

Author replies to comments on “soild-1-267-2014”

Page 269, Lines 14-18, I think it is important to acknowledge here that the idea of organic matter composition/chemistry controlling decomposition (esp. in the long-term) is currently challenged in the literature (ex. see Schmidt et al. 2011, Nature).

Ok, we changed (see P. 3, L. 51-53 of the revised Word file) to: “The current common belief is that environmental and biological factors predominate on the molecular structure in controlling SOM stability (Kleber, 2010; Kleber et al., 2011; Schmidt et al., 2011); however, in some environments, vegetation cover is a good proxy for soil C dynamics, since it controls the input and quality of litter (De Deyn et al., 2008).”

Page 269, line 17: Please replace humification with 'decomposition', 'transformation' or 'processing' (see comment above).

“Humification processes” were replaced by “SOM transformation” (see P. 3, L. 55 of the revised Word file).

Page 271, lines 1-4: here and later in discussion you have to address how measuring changes in rate of decomposition (and chemistry of decomposing litter) over 1 yr will give reliable information of SOM quality and dynamics? This is important in order to establish a logical link between the short-term dynamics and long-term dynamics when it comes to amount, composition, and decomposability of the litter that grows and decomposes on the surface, and the portion of the litter that actually enters the soil organic matter pool (even in the near-surface soil layers).

More prudentially, now we inserted the terms “initial” or “early stages of” before litter decomposition rate. Furthermore, hereafter we clearly specified that our short-term study only provides indications (and nothing more) on the long-term fate of SOM.

Page 273, line 4: please include information on the type of filter was used for filtration of dissolved OM.

The used filter was a 0.45 µm membrane filter (Millipore). This is now included in the text, here and some lines above (see P. 7, L. 163 and 175 of the revised Word file).

Page 275, lines 15-18: was there any soil material that couldn't be cleaned (i.e. was too strongly attached to the remaining litter)? If so, how was it handled?

No, everything was quite easy to remove from the bags by using a soft brush. Such information is now included in the text (see P. 9, L. 228 of the revised Word file).

276, line 6: Please replace 'intrinsic recalcitrance' (see comment above and Kleber 2010, Environmental Chemistry in addition to Schmidt et al 2011), possibly you can say 'short-term resistance to decomposition'?

41 We understand this criticism, which is in line with the first one. In that case we
42 acknowledged that the persistence of soil organic matter is partly an ecosystem property and
43 in fact we quoted the paper by Schmidt et al. 2011 published in Nature, as the Reviewer
44 suggested, and two valuable others. However, here we just wanted to say “intrinsic
45 recalcitrance”, which means the ability to resist degradation due to its chemical nature.
46 Recent findings and new concepts cannot lead to deny that a simple sugar is less resistant to
47 degradation than lignin and that high C/N ratios are symptomatic of more difficult
48 degradation. Anyway, the sentence in question, which is not a result, was deleted.
49 Additionally, please note that after your comment, at P. 15, L. 413 we changed “...intrinsic
50 resistance of Sphagnum” to “...short-term resistance of Sphagnum to degradation”.

51

52 *276, line 6-9: this statement is not supported by any data or conclusive evidence and should*
53 *be taken out.*

54 Ok, we removed this sentence.

55

56 *Table 1: please mark which of the differences in means are statistically significant. Does the*
57 *statement in line 15-17, page 276 suggest that all the values are insignificant or just the C:N*
58 *ratio?*

59 Correct comment: this part was in fact unclear. Significant differences between the plant
60 species with respect to C, N and C/N ratios have been included in Table 1 and the
61 reorganized text to account for significant differences only (see P. 10 in the Results of the
62 revised Word file).

63

64 *Please revise the statement in Page 278, lines 26-28, what does 'side-effect' refer to?*

65 The term we used is “site” effect, not “side” effect. It is largely used in the literature and we
66 think it does not require further explanation in the text.

67

68 *Pages 280 (last line) -281 line 1, alkyl/O-alkyl ratio as per Baldock and co-workers and*
69 *others is an index of SOM transformation/processing, not 'recalcitrance' per se (even though*
70 *in some cases these two can be related). Please either provide a correlation coefficient or at*
71 *least prior reference to support this statement.*

72 Actually, the Reviewer is right in saying it. We adjusted this sentence accordingly, based also
73 on the enlightening papers by Kleber and co-authors now cited in the manuscript.

74

75 *Page 281, lines 5-8: this statement can also be valid only if supported by a qualifying*
76 *statement as to the short-term (1yr) nature of this study and findings there in. It is possible*
77 *that after a year (possibly after a year of processing by meso- and macroorganisms), it could*
78 *be rendered more decomposable.*

79 To be honest, everything is possible after the period we investigated, i.e. after 1yr. However,
80 we do not like to make any hypothesis beyond the monitored time lapse. Also, we do not feel
81 the necessity to further specify, here, what requested by the Reviewer. In fact, in the
82 statement in question we were talking about the chemical capacity of the material in the

83 litterbags to resist decomposition (of course, up to the time when we removed it from the
84 ground for analysis).

85

86 *Page 283, line 1, please consider revising 'parent vegetation'*

87 Ok, "...quality of the parent vegetation" was changed to "...litter quality" (see P. 16, L. 487
88 of the revised Word file).

89

90 *Page 283, line 2: please revise 'refractory' (see comment above)*

91 The term "early stage of" decomposition was actually included in this conclusion, to clarify
92 that it just refers to a short-term fate. Moreover, we replaced the term "refractory" with the
93 more comprehensive and less compromising "stable", which refers to the capacity to resist
94 degradation not only due to the intrinsic molecular recalcitrance.

