Four corresponding subsamples of peat per
196 treatment were extracted following the Mehlich III method (Mehlich 1984) to quantify P,

197 K, Ca, Mg, Al, Cu, Fe, Mn, Zn, B, and Na. Soil subsamples were diluted 1:1 in water to
198 measure pH. Statistical analyses were performed using Statistix 9 Analytical Software
199 (Tallahassee, FL): degree of decomposition and sex were used as fixed factor in the
200 ANOVA and protected LSD comparisons.

201

RESULTS

202 **Field Experiment**

203 Soil properties in the different sites varied in terms of degree of decomposition on the
204 Von Post scale (H2 to H5), but also in terms of bulk density (mean: 0.078 g cm⁻³; range:
205 0.056 to 0.128 g cm⁻³), and pH (mean: 3.75; range: 3.4 to 4.1). Survival during Year 1
206 varied greatly among plots with a 52 to 98% survival. Five weeks after planting,
207 cloudberry establishment was independent of restoration application (two-sample *T* test;
208 *P* = 0.26), degree of peat decomposition (ANOVA, *P* = 0.80), bulk density (correlation, *P*
209 = 0.40), pH (correlation, *P* = 0.79), or nutrients (correlations). No difference in number of
210 ramets per plot was detected in the three following years (2 to 4) either as a function of
211 restoration application, bulk density, pH, or nutrient availability. The number of ramets
212 decreased with an increase in the degree of peat decomposition but only in the second
213 year (*P* = 0.03; Fig. 1; note that restored and unrestored plots were pooled to test for the
214 degree of decomposition). Four years after planting, ramet density was still very low and
215 the density even dropped below the initial density at planting, with an average of 46
216 ramets per plot. Nevertheless, two plots had 4 times more ramets than the average, both
217 in unrestored, weakly decomposed peat (H2 and H3), with a bulk density of 0.058 and
218 0.072 g cm⁻³, and a pH of 3.9. Yet, an ANOVA performed on unrestored plots only, with

219 degree of peat decomposition as fixed factor, did not indicate statistical differences in the
220 number of ramets per plot in Year 4 ($F = 0.68$, $P = 0.54$).

221 **Greenhouse Experiment**

222 *Vegetative Growth and Flowering*

223 In the second growing season, survival was good (93%) as only 9 out of the 128 rhizomes
224 had died: 1 in Fib_Fem, 7 in Mes_Fem and 1 in Mes_Mal. Male clones grew bigger than
225 female ones (Table 1; Fig. 2). Each male rhizome initially planted produced 34% more
226 ramets compared to females after three growing seasons with, on average, the same
227 number of leaves per ramet. Male clones also produced leaves that were 11% larger
228 compared to female clones. Percent flowering was similar for both sexes. Biomass
229 produced by male clones was higher than for female clones except for leaf biomass and
230 consequently for total aerial biomass in mesic peat, where male and female clones did not
231 differ, and produced on average, only 0.25 ± 0.4 g of aerial tissues per container (Fig. 3).

232 Cloudberry grew better, and consequently produced more flowering ramets, in fibric
233 than in mesic peat (Table 1; Fig. 4). Cloudberry clones grown in fibric peat produced five
234 times more ramets than in mesic peat (Fig. 5): respectively 6.2 compared to 1.2 ramets
235 produced per rhizome initially planted. Each of the ramets produced in fibric peat had on
236 average two leaves, and each leaf had an area 3 to 4 times larger (diagonal of 5.3 cm)
237 than the single leaf produced on average by ramets in mesic peat (2.5 cm). Plants
238 produced more leaf, petiole, rhizome and root biomass in fibric peat than in mesic peat
239 (Table 1; Fig. 3). Belowground/aerial biomass ratio was decreased by growing plants in
240 fibric rather than in mesic peat. Twice the proportion of ramets flowered in fibric (44%)

241 compared to mesic (19%) peat. As expected, no fruit was produced in greenhouse, due to
242 the lack of pollination (Pelletier et al. 2001).

243 *Nutrients*

244 Male and female leaves differed in their mineral composition for seven out of 11
245 elements tested (Table 2, Fig. 6). Female leaves had higher concentrations of nitrogen
246 and lower concentrations of sodium and zinc than male leaves. Female leaves also
247 exhibited higher concentrations of phosphorus and lower concentrations of calcium and
248 magnesium than male leaves, but only for plants grown in fibric peat (Table 2). This led
249 to reduced N:P ratios in female compared to male plants grown in fibric peat and to
250 increased N:P ratios in female compared to male plants grown in mesic peat.

