

1 The *n*-alkane and sterol composition of living fen plants as a potential
2 tool for palaeoecological studies

3 Tiina Ronkainen ^{a*}, Erin L. McClymont ^b, Minna Väiliranta ^a, Eeva-Stiina Tuittila ^{c,d}

4 ^a *Environmental Change Research Unit (ECRU), Department of Environmental Sciences, PO*
5 *Box 65, FI-00014 University of Helsinki, Finland*

6 ^b *Department of Geography, Durham University. South Road, Durham DH1 3LE, UK*

7 ^c *Current address: School of Forest Sciences, University of Eastern Finland, PO Box 111,*
8 *80101 Joensuu, Finland*

9 ^d *Peatland Ecology Group, Department of Forest Sciences, PO Box 27, FI-00014 University*
10 *of Helsinki, Finland*

11 * Corresponding author. *E mail addresses:* tiina.m.ronkainen@helsinki.fi (Tiina Ronkainen).

12

13 ABSTRACT

14 In groundwater-fed fen peatlands, the surface biomass decays rapidly and as a result, highly
15 humified peat is formed. A high degree of humification constrains palaeoecological studies
16 because reliable identification of plant remains is hampered. Organic geochemistry
17 techniques as a means of identifying historical plant communities have been successfully
18 applied in bog peats. The method has also been applied to fen peat, but without reference to
19 the composition of fen plants. In this study we have applied selected organic geochemistry
20 methods to determine the composition of neutral lipid fractions from 12 living fen plants, to
21 investigate the potential for the distributions to characterize and separate different fen plants

22 and plant groups. Our results show correspondence with previous studies, e.g. C₂₃ and C₂₅ *n*-
23 alkanes dominate *Sphagnum* spp. and C₂₇ to C₃₁ alkanes dominate vascular plants. However,
24 we also found similarities in *n*-alkane distributions between *Sphagnum* spp. and the below
25 ground parts of some vascular plants. We tested the efficiency of different *n*-alkane ratios to
26 separate species and plant groups. The ratios used in bog studies (e.g. *n*-C₂₃/*n*-C₂₅ and *n*-
27 C₂₃/*n*-C₂₉) did not work as consistently on fen plants. Some differences in sterol distribution
28 were found between vascular plants and mosses; in general vascular plants had a higher
29 concentration of sterols. When distributions of *n*-alkanes, *n*-alkane ratios and sterols were all
30 included as variables, redundancy analyses (RDA) separated different plant groups into their
31 own clusters. Our results imply that the pattern in bog biomarkers cannot directly be applied
32 to fen environments. Nevertheless, they encourage further testing to determine whether or not
33 the identification of plant groups, plants or plant parts from highly humified peat is possible
34 by applying fen species-specific biomarker proxies.

35 **Keywords:** biomarker, geochemistry, palaeoecology, peatland, fen, *Sphagnum*, vascular
36 plant.

37 1. Introduction

38 Northern peatlands comprise a large store of carbon, 547 (473—621) Pg (Yu et al., 2010);
39 acute and contemporary questions related to human-induced changes in climate have
40 therefore emphasized the importance of thoroughly understanding peatland dynamics, past
41 and present. Peatland carbon balance is highly sensitive to moisture conditions (e.g. Alm et
42 al., 1999; Waddington and Roulet, 2000): the water table depth defines both the prevailing
43 plant assemblages and the thickness of the oxic layer, where most biological production and
44 decomposition take place. Hydrology and the source of nutrients are the main factors
45 controlling the type of peatland and the occurrence of species (Wheeler and Proctor, 2000,
46 Økland et al., 2001). The nutrient (trophic) level of a peatland is described as a gradient from

47 nutrient rich to nutrient poor: eutrophic, minerotrophic, mesotrophic, oligotrophic and
48 ombrotrophic. Nutrient poor bogs receive water and nutrients only through precipitation
49 while nutrient rich fens receive water and nutrients from atmospheric input, groundwater and
50 underlying and surrounding mineral soils (Rydin et al., 2006). Bogs are characterized by dry
51 and wet microhabitats: hummocks (surface 20-50 cm above the water table), intermediate
52 lawns (5-20 cm above the water table) and wet flarks where the water table is at the surface,
53 each maintaining specific plant assemblages. Fens on the other hand do not have such
54 distinguishable microhabitat formation (Rydin et al., 2006, Laine et al., 2009). Given the vital
55 role of vegetation in the peatland carbon budget (e.g. Riutta et al., 2007) and the fact that
56 bryophyte and vascular plant dominated communities differ in their CO₂ and CH₄ dynamics
57 (Laine et al., 2007, Levy et al., 2012) it is important to understand past mechanisms that have
58 controlled the vegetation dynamics. Historical variations in climate and hydrology are
59 preserved in peat layers as alterations in the assemblages of different biological organisms. In
60 particular, past vegetation assemblages have been a key proxy for reconstructing past
61 moisture conditions in a range of sites (e.g. Barber et al., 1998; Mauquoy et al., 2002; Tuittila
62 et al., 2007; Väiliranta et al., 2007). This reflects the slow and incomplete decomposition of
63 peat in bog environments, meaning that bogs usually contain relatively well preserved plant
64 material for palaeoecological examination. In contrast, in fen environments surface decay is
65 rapid and a major part of the peat below the surface layer is highly humified (Moore et al.,
66 2007). Fen peats thus tend to lack identifiable plant remains. Given that all bogs are underlain
67 by a fen peat phase and a major proportion of the northern peatlands are still in a fen phase,
68 there is considerable spatio-temporal restriction for palaeoecological applications based on
69 identifiable plant remains alone.