95

96 *Page 283, line 7: please revise 'higher concentration of rainfall' ... do you mean higher
97 amount or distribution of rainfall?*

98 Such a long and a bit confused sentence was changed to "...assessing the consequences of
99 climate change on SOM stocks and dynamics".

100

101 *Page 283, line 9: please note that your study can only suggest this conclusion for short-term.
102 You haven't presented any model fitting (or other approaches) to demonstrate what can be
103 expected in the long-term.*

104 Right. The "short-term" perspective of our study was acknowledged.

105

106 *Other general comments:*

107 *Please address how use of litterbags could have affected your results. This is important
108 because several studies have shown there is a significant effect of litterbags in affecting rate
109 of organic substrate decomposition and chemistry of decomposing litter, in addition the effect
110 of litterbags is also modulated by soil hydrologic conditions (ex. see Berhe 2013, Journal of
111 Soils and Sediments).*

112 We honestly admitted, at P. 16, L 464-467, what types of bias from using litterbags could
113 have affected our results. The enlightening paper by Berhe was of course quoted.
114 Nevertheless, we may assume that such a bagging effect in our experiment is uniform
115 through the samples, also because the bags are very similar.

116

117 *Please include error bars in Figures 3 and 4.*

118 We included error bars in Figure 3, but preferred to not do the same with Figure 4, because
119 otherwise it would become unreadable. On the other hand, there are papers where quite
120 similar patterns are devoid of error bars (e.g., Rezig et al., 2014. International Journal of
121 Recycling of Organic Waste in Agriculture, 3: 1-9).

122

123 *I think it is important to fit the litter decomposition data with one or two pool decomposition*
124 *models to derive important indices (ex. long-term rate of decomposition) that would make*
125 *findings of this study even more significant and easier to interpret. Please see works of Mark*
126 *Harmon and co-workers (1992, 1999, 2009) as a reference for this.*

127 We read the suggested papers by Harmon and co-workers and some others dealing with long-
128 term patterns of mass loss during litter decomposition. Nevertheless, we feel that one year of
129 investigation is not enough to reveal dynamics in the later phases. As a consequence, we
130 prefer to avoid using decomposition models and making inferences concerning the long-term
131 fate of litter. Actually, what we were interested to in this paper was to check the site- and
132 litter-quality effects in the early stage of decomposition.

133

134 *RE: Author responses to reviewer comments*

135 *The authors are strongly encouraged to address EACH comment (separated by start of the*
136 *new line, or page and line numbers proceeding comments) raised by the reviewers*
137 *individually. Please add your response to each comment in bold, italic, indented (or other*
138 *format) text for the sake of clarity.*

139 Done.

140

141

142 We'd like to thank the Editor and Reviewers for their help in improving the quality of our
143 manuscript with their useful comments.

144 The Authors

145

**Litter decomposition rate and soil organic matter quality in a patchwork heathland of
Southern Norway**

Giacomo Certini^{1*}, Live S. Vestgarden^{2,3}, Claudia Forte⁴, Line Tau Strand²

¹*Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente (DISPAA),
Università degli Studi di Firenze, Firenze, Italy*

²*Department of Environmental Sciences, Norwegian University of Life Sciences, Ås, Norway*

³*Department of Environmental and Health Studies, Telemark University College, Bø, Norway*

⁴*Istituto di Chimica dei Composti OrganoMetallici (ICCOM), UOS Pisa, CNR, Pisa, Italy*

* Corresponding author: certini@unifi.it

159 **Abstract**

160 Norwegian heathland soils, although scant and shallow, are major reservoirs of carbon (C).
161 We aimed at assessing whether vegetation cover and, indirectly, its driving factor soil
162 drainage are good proxies for soil organic matter (SOM) composition and dynamics in a
163 typical heathland area of Southern Norway consisting in a patchwork of three different types
164 of vegetation, dominated by Calluna (*Calluna vulgaris* (L) Hull), Molinia (*Molinia caerulea*
165 (L) Moench), or Sphagnum (*Sphagnum capillifolium* (Ehrh.) Hedw.). Such vegetation covers
166 were clearly associated to microtopographic differences, which in turn dictated differences in
167 soil moisture regime, Calluna growing in the driest sites, Sphagnum in the wettest, and
168 Molinia in sites with intermediate moisture.

169 Litter decomposition was followed over a period of 1 year, by placing litterbags filled with
170 biomass from each dominant species in each type of vegetation cover. The composition of the
171 plant material and SOM were investigated by chemical methods and solid-state ¹³C nuclear
172 magnetic resonance (NMR) spectroscopy.

173 Litter decomposition was faster for Molinia and Calluna, irrespective of the vegetation cover
174 of the site where they were placed. Sphagnum litter decomposed very slowly, especially
175 under Calluna, where the soil environment is by far more oxidising than under itself. In terms
176 of SOM quality, Calluna covered areas showed the greatest differences from the others, in
177 particular a much higher contribution from lipids and aliphatic biopolymers, apparently
178 related to biomass composition.

179 Our findings showed that in the studied environment litter decomposition rate and SOM
180 composition are actually dependent on vegetation cover and/or soil drainage. On this basis,
181 monitoring changes in the patchwork of vegetation types in boreal heathlands could be a
182 reliable cost-effective way to account for climate change induced modifications to SOM and
183 its potential to last.