251 Nutrient profile of cloudberry was clearly different after three years of growth on the
252 two different peat substrates. Differences were recorded for all nutrients, except for Cu
253 (Table 2; Fig. 6). Nitrogen (which is not presented; Fib_Fem : $21.4 \pm 1.0 \times 10^3$ (SE) $\mu\text{g g}^{-1}$,
254 Fib_Mal : $16.8 \pm 1.4 \times 10^3 \mu\text{g g}^{-1}$, Mes_Fem : $13.0 \pm 0.9 \times 10^3 \mu\text{g g}^{-1}$, Mes_Mal :
255 $9.8 \pm 0.7 \times 10^3 \mu\text{g g}^{-1}$), phosphorus, calcium, magnesium, iron, manganese and zinc foliar
256 concentrations were all increased when plants were grown in fibric peat compared to
257 those grown in mesic peat. For calcium and magnesium, foliar concentration was
258 increased after three years of growth in fibric peat, even though concentration of these
259 nutrients was clearly lower in fibric than in mesic peat. In contrast, potassium, aluminium
260 and sodium concentrations were reduced in leaves of cloudberry grown in fibric peat
261 compared to those grown in mesic peat, even though there were more of those nutrients
262 in fibric compared to mesic peat at the end of the experiment. Leaf N:P ratio was lower in

263 fibric peat (11.6 ± 0.6 (SE) for female and 14.7 ± 1.3 for male) than in mesic peat
264 (25.2 ± 1.2 for female and 21.6 ± 2.6 for male). After the third growing season, pH was
265 on average 3.40 ± 0.03 (SE) in fibric peat and 4.20 ± 0.02 in mesic peat ($F = 489.8$, $P <$
266 0.001).

267 Plants grown in mesic peat had more leaves colored in red at the end of the third
268 season than plants grown in fibric peat. After ranking the 32 units, average rank was 28
269 for male clones grown in mesic peat, which was higher than for female clones in mesic
270 peat (18), which was also higher than for both female (11) and male (9) clones grown in
271 fibric peat.

272

273

DISCUSSION

274 **Effect of Substrate**

275 To better understand how the degree of peat decomposition impacts on cloudberry
276 production, we conducted two substrate experiments, one in the field and one under
277 controlled conditions in a greenhouse. Even if the degree of peat decomposition had no
278 significant effect on cloudberry propagation in the field experiment, the greenhouse
279 experiment showed contrasting results consistent with our hypotheses. Indeed, cloudberry
280 grew bigger and produced much more ramets and flowers in less decomposed peat
281 (fibric) than in more decomposed one (mesic). In mesic peat, growth was so low that it
282 was equivalent to virtually no propagation: having a single ramet per initial rhizome
283 planted following three growing seasons is not sustainable. Ramets flowered more

284 frequently in fibric than in mesic peat, which is consistent with the generally better
285 healthiness and larger plant size of the cloudberry grown in fibric peat. These results are
286 also consistent with a larger scale experiment where rooted ramets were planted in fibric
287 peat in unrestored sites, and the number of ramets doubled every year at least up to 7
288 years after the planting (J. Boulanger-Pelletier, unpublished data). The degree of peat
289 decomposition appears to be one of the key factors in the planting success of cloudberry.
290 Less decomposed fibric peat presents better physical properties for plants grown in
291 containers than more decomposed mesic peat (Caron and Rivière 2003). Mesic peat has
292 better water retention capacity which in some cases can be deleterious to plant growth, as
293 it can reduce soil aeration. However, in both field and greenhouse experiments, drainage
294 was sufficient to avoid water logging conditions. The large difference between
295 cloudberry clones grown in the two types of peat is probably also due to differences in
296 chemical properties. Leaf nitrogen and phosphorus concentration of plants grown in fibric
297 peat was in the range reported from natural sites (Ågren 1987; Hébert-Gentile et al.
298 2011). The reduced leaf nitrogen and phosphorus concentrations observed in plants
299 grown in mesic peat could explain their high production of anthocyanins, which is a
300 typical symptom of deficiencies of these nutrients (Close and Beadle 2003). Furthermore,
301 female plants had on average higher nitrogen and phosphorus concentration in their leaf
302 tissue than male plants in mesic peat, and they also had on average diminished percentage
303 of red tissue in their leaves. Leaf N:P ratios in fibric peat (12 – 15) suggest that both N
304 and P co-limit plant growth whereas in mesic peat, N:P ratios were strongly biased in
305 favour of N (22 – 25), suggesting significant P limitation (Aerts and Chapin 2006).
306 Previous studies on natural sites in Europe have reported slight to intermediate N

307 limitation in cloudberry based on leaf N:P ratios (N:P ratios of 7 – 14 (Ågren 1988b;
308 Marks and Taylor 1972;; Sæbø 1968; 1970), whereas in Canada, it appears that the two
309 nutrients are co-limiting (Hébert-Gentile et al. 2011). Concentrations of the other
310 nutrients were in the same range as those reported previously (Hébert-Gentile et al.
311 2011), except for calcium and manganese which were much higher, and iron and zinc
312 which were lower in the present study. Lower iron and zinc concentrations could partially
313 explain enhanced manganese absorption (Thomine et al. 2003).

314 One of the potential explanations for the poor performance of cloudberry in mesic peat
315 may be the toxic effect of an accumulation of aluminium in plants already deficient in
316 some nutrients. Indeed, Parent and collaborators (2013) concluded that an excess of Al
317 and S in female cloudberry leaves is associated with low fruit yield in natural habitats.
318 Concentration of aluminium in leaves of cloudberry grown in mesic peat was on average
319 more than twice that recorded in leaves of plants grown in fibric peat or in natural stands.
320 However, it is worth mentioning that cloudberry leaf aluminium concentrations are much
321 lower than those recorded in plants grown in acidic mineral soils where soluble
322 aluminium is readily available (Jansen et al. 2002). The pH data confirmed that
323 cloudberry is very well adapted to low pH as it exhibited much better growth in the more
324 acidic fibric peat than in the lesser acidic mesic peat.

325 **Effect of Restoration in Cutover Peatlands**

326 Contrary to our prediction, planting five years after restoration was not better than
327 planting on unrestored sites. Nevertheless, two plots performed well: both in weakly
328 decomposed unrestored peat. Cloudberry might require a thick layer of newly

329 accumulated fibric peat layer to survive and propagate, which would require planting
330 rhizomes 15 to 20 years after restoration took place (Lucchese et al. 2010). This would be
331 the closest thing to natural habitat. Overall, the initial survival in the field experiment was
332 good (36 to 90%) compared to what has been reported in previous work (Th eroux-
333 Rancourt et al. 2009). Nevertheless, the technique needs further improvements since the
334 number of ramets dropped with time, despite potential vegetative propagation. The poor
335 survival (around 20%) reported by Th eroux-Rancourt and collaborators (2009) was
336 independent of the treatment (unrestored vs. restored a few days prior to cloudberry
337 planting) and also decreased with time. They suspected that the combination of deep
338 planting (10 cm) and application of restoration technique was responsible for the high
339 mortality and low propagation rate. Rhizomes planted deeper might exhaust their reserves
340 before buds reach the ground, whereas the presence of a straw mulch in restored plots
341 might have maintained cold soil temperature in spring (Price et al. 1998), slowing plant
342 growth each year. Although these two factors most likely explain the difference in
343 mortality rates observed between the two studies, the degree of peat decomposition
344 appears to strongly influence the capacity of cloudberry to get established in abandoned
345 peatlands.

346 **Performance of Male and Female Clones**

347 The better growth of male compared to female in greenhouse is consistent with previous
348 work ( gren 1988b). The higher number of male ramets produced is also consistent with
349 the male biased ratio generally observed between flowering ramets in the field (Dumas
350 and Maillette 1987; Karst et al. 2008; Korpelainen 1994). Male clones would produce
351 more ramets which, with time, would translate into a biased ratio in favour of males. A

352 1:10 male to female ratio at planting was suggested as being sufficient to insure efficient
353 pollination. We also recommend this ratio in order to avoid an overabundance of male
354 ramets after 15 or 20 years of cultivation. The proportion of flowering ramets was the
355 same in male and female clones in the present study. In natural habitat, Ågren (1988b)
356 reported higher proportion of flowering ramets in male compared to female clones, but
357 this was estimated at a single site. Better growing conditions in the greenhouse than in the
358 field most likely explain the high flowering capacity reported here compared to what has
359 been reported in natural peatlands (Ågren 1988a; Bellemare et al. 2009b); good growing
360 conditions could mask potential male-female differences in terms of flowering capacity.