184

185 1. Introduction

186 Heathland vegetation covers approximately 60% of Norway's land area. Norwegian
187 heathland soils, although scant and shallow, are so rich in organic matter that they represent a
188 stock of carbon (C) at least one order of magnitude larger than the aboveground vegetation
189 they sustain (Rosberg et al., 1981). To predict the ecological effects of climate and land use
190 changes, it is essential to understand the nature and environmental dependencies of soil
191 organic matter (SOM) in these widespread systems. In fact, any change influencing their
192 SOM stocks and dynamics may have major consequences for both C balance and the water
193 quality of lakes and rivers (Stuanes et al., 2008).

194 Following changes in SOM stocks is not a simple task, and several approaches have been
195 proposed for this purpose (e.g., Johnson and Curtis, 2001; Trumbore, 2009; Chiti et al.,

196 2011). The current common belief is that environmental and biological factors predominate
197 on the molecular structure in controlling SOM stability (Kleber, 2010; Kleber et al., 2011;
198 Schmidt et al., 2011); however, in some environments, vegetation cover is a good proxy for
199 soil C dynamics, since it controls the input and quality of litter (De Deyn et al., 2008). In
200 turn, vegetation depends, among other factors, on soil drainage, which also influences litter
201 decay and SOM transformation (Wickland et al., 2010), so representing another possible
202 proxy for SOM storage.

203 Although present-day vegetation may be different from the one the underlying SOM
204 originated from (Chambers et al., 1999; Hjelle et al., 2010), many studies have demonstrated
205 that the most active part of SOM is the youngest (e.g., Leavitt et al., 1996; Trumbore, 2000;
206 Chiti et al., 2009). Trumbore (2000) found that the average age of the carbon dioxide (CO₂)
207 released by decomposition processes in boreal forest soils is 30 years, and 50-60% of total
208 soil respiration arises from SOM with mean residence time less than 1 year. The dominant

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220 contribution of recently synthesized organic matter to soil respiration was also assessed by
221 Certini et al. (2003) for forests in temperate regions. Theoretically, the moister and colder the
222 pedoclimate, the better preserved the dead biomass in soil (Hobbie et al., 2000; Hicks Pries et
223 al., 2013). Hence, the wet boreal heathlands are environments where the investigation of a
224 possible relationship between vegetation covers and SOM dynamics is particularly
225 meaningful. Here, due to the intense leaching, lost dissolved organic C (DOC) may be much
226 older than the respired C (Karlton et al., 2005), rendering any possible relationship between
227 present day vegetation and bulk SOM quality less clear. Nonetheless, in the uppermost soil,
228 where SOM is younger and less degraded than below, such relationship is expected to be
229 strong enough.

230 In Southern Norway, heathland areas are in most cases characterised by the alternate
231 occurrence – essentially dictated by the soil drainage, in turn controlled by topography,
232 particle size distribution, and soil depth to bedrock – of three vegetation types, which are
233 dominated by the heather *Calluna* (*Calluna vulgaris* (L.) Hull), the moor grass *Molinia*
234 (*Molinia caerulea* (L.) Moench), and the peat moss *Sphagnum* (*Sphagnum capillifolium*
235 (Ehrh.) Hedw.). Such different vegetation types are cause and effect of the properties and
236 behaviour of the underlying soil. This is undoubtedly true for the soil profile morphology and
237 the sequence of horizons, generally ranging from the O-E-Bhs soil sequum of *Calluna*-
238 sustaining podzols to multiple H horizons consisting histosols where *Sphagnum* grows
239 (Strand et al., 2008).

240 In this study we report an *in situ* investigation of the relationships between vegetation cover,
241 initial litter decay rate and soil organic matter composition for a typical montane heathland
242 area in Southern Norway where the alternation between *Calluna*, *Molinia*, and *Sphagnum*
243 occurs on decametric scale. The objective of the study was to assess whether in this
244 environment the current vegetation cover is a good proxy for SOM quality and dynamics. To

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246 this end, litter decomposition was followed over a period of 1 year, by placing litterbags
247 filled with biomass from each dominant species under each type of vegetation cover, so as to
248 simulate the effects of possible climate change induced shift of vegetation on early stages of
249 litter decomposition. Furthermore, the composition of the aboveground biomass and the bulk
250 SOM were investigated by chemical methods and solid-state ^{13}C nuclear magnetic resonance
251 (NMR) spectroscopy.

252

253 2. Materials and Methods

254 2.1. Study site

255 The study area, Storgama (59°02'47"N, 8°39'37"E), is located in the Telemark county,
256 southern Norway, at an elevation of 560-m above sea level. The mean annual precipitation in
257 Storgama for the period 1961-1990 was 994 mm, the mean annual air temperature for the
258 same period was 5.0 °C. Approximately 30 % of the area is barren granite bedrock and
259 boulders, and soil often occurs as pockets in depressions in the bedrock surface (Fig. 1a). The
260 average soil depth generally varies between 10 and 35 cm but greater thicknesses, up to 100
261 cm, do occur. According to the U.S. Soil Taxonomy (Soil Survey Staff, 2010) and moving
262 from drier to wetter locations, soils are Lithic Haplorthods, Lithic Udipsamments, Lithic
263 Endoaquents, and Lithic Haplosaprists. Although there are some scattered or vaguely
264 grouped Scots pines (*Pinus sylvestris* L) and Downy birch trees (*Betula pubescens* Ehrh), the
265 vegetation is largely dominated by heather (*Calluna vulgaris* (L) Hull) at well drained sites,
266 peat moss (*Sphagnum capillifolium* (Ehrh.) Hedw.) at poorly drained sites, and moor grass
267 (*Molinia caerulea* (L) Moench) at intermediately drained sites (Figs. 1a and 1b). These
268 dominant vegetation types are interspersed in the area, forming a patchwork dictated by
269 topography, which in turn is a driving factor of water supply. At the Calluna sites *Calluna*
270 *vulgaris* was virtually 100 % of the vegetation cover. At the Molinia sites some Calluna,