361 Female clones had on average smaller leaves than male clones. Ågren (1988b)
362 concluded that the smaller leaves of female clones in cloudberry was due to the
363 investment in fruits, but the present study indicated that the leaf size difference was
364 present even in the absence of fruit production. Furthermore, flower investment during
365 flowering is more demanding in cloudberry male than in female ramets because of pollen
366 production (Rameau and Gouyon 1991). Nitrogen and phosphorus were higher in non-
367 fruiting female than in male leaves (for phosphorus, differences are significant only in
368 fibric peat), whereas no difference in terms of nitrogen and phosphorus concentration
369 between leaves of female fruiting ramets and of male ramets has been reported from a
370 field study (Ågren 1988b). Female ramets might require more nutrients in order to
371 flower, considering that part of the nitrogen and phosphorus appears to be translocated to
372 the developing fruit.

373 **Conclusion**

374 The present data clearly demonstrates that cloudberry should be planted on fibric (H1
375 to H3) rather than on mesic peat. A low proportion of former cutover peatlands present
376 weakly decomposed peat (Graf et al. 2012). Future research should thus focus on the
377 possibility to plant cloudberry on restored sites that have accumulated a significant moss
378 layer in order to maximise both restored areas in cutover peatlands and cloudberry
379 cultivation success. Field and greenhouse experiments confirmed the necessity of
380 conducting experiments on cloudberry over more than one growing season due to the
381 slow growth of the species. Further studies should also address aluminium assimilation
382 and toxicity in cloudberry as the plant appears to be very sensitive to this element.
383 Greenhouse experiments confirmed that male clones produce larger leaves and more
384 ramets than female clones. The proportion of male to female clones at planting should be
385 low in order to avoid a disproportion of male clones after many years of cultivation.

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500 **Table 1. Analysis of variance testing the effect of the degree of decomposition and sex of the clones on vegetative growth,**
 501 **flowering and dry biomass of aerial and belowground tissues of cloudberry after three growing seasons in greenhouse**

Variable	Degree of decomposition		Sex		Degree of decomposition × Sex	
	<i>F</i> ^z	<i>P</i>	<i>F</i> ^z	<i>P</i>	<i>F</i> ^z	<i>P</i>
Number of ramets / rhizome planted ^y	114.3	< 0.001	8.3	0.008	0.6	0.43
Number of leaves / ramet	236.1	< 0.001	0.5	0.51	0.5	0.49
Individual leaf diagonal (cm)	292.4	< 0.001	5.8	0.02	1.0	0.33
Percent flowering per container	17.7	< 0.001	0.3	0.58	0.1	0.81
Aerial biomass ^x	615.1	< 0.001	19.3	< 0.001	6.0	0.02^w
Leaf biomass ^x	572.5	< 0.001	17.9	< 0.001	6.3	0.02^w
Petiole biomass ^y	144.3	< 0.001	18.6	< 0.001	1.3	0.3
Belowground biomass ^x	280.5	< 0.001	23.4	< 0.001	0.2	0.7
Rhizome biomass ^x	171.1	< 0.001	31.3	< 0.001	0.7	0.4
Root biomass ^x	368.4	< 0.001	8.1	0.008	1.5	0.2
Belowground/aerial biomass ^x	252.7	< 0.001	0.8	0.4	0.0	1.0

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504 ^zDf is 1 for degree of decomposition, 1 for sex, 1 for the interactive term degree of decomposition × sex and 28 for error except for
 505 aerial and belowground/aerial biomass variables for which df_{error} is 27.

506 ^y rank transformed

507 ^x log transformed; all biomass were estimated on a per plant basis

508 ^w LSD comparisons for interactions for aerial biomass and for leaf biomass is: Fib_Mal a, Fib_Fem b, Mes_Mal c and Mes_Fem c.

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512 **Table 2. Analysis of variance testing the degree of decomposition and sex effects on cloudberry leaf nutrient concentration and**
 513 **leaf red coloration after three growing seasons in greenhouse**

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Variables	Degree of decomposition		Sex		Degree of decomposition × Sex		LSD comparisons for interactions
	<i>F</i> ^z	<i>P</i>	<i>F</i> ^z	<i>P</i>	<i>F</i> ^z	<i>P</i>	
N	55.8	< 0.001	14.4	0.003	0.55	0.47	
P ^y	203.8	< 0.001	15.8	0.002	5.8	0.03	Fib_Fem a Fib_Mal b Mes_Fem c Mes_Mal c
K	20.2	< 0.001	0.1	0.76	1.2	0.28	
Ca	59.4	< 0.001	0.4	0.54	9.5	0.01	Fib_Mal a Fib_Fem b Mes_Fem c Mes_Mal c
Mg	126.0	< 0.001	8.0	0.02	11.6	0.005	Fib_Mal a Fib_Fem b Mes_Fem c Mes_Mal c
Cu	0.2	0.67	3.6	0.08	6.1	0.03	Fib_Mal a

							Mes_Fem	ab
							Mes_Mal	ab
							Fib_Fem	b
Al	17.6	0.001	0.1	0.77	4.0	0.07		
Fe ^x	17.2	0.001	3.5	0.09	3.2	0.10		
Mn	55.1	< 0.001	1.6	0.23	0.5	0.51		
Na	12.1	0.005	14.6	0.003	0.04	0.84		
Zn	92.5	< 0.001	5.9	0.03	0.02	0.89		
N : P ratio	149.0	< 0.001	0.1	0.79	15.7	0.002	Mes_Fem	a
							Mes_Mal	b
							Fib_Mal	c
							Fib_Fem	d
Leaf red coloration ^w	39.2	< 0.001	2.8	0.10	9.1	0.006	Mes_Mal	a
							Mes_Fem	b
							Fib_Fem	c
							Feb_Mal	c

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516 ^z Df is 1 for degree of decomposition, 1 for sex, 1 for degree of decomposition × sex and 12 for error.

517 ^y log transformed

518 ^x rank transformed

519 ^w based on ranked data

521

522 **Figure legends**

523 **Fig. 1.** Number of ramets per plot produced during four years in restored and unrestored
524 cutover peatland as a function of the degree of peat decomposition. Fifty rooted
525 cloudberry plants were initially planted per plot in Year 1. Note that there was no H5 in
526 unrestored plots.

527 **Fig. 2.** Vegetative growth and flowering of male and female cloudberry grown in
528 greenhouse. Data (mean \pm SE) are combined results of plants grown in fibric and mesic
529 peat after three growing seasons. As each ramet bears a single flower, the percentage of
530 flowering reflects the number of flowering / total number of ramets per container.

531 **Fig. 3.** Dry biomass of aerial and belowground tissues produced by surviving female
532 (Fem) and male (Mal) clones of cloudberry grown in fibric (Fib) and mesic (Mes) peat
533 for three seasons in greenhouse. Four rhizomes were initially planted per container.
534 Survival was 97% for Fib_Fem, 100% for Fib_Mal, 78% for Mes_Fem, and 97% for
535 Mes_Mal after two growing seasons.

536 **Fig. 4.** Cloudberry grown for three seasons in greenhouse. Upper six plates are plants
537 grown in mesic peat (A) and lower six plates in fibric peat (B). Four rhizomes were
538 initially planted in each container.

539 **Fig. 5.** Vegetative growth and flowering of cloudberry grown in fibric and mesic peat in
540 greenhouse. Data (mean \pm SE) are combined results of male and female clones after three

541 growing seasons. As each ramet bears a single flower, the percentage of flowering
542 reflects the number of flowering / total number of ramets per container.