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Erica (*Erica tetralix* L), and Nartecium (*Nartheicum ossifragum* (L) Huds) were associated with *Molinia caerulea* but, on a visual basis, amounting to no more than 5% of the total cover. At the Sphagnum sites, *Sphagnum capillifolium* covered the entire surface except for a few scattered individuals of Molinia, Erica and Calluna. Hereafter, we will refer to such vegetation assemblages simply as Calluna, Molinia and Sphagnum, respectively. Further pictures and information on vegetation and soils at Storgama are reported in Strand et al. (2008).

2.2. Vegetation sampling and analysis

Three sampling sites per dominant vegetation were chosen within an area of approximately one hectare. At each location, three soil pits were dug down to bedrock, which was 35 to 50 cm deep. All the vegetation above the pit had been previously sampled and divided according to species. In the case of Calluna, the woody stems and branches were separated from the leaves and flowers. Capitula and the five upper centimetres were used to represent the whole Sphagnum material. Visible roots were picked out from the soil samples and separated according to species when possible. The aboveground biomass and the roots were analysed for C and N by dry combustion on oven-dried (60 °C to constant weight) and finely ground samples using a LECO® CHN1000 Analyser. The aboveground biomass also underwent NMR investigation.

2.3. Soil sampling and analysis

We focused our attention on the uppermost soil layer, where we expected the closest relationship between SOM quality and current vegetation. Two undisturbed soil samples, to be used for soil solution extraction, were taken by completely inserting 7.0 cm high and 4.6 cm in inner diameter, rigid cylinders at about five cm depth in each soil profile. The filled

299 cylinders were carefully extracted from the soil and placed in a cooling box after sealing the
300 ends with plastic lids. The samples were stored at 4 °C, for a maximum of one week, until
301 they were processed further. Two standard disturbed soil samples were taken near the holes
302 left by the cylinders and used for C, N, and pH determination, and NMR analysis. As for the
303 plant material, soil C and N concentrations were measured by dry combustion on oven-dried
304 and ground samples, while soil pH was determined potentiometrically in a 1:2.5 V/V distilled
305 water suspension.

306 The soil-containing cylinders were inserted in two-compartment buckets and centrifuged at
307 4620 g for 20 min, following the method described by Giesler et al. (1996). The obtained
308 solution was filtered through a 0.45 µm membrane filter (Millipore). An aliquot of the filtrate
309 was analysed for total C (Shimadzu TOC-V element analyser) and, after oxidation by
310 peroxodisulphate (NS4743 1975), for total N (FiaSTAR, Tecator Spectrophotometer system).
311 Another aliquot of the filtrate was used to measure hydrophobicity, by determining the ratio
312 between the absorbances of the solution at 285 and 254 nm using an UV-VIS
313 spectrophotometer (UV-1201 Shimadzu). These two absorbances are, in fact, correlated to
314 hydrophobic C (π - π^* electron transitions occur at ~285 nm for a number of aromatic
315 substances, as described in Chin et al. 1994) and total C (Brandstetter et al., 1996),
316 respectively.

317 After centrifugation the soil was immediately passed through a 2 mm-mesh sieve. Two grams
318 of the moist sieved soil was treated as in the second step of the procedure proposed by Ghani
319 et al. (2003) to obtain hot-water extract (80 °C for 16 h). After centrifugation for 20 min at
320 2,000 g and filtration through 0.45 µm membrane filters (Millipore), the extract was analysed
321 for total C (HWC), total N (HWN), and carbohydrate C (Carb-C). HWC and HWN were
322 determined by the same method as DOC and total dissolved nitrogen (TDN), while the
323 analysis of Carb-C was done according to the “direct determination” method proposed by

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325 Safarik and Santrucková (1992). In brief, 1 mL of the extract was combined in a polyethylene
326 tube with 1 mL 5% phenol solution and 5 mL concentrated sulphuric acid and immediately
327 shaken on a vortex mixer. The absorbance of the mixture was read after 1 h at 485 nm on a
328 UV-VIS spectrophotometer (UV-1201 Shimadzu). A calibration curve was built with the
329 following standards: 0.00, 0.05, 0.10, 0.25, 0.40 g L⁻¹ of α -D glucose ($R^2=0.9907$).

330

331 2.5. Nuclear magnetic resonance spectroscopy

332 The chemical structure of the aboveground vegetation (one composite sample per dominant
333 species, after removal of stems and coarse branches in the case of Calluna) and SOM (one
334 composite sample per soil pit, hence, three samples per vegetation type) was investigated by

335 solid-state ¹³C nuclear magnetic resonance (NMR) spectroscopy using the cross polarization
336 with magic angle spinning (CP MAS) technique. Prior to analysis, soil samples underwent 2
337 % HF treatment according to Skjemstad et al. (1994) in order to remove possible
338 paramagnetic oxides, which cause broadened resonances and signal loss. NMR spectra were
339 obtained by a Bruker AMX 300-WB spectrometer equipped with a 4 mm CP MAS probe.