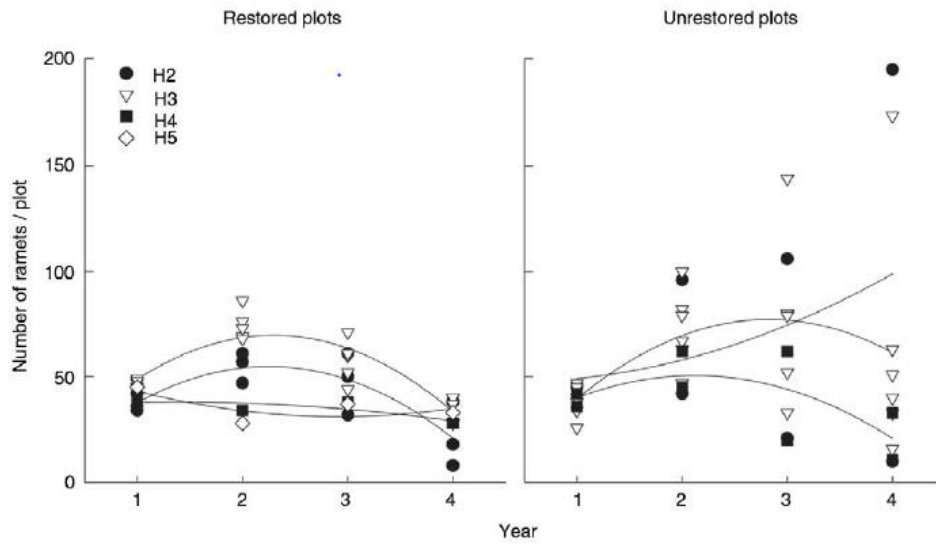
543 **Fig. 6.** Soil and foliar concentration of phosphorus, potassium, calcium, magnesium,
544 manganese, copper, iron, zinc, aluminium, and sodium for male (Mal) and female (Fem)
545 clones of cloudberry grown for three seasons in fibric (Fib) and mesic (Mes) peat in
546 greenhouse.

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549 Figure 1

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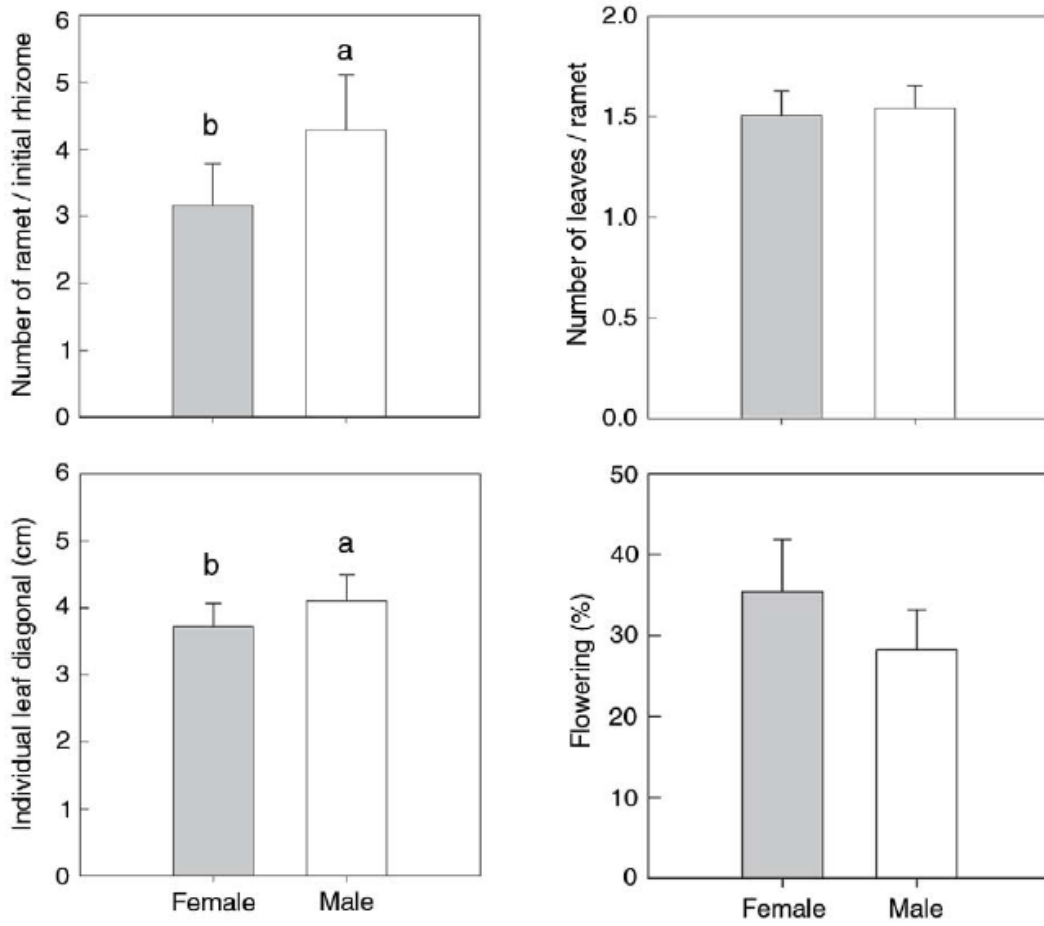
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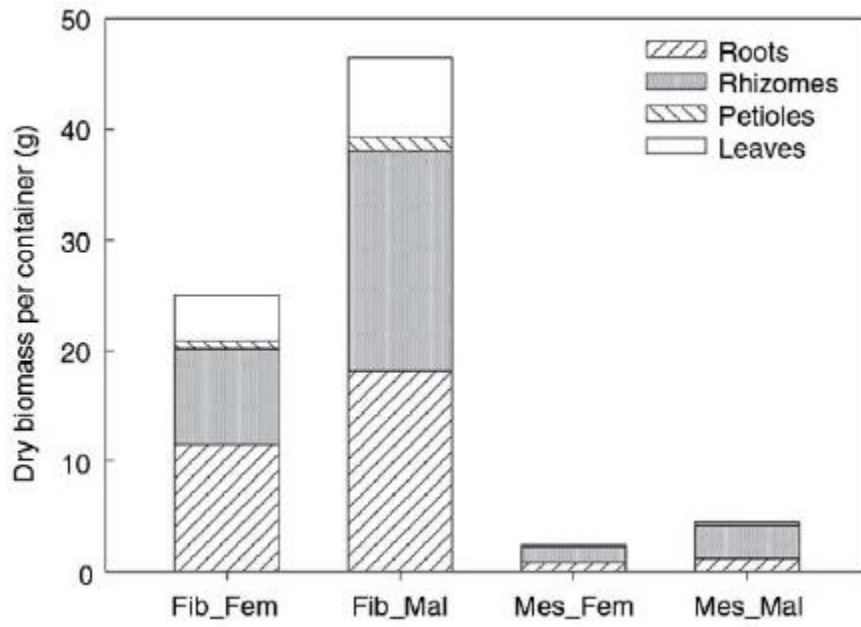


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559 Figure 3

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570 Figure 4

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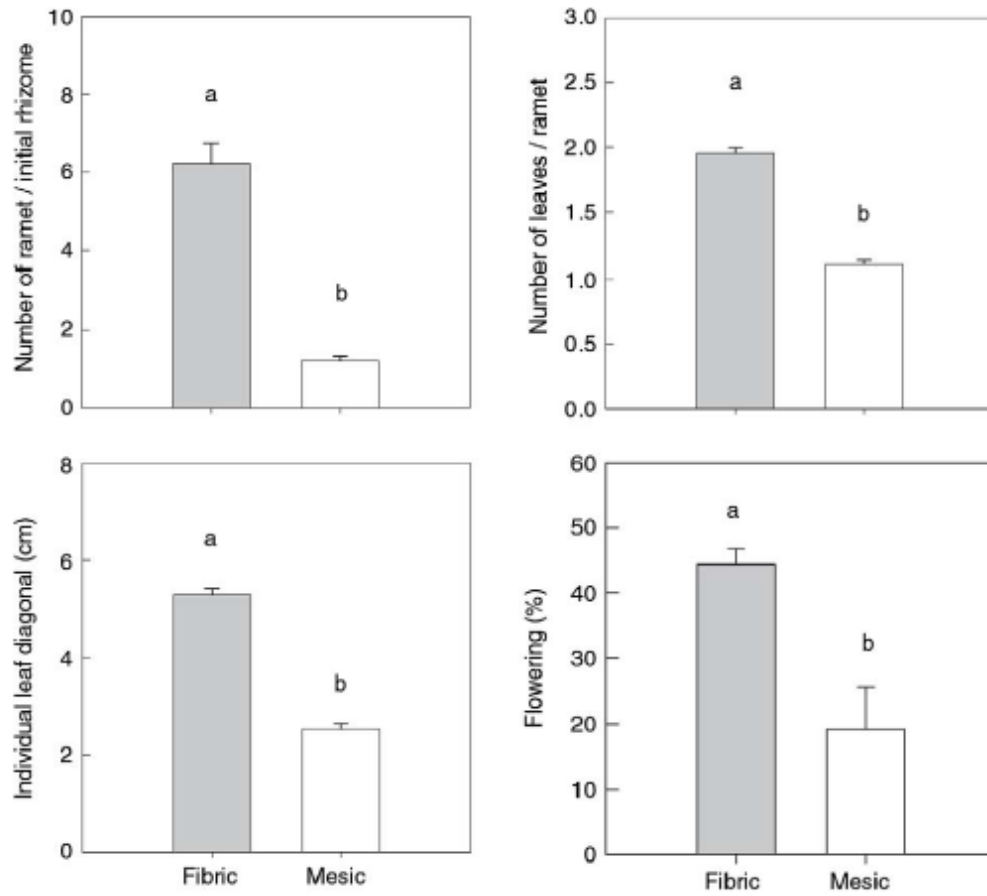