340 The operating frequencies were 300.13 and 75.47 MHz for ¹H and ¹³C, respectively; the $\pi/2$
341 pulse was 3.4 μ s on the ¹H channel. A contact time of 2 ms and a relaxation delay of 4 s were
342 used. The MAS speed was set to 8 kHz and the number of scans recorded ranged between
343 4800 and 40000, depending on the sample. The chemical shifts were referenced to

344 tetramethylsilane (TMS) using adamantane as external standard. Seven chemical-shift regions
345 of the NMR-spectra, corresponding to the main C forms, were integrated and expressed as
346 percent contribution to total area subtended by the spectrum between 0 and 220 ppm. The

347 seven regions account for alkyl C (0-45 ppm, mainly comprising lipids, waxes, resins,
348 suberin), methoxyl and N-alkyl C (45-60 ppm, comprising the methoxy group of guaiacyl
349 and the two methoxy groups of syringyl lignin moieties at ~56 ppm), O-alkyl C (60-90 ppm,

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355 carbohydrates, mainly cellulose and hemicellulose, with contributions from carbohydrate
356 carbons bonded to one oxygen), di-O-alkyl C (90-110 ppm, mainly from polysaccharides,
357 with contributions from anomeric carbons of carbohydrates, *i.e.*, bonded to two oxygens), H-
358 and C-substituted aromatic C (110-140 ppm), O-substituted aromatic C (140-162 ppm,
359 mainly from lignin structures, tannins, polyphenols), and carboxyl C (162-190 ppm, esters,
360 acids and amides); no carbonyl intensity in the 190-220 ppm region, ascribable to aldehydes
361 and ketones, was detected.

362

363 2.6. Litter decomposition

364 Litter decomposition was determined *in situ* by the litterbag technique. Recently formed
365 aboveground biomass of Calluna, Molinia, and Sphagnum (approximately, the top 5 cm),
366 were collected at the end of the growing season in late September. This material was oven
367 dried (35 °C to constant weight) and used for filling 10 x 12 cm nylon mesh bags (0.5-1 mm
368 mesh), with 3.0 g Calluna, 2.0 g Molinia, or 1.0 g Sphagnum. In November, 32 litterbags of
369 each vegetation type were installed on the surface of each sampling site, except Calluna
370 under Sphagnum, since a substitution of Calluna by Sphagnum was judged to be highly
371 improbable. Eight to ten litterbags per type of content were sampled from each site after 6, 9
372 and 12 months of decomposition. The removed litterbags were cleaned of plant remnants and
373 other minor foreign material by gently using a soft brush, oven dried (35 °C to constant
374 weight) and weighed for determining mass loss. Their content was thus ground and analysed
375 for carbon and nitrogen as described for the vegetation and soil samples.

376

377 2.7. Statistics

378 All statistical analyses were performed using the software program SAS (SAS Institute, Inc.,
379 1990, Cary, NC). After checking the dataset for normality and variance heterogeneity, effects

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of vegetation and decomposition site on soil pH and SOM were tested by one-way analysis of variance (General Linear Model, GLM). Two separate one-way ANOVAs were performed on litterbags data: one assessing differences in decomposition rate between the three litter types disregarding the dominant vegetation effect, the other assessing differences between the vegetation types disregarding the litter effect. Pairwise comparisons were done by the Tukey's Simultaneous test.

3. Results

The experimental data set indicated marked differences in composition among the dominant plant species. The C concentration in the aboveground biomass increased in the order Sphagnum<Molinia<Calluna, whereas the C/N ratio increased in the order Molinia<Sphagnum<Calluna, with most interspecific differences being significant (Table 1). Belowground, Calluna, and Molinia also showed different composition, hence reflecting the aboveground biomass (Table 1).

Concerning the soil, the measured pH values, all much below neutrality (Table 2), ensured that all C there present was in organic forms. The N content of Sphagnum was significantly higher than that of Calluna, although there were no differences in terms of C/N ratio. The latter, clustering around 20, was in all cases much smaller than the C/N ratio of the respective dominating plant species.

There was a large variability in soil DOC and TDN concentrations, and vegetation types did not show any significant difference with respect to these two variables (Table 2). On the contrary, the hydrophobicity index was significantly different in soils under the three types of vegetation, being highest for Calluna and lowest for Molinia. This difference indicates that a greater proportion of DOC under Calluna was hydrophobic. For the rest, the only other significant differences were in terms of HWN and HW-C/N ratio between Calluna, on the

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431 | one hand, and Molinia and Sphagnum, on the other (Table 2).

432 The ^{13}C CPMAS NMR spectra of the aboveground biomass and soil are shown in Fig. 2, and
433 the relative contributions of the different chemical shift regions are reported in Table 3. The
434 NMR spectra of the aboveground vegetation suggested more similar compositions for
435 Molinia and Sphagnum with respect to Calluna. The spectrum of the Calluna biomass was
436 dominated by signals between 60 and 104 ppm, characteristic of polysaccharides; the
437 relatively high intensity in the alkyl C region (0-50 ppm) was due to lipids and aliphatic
438 biopolymers. The spectrum also revealed the presence of lignin and tannins, as indicated by
439 the lignin methoxyl carbon signal at 56 ppm, and the distinct aromatic peaks at 145 and 155
440 ppm, typical of condensed tannins. The sharp peak at 172 ppm is normally assigned to the
441 carboxyl C of hemicellulose esters, but may also have contributions from amides (Forte et al.,
442 2006). The spectra of Molinia and Sphagnum aboveground biomasses showed the same
443 dominant polysaccharide features of Calluna in the 50-110 ppm range, but a significantly
444 lower intensity of signal in the alkyl and aromatic C regions, which means lower contribution
445 of lipids and lignin/tannins, respectively. In the case of Molinia, the slightly narrower signals
446 in the 60-100 ppm region and the relatively smaller peak shoulder at about 103 ppm
447 compared with both Calluna and Sphagnum, suggested the occurrence of less hemicellulose
448 and some crystalline cellulose, respectively. Sphagnum did not show the typical lignin
449 signals, in agreement with the common lignin-free composition of bryophytes (Kļaviņa et al.,
450 2012). The only aromatic signals in the sphagnum spectrum were due to unsubstituted or C-
451 substituted aryl C at 130 and 117 ppm, while the signal at 158 ppm was ascribable to
452 phenolic structures. In the case of Calluna, the two sharp tannin peaks at 145 and 155 ppm
453 observed in the aboveground biomass spectrum were totally absent in the SOM spectrum. In
454 the case of Molinia, differently from the other two vegetation types, the relative contribution
455 of aromatic C significantly increased in soil compared to that observed in the aboveground

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458 biomass. In the case of Sphagnum, no major changes occurred in the aromatic region, except
459 for the absence in the soil spectrum of the signal at 158 ppm detected for the aboveground
460 vegetation (Fig. 2). The alkyl C/O-alkyl C ratio increased for all vegetation types on passing
461 from the intact biomass to its decomposition products in soil (Table 3), with large differences
462 in absolute values between Calluna, on the one side, and Molinia and Sphagnum, on the other
463 side.

464 The *in situ* decomposition study using litterbags showed that the litter mass remaining after
465 one year of decomposition varied between 62 and 66% in the case of Molinia and Calluna
466 and 83 and 94% for Sphagnum (Fig. 3). The discrepancy between the couple Calluna-Molinia
467 and Sphagnum was lower, although significant, in the intermediate stages of the experiment.

468 After six months, Calluna showed significantly lower mass loss than Molinia under itself,
469 while at the end of the experiment Calluna resulted to be better preserved than Molinia only
470 under Molinia (Fig. 3).

471 In terms of relative C content of the residual litter, Calluna did not change throughout the 12
472 months of the experiment, while Molinia and Sphagnum experienced a marked decrease
473 compared to the original value (Fig. 4). Relative concentrations of N in the litter changed
474 more than the C ones. Except for Sphagnum under itself or under Molinia, all litters increased
475 their N content from November to May; later, all of them increased until August, with the
476 exception of Sphagnum under Calluna and Molinia under itself; finally, in the period from
477 August to November, N concentration continued to increase in Calluna, whereas it decreased
478 in Molinia and showed an irregular trend in Sphagnum (Fig. 4). These C and N trends
479 implied progressive, although slight, decrease in C/N ratio for Calluna and Sphagnum, and a
480 sharper decrease for the same ratio for Molinia until August, after which it increased (Fig. 4).

481 Contrary to Calluna and Sphagnum, Molinia degraded maintaining significantly higher
482 values of C/N ratio under Sphagnum than under the other types of vegetation (Molinia and

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496 | Calluna). At the end of the experiment, in November, the C/N ratio in Molinia under
497 | Sphagnum was even higher than the original value.

498

499 | 4. Discussion

500 | In the heathland environment of Storgama, the composition of SOM appeared to partly reflect
501 | that of the parent vegetation. Hence, for example, the abundance in alkyl C in the Calluna
502 | biomass relative to the other two vegetation types was transferred to the SOM. Nevertheless,

503 | SOM accumulated over a long period of time; as a consequence, it could be the result of
504 | multiple changes in vegetation cover in the area and, thus, be partly unrelated to the current
505 | vegetation cover. Actually, there was no direct or indirect evidence in this regard, but, inputs
506 | of wind-blown or water-transported material cannot be excluded at any site.

507 | Sphagnum showed a composition potentially more prone to decay than Calluna and Molinia.
508 | Nevertheless, there were no significant differences in the SOM content of the topsoil of the
509 | three vegetation covers. Evidently, the prevailing anoxic conditions limited decomposition at
510 | the Sphagnum sites. This is in accordance with several studies that used the type of
511 | vegetation cover as a proxy for carbon dynamics, based on the consideration that vegetation
512 | chiefly reflects the soil moisture regime (Bridgham et al., 2008; Couwenberg et al., 2011;
513 | Delarue et al., 2011), which is in turn a driving factor of litter decomposition (Hobbie et al.,
514 | 2000; Laiho 2006). Large variability in DOC concentrations and no significant effect of
515 | vegetation was observed (Table 2). It must be noted, however, that our study shows the
516 | conditions only at one sampling occasion, *i.e.* at the end of the growing season, when DOC
517 | concentrations are affected by a considerable contribution from senescing plant material. The
518 | measured DOC concentrations were generally in agreement with those recorded in autumn
519 | using zero tension lysimeters in soils at Storgama and other Norwegian heathland areas
520 | (Strand et al., 2002; Vestgarden et al., 2010), although DOC concentrations in centrifuged

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528 and freely drained soil solutions are not directly comparable (Giesler et al., 1996). Similarly
529 to DOC, TDN showed a large variability and no apparent correlation with vegetation. The
530 relatively small amount of water extracted by centrifugation limited the number of possible
531 analyses, preventing N speciation. TDN therefore included both organic N and inorganic N,
532 the latter amounting to 25-50% of TDN in soil water from southern Norway (Austnes et al.,
533 2008; Kaste et al., 2008).

534 The hydrophobicity index of soil water differed significantly among vegetation types.
535 Apparently, Calluna released DOC with the highest proportion of hydrophobic organic
536 compounds, perhaps mostly arising from tannins and decomposition of lignin (Dilling and
537 Kaiser, 2002), which are indeed important components of the Calluna litter (Fig. 2).