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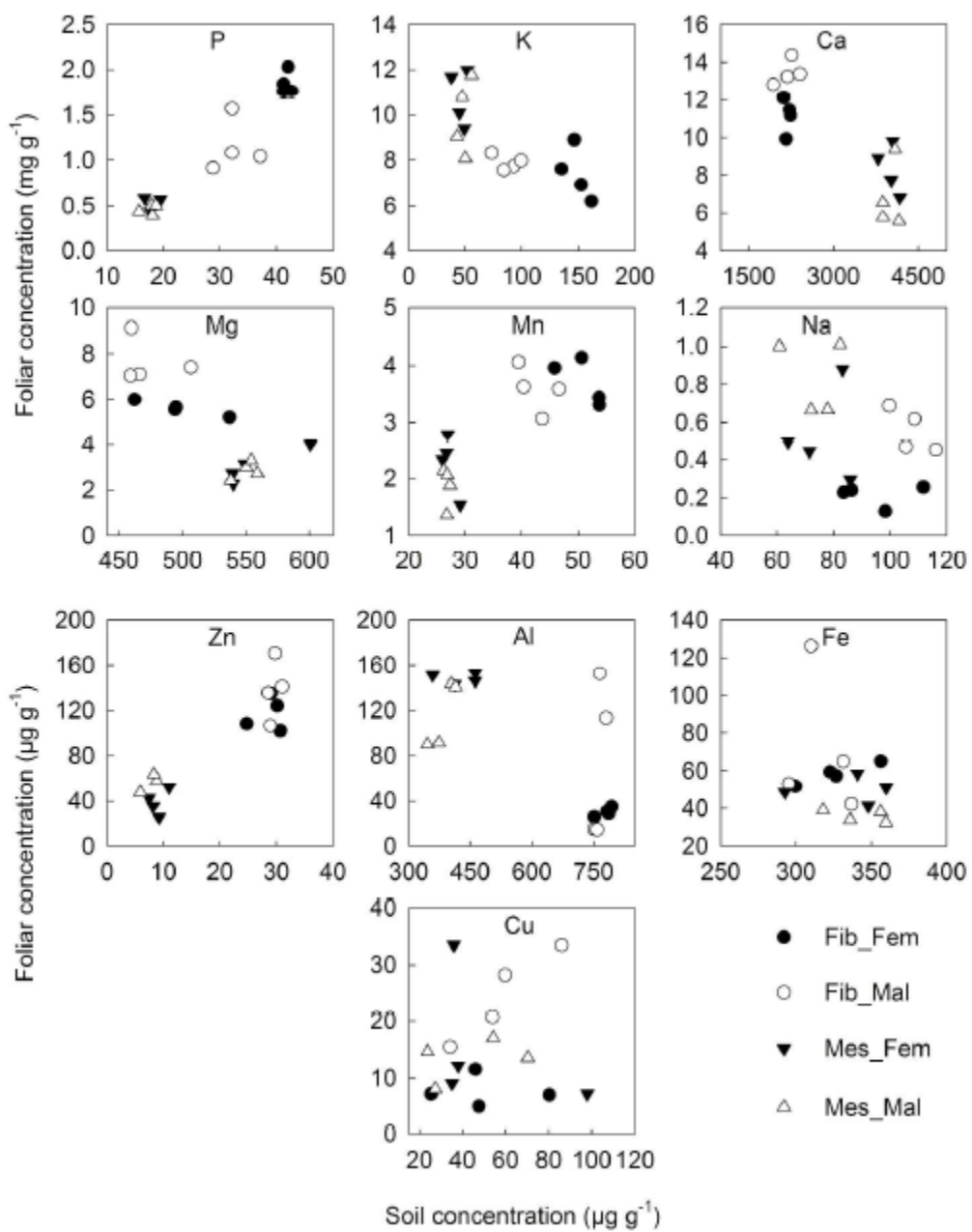
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577 Figure 6

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