538 Hot water C approximately amounted to 4.5% of SOC in all samples, irrespective of
539 vegetation. This percentage is in the range reported by von Lützow et al. (2007). Significantly
540 lower amounts of HWN were extracted from the Calluna soils compared to the Molinia and
541 Sphagnum ones, which also implied significantly higher HWC / HWN-ratio for Calluna
542 (Table 2). We did not partition HWN, however Curtin et al. (2006) demonstrated that it is
543 mainly organic and, in suborder, NH₄-N generated by hydrolysis of heat-labile organic N.
544 The quality of the hot water extract rather well discriminated Calluna from Molinia and
545 Sphagnum. Some authors have proposed hot water extraction of SOM as a method to
546 measure the labile SOM pool (Chodak et al., 2003; Ghani et al., 2003; Curtin et al., 2006);
547 however, other authors consider this method not selective enough for this purpose (Landgraf
548 et al., 2006; von Lützow et al., 2007). In our case, approximately half the C extracted by hot
549 water belonged to carbohydrates.

550 The NMR spectra showed clear structural differences in aboveground plant material (Fig. 2
551 and Table 3). In particular, Calluna was richer in alkyl C and poorer in O-alkyl C than
552 Molinia and Sphagnum, as well reflected in the alkyl C / O-alkyl C ratio. The richness in

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alkyl C has been correlated to slow decomposition rates in heathland ecosystems (van Vuuren and van der Eerden, 1992; van Vuuren and Berendse, 1993). However, in our litterbags experiment there were little and variable differences between the mass losses of Calluna and Molinia, and both of them were much higher than the one in Sphagnum wherever the latter was placed (Fig. 3). A possible explanation for such short-term resistance of Sphagnum to degradation could be that this type of vegetation is particularly rich in sphagnum pectin-like polysaccharides, which, unlike the other types of polysaccharides, induce processes that prevent organic matter decay (Hájek et al., 2011; Ballance et al., 2012). Moreover, it must be considered that Sphagnum might have experienced a “non-additive” pattern of mass loss, *i.e.* a decomposition behaviour sometimes observed in litter mixes that deviates from the response predicted for the individual species because of the influence of the other species present in the mix (Gartner and Cardon, 2004). In this case, the necromasses of Molinia and, in particular, Calluna could have partly inhibited the decomposition of the Sphagnum in the litterbags.

In addition to a “vegetation effect”, the litterbags experiment showed some “site effect”, *i.e.* more rapid decomposition when litter was placed beneath the parent vegetation rather than beneath other species (Ayres et al., 2009; Perez et al., 2013; Wang et al., 2013). In fact, for Sphagnum the mass loss was significantly lower when it decayed under Calluna than under Molina or Sphagnum (Fig. 3). Calluna was better preserved under Molinia than under itself at the end of the trial, while, after six months only, Molinia litter showed significant environment-induced advantage under Sphagnum compared to under itself (Fig. 3). Unexpectedly, the well drained Calluna soils preserved Sphagnum and Molinia from decay better than the moister soils where they were growing, perhaps as an effect of a seasonal drought.

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Eliminato: led us to expect differences in decomposition rates among the three vegetation types. Overall, the NMR investigation revealed that Calluna and the related SOM were richer in alkyl C and poorer in O-alkyl C than the corresponding specimens from Molinia and Sphagnum. Actually, the apparent richness in alkyl C has been correlated to slow decomposition rates of Calluna biomass

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Eliminato: NMR spectra also revealed that the residues of all three dominant plants, once in soil, experienced a significant increase in the alkyl C contribution and a concomitant decrease in the O-alkyl C one, most probably as a result of a faster decay of carbohydrates than of other C forms and the synthesis of lipids from the biodegradation of carbohydrate and aromatic fractions (Baldock et al., 1992). Less important but noteworthy differences in the spectral features between the aboveground biomass and soil were observed in the aromatic region as well (Fig. 2 and Table 3). The alkyl C / O-alkyl C ratio, which generally increases as decomposition proceeds, was significantly higher under Calluna than under Molinia and Sphagnum. Hence, on the basis of the NMR spectra, Calluna appeared to be potentially more recalcitrant to decomposition than Molinia and Sphagnum.

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609 The enclosure of litter inside mesh bags may actually change its overall decomposition rate
 610 and its C and N percent concentrations compared to non-bagged substrate (Berhe, 2013).
 611 However, we must confidently assume that in our experiment such a bagging effect is
 612 uniform through the samples, also because the bags are very similar. On this basis, Molinia
 613 showed an initial C/N ratio much higher than the ones of Calluna and, especially, Sphagnum
 614 (Fig. 4), which suggested a more marked intrinsic resistance of Molinia to decay. Noteworthy
 615 is the difference in C/N ratio between the aboveground Molinia biomass analysed for basic
 616 characterisation (data of Table 1) and the Molinia used in the litterbags experiment (30 vs.
 617 circa 80). Actually, Molinia is a grass that wilts at the end of the growing season, when we
 618 sampled the material to be inserted in the bags, while the Molinia sampled for basic
 619 characterisation was still with active photosynthesis, when the C/N ratio is relatively low
 620 (Taylor et al., 2001). On the other hand, Calluna is an evergreen and no great seasonal
 621 changes in C and N concentrations occur, while Sphagnum, although it is not an evergreen,
 622 does not wilt and its C/N ratio is rather constant throughout the year. Our litterbags
 623 experiment showed that the C/N ratio is a poor predictor of decay in this environment. The
 624 anoxic conditions imposed by prolonged water saturation, commonly occurring in the
 625 Sphagnum soils and expected to have considerable influence in slackening litter
 626 decomposition, on the contrary appeared to be irrelevant in preserving organic residues
 627 during a 1-year long experiment (Fig. 3). In this regard, during a 3-year study in heathlands
 628 on *Molinia caerulea* and *Erica tetralix*, van Vuuren and Berendse (1993) did not find any site
 629 effect and litter quality appeared to be the sole driving factor. Also Scheffer et al. (2001),
 630 studying the decomposition process in fens dominated by Sphagnum species or without
 631 Sphagnum, concluded that decomposition was controlled more by intrinsic differences in
 632 litter quality than by the environment.

Claudia Forte 21/1/y 10:17
 Eliminato: rate of
 Claudia Forte 21/1/y 10:16
 Eliminato: overall
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 Eliminato: percent
 Claudia Forte 21/1/y 10:17
 Eliminato: t
 giacom 17/1/y 22:10
 Eliminato: In our litterbag experiment,
 giacom 16/1/y 08:27
 Eliminato: its tissues

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 Eliminato: sphagnum
 giacom 18/1/y 11:07
 Eliminato: The
 giacom 18/1/y 11:06
 Eliminato: not a highly reliable

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644 The NMR investigation revealed that soils, compared to the litter they receive, showed
645 significantly higher contribution of alkyl C and lower contribution of O-alkyl C (Fig. 2 and
646 Table 3), most probably as a result of a faster decay of carbohydrates than of other C forms
647 and the synthesis of lipids from the biodegradation of carbohydrate and aromatic fractions
648 (Baldock et al., 1992). The alkyl C / O-alkyl C ratio, which generally increases as
649 decomposition proceeds, was significantly higher in the Calluna soil than under Molinia and
650 Sphagnum.

651

652

653 5. Conclusions

654 We found that in the varied heathland of Storgama there were many significant differences in
655 terms of SOM composition between the Calluna dominated areas and the interspersed
656 Sphagnum-covered areas. Most differences were clearly due to the litter quality. A
657 “vegetation effect” on the early stage of litter decomposition rate was clear, Sphagnum
658 remnants being much more stable independently of the environmental conditions they
659 underwent, which differed especially in terms of soil drainage. Hence, overall, vegetation
660 appeared to be a good proxy for SOM quality. On this basis, monitoring the distribution of
661 vegetation types in heathlands of Norway and elsewhere could be of particular interest for
662 assessing the consequences of climate change on SOM stocks and dynamics. In the plausible
663 scenario of a less continuous rainfall supply and a consequent contraction of Sphagnum-
664 covered areas, the Sphagnum-released litter seems to have good short-term ability to resist
665 decomposition under the two replacing types of vegetation, Molinia and Calluna. Long-term
666 experiments addressing this issue are needed.

667

668

Live Semb Vestgarden 13/11/y 14:52
Eliminato: quality of the parent vegetation.

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Eliminato: refractory

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Eliminato: varied

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Eliminato: environmental changes such as global warming and higher concentration of rainfall higher precipitation on litter

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Eliminato: SOM

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Eliminato: Medium to l

677 **Acknowledgements**

678 We thank Irene Eriksen Dahl, Grete Bloch, and Ivan Digernes for laboratory assistance at the
679 Department for Plants and Environmental Sciences, Norwegian University of Life Sciences.

680 We also thank Silvia Pizzanelli of ICCOM-CNR, for performing part of the NMR analyses.

681 The study was carried out in close cooperation with the CLUE project (NFR 155826/S30).

682 This specific investigation was made possible by a grant from the Research Council of
683 Norway (NFR 164903/S30) enabling the first author to cooperate with researchers from the
684 Norwegian University of Life Sciences.

685

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833

834 **Figures captions:**

835

836 **Figure 1. a)** A general view of the study area, Storgama, showing soil occurring in pockets
837 and small depressions at the bedrock surface; note that close up vegetation at the bottom right
838 is dominated by *Molinia caerulea* (L), the understorey of pines beyond is *Calluna vulgaris*
839 (L) Hull, the basin in the background is covered by *Sphagnum* spp. **b)** A rare coalescence of
840 the three dominant species, *Calluna vulgaris*, on the left, *Sphagnum* spp. L, at the bottom,
841 and *Molinia caerulea*, on the right.

842

843 **Figure 2.** ^{13}C CPMAS NMR spectra of the aboveground biomass of the dominant plant
844 species and soil.

845

846 **Figure 3.** Residual mass in the litterbags as a function of time for different combinations of
847 litter and vegetation cover. Cal in Cal means *Calluna* litter decomposing under *Calluna*, Cal
848 in Mol means *Calluna* litter decomposing under *Molinia*, and so on. Error bars are standard
849 deviations, while lower case letters indicate significant differences ($p < 0.05$) between same
850 litters decomposing under different types of vegetation. The trial was one year long.

851

852 **Figure 4.** Carbon and nitrogen concentrations and C/N ratio in decaying biomass in the
853 litterbags as a function of time for different combinations of litter and vegetation cover. Cal
854 in Cal means *Calluna* litter decomposing in soil under *Calluna*, Cal in Mol means *Calluna*
855 litter decomposing under *Molinia*, and so on. Upper case letters indicate significant
856 differences ($p < 0.05$) between different litters, whereas lower case letters indicate significant
857 differences between same litters decomposing in soils covered by different types of
858 vegetation.

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Eliminato: the underlying

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Eliminato: Lower