70

71 Studies of bog peats have shown that plant biomarkers, i.e. species-specific compounds, can
72 be successfully applied to less-humified peat to identify fossil plant groups (e.g. Xie et al.,
73 2000; Avsejs et al., 2002; Pancost et al., 2002, 2003; Nichols et al., 2006; Jia et al., 2008;
74 McClymont et al., 2008; Bingham et al., 2010). Different plant groups can be separated, for
75 instance by comparing *n*-alkane distributions and ratios, e.g. the difference in concentration
76 of low molecular weight (LMW) *n*-C₂₃ and *n*-C₂₅, and high molecular weight (HMW) *n*-C₂₉
77 and *n*-C₃₃ can be used to separate contributions from *Sphagnum* and non-*Sphagnum* species
78 (Pancost et al., 2002, Nichols et al., 2006, Vonk and Gustafsson, 2009, Lopez-Diaz et al.,
79 2010, Ortiz et al., 2011, Andersson et al., 2011). Studies have also shown that some moss
80 species can be distinguished down to species level (Jia et al., 2008; Bingham et al., 2010),
81 e.g. *n*-C₂₃/*n*-C₂₅ alkane ratio in bog peat may track changes in *Sphagnum fuscum* abundance
82 (Bingham et al., 2010).

83

84 A thorough investigation of the lipid distributions in fen plants has not, to our knowledge,
85 been performed. As a result, it is not clear whether or not the application of biomarker ratios
86 from ombrotrophic peat plants would be a robust approach for the characterization of
87 peatlands including fens (Andersson et al., 2011). In this study we have applied selected
88 organic geochemical analyses to living fen plant species, excluding the litter. Specifically, we
89 aimed to define whether or not (i) the analyses could separate bryophytes from vascular
90 plants and (ii) there are specific fen plant proxies.

91

92 **2. Material and methods**

93 *2.1. Sampling*

94 Samples of living plants were collected from three individual but closely located fens from
95 the Siikajoki commune (64°45'N, 24°42'E) in the mid-boreal bio-climate zone in Finland

96 (Fig. 1). The water level of fens is on average 10 cm below the soil surface and the pH of the
97 water squeezed from the mosses is between 4 and 4.3. A detailed description of the sites
98 (SJ2-4) is given by Leppälä et al. (2011) and Laine et al. (2011).

99

100 Twelve plant species typical of fens were chosen: five bryophyte species and seven vascular
101 plant species (Table 1). Bryophytes were treated as whole plants. Vascular plants, sedges and
102 forbes were divided into above and below ground parts because previous studies had shown
103 that the *n*-alkane concentration might vary between different plant parts (Dawson et al., 2000,
104 Jansen et al., 2006). In fen environments sedge and forb roots may also form a substantial
105 contribution to the organic matter (OM) input to the upper peat (cf. Saarinen, 1996, Moore et
106 al. 2002, Andersson et al., 2011, Huang et al., 2011). To assess methodological
107 reproducibility we repeated the analyses with six randomly selected samples [*Sphagnum*
108 *papillosum*, *Warnstorfia exannulata*, *Carex rostrata* (above and below ground parts),
109 *Potentilla palustris* and *Menyanthes trifoliata* (above ground parts). Compound
110 concentrations are as mean values, and the standard error of the mean (SE) is reported when
111 the compound was found in both the original and repeated analyses. Moreover, we collected
112 and analyzed a selection of species (*W. exannulata*, and the below ground parts of *C.*
113 *rostrata*, *C. livida*, *C. nigra*, *C. lasiocarpa*, *E. angustifolium* and *M. trifoliata*) from a nearby
114 peatland. This procedure was executed in order to test for location-related variation in
115 compositions. Total organic carbon (TOC) was measured to test whether or not the lipid
116 concentration between sampled plants/plant parts differed because of TOC content or
117 concentration calculated from dry weight.

118

119 2.2. Solvent extraction

120 The plant parts were separated and washed with distilled water. Lipids were extracted from
121 ca. 0.2 g of the freeze dried and ground samples using repeated ultrasonication (20 min) with
122 6 ml CH₂Cl₂/MeOH (3:1, v/v). Samples were saponified with 0.5 M methanolic (95%) NaOH
123 for 2 h at 70 °C and the neutral lipids extracted using hexane. The neutral lipids were further
124 separated into apolar and polar compounds using activated Al₂O₃ columns, eluting with
125 hexane/CH₂Cl₂ (9:1, v/v) and CH₂Cl₂/MeOH (1:2, v/v), respectively. Prior to analysis using
126 gas chromatography (GC) and GC-mass spectrometry (GC-MS) the polar fractions were
127 derivatised using bis(trimethylsilyl)trifluoroacetamide (Sigma Aldrich).

128

129 2.3. GC-MS

130 Apolar and polar fractions were analyzed using GC-MS with the gas chromatograph
131 equipped with flame ionisation detection (GC-FID) and split/splitless injection (280 °C).
132 Separation was achieved with a fused silica column (30 m x 0.25 mm i.d) coated with
133 0.25µm 5% phenyl methyl siloxane (HP-5MS), with He as carrier gas, and the following
134 oven temperature programme: 60 – 200 °C at 20 °C/min, then to 320 °C (held 35 min) at
135 6°C/min. The mass spectrometer was operated in full scan mode (50-650 amu/s, electron
136 voltage 70eV, source temperature 230 °C). Compounds were assigned using the NIST mass
137 spectral database and comparison with published spectra (e.g. Goad and Akihisa, 1997;
138 Killops and Frewin, 1994). Quantification was achieved through comparison of integrated
139 peak areas in the FID chromatograms and those of internal standards of known concentration
140 (5- α -cholestane for apolars and 2-nonadecanone for polars). Concentration values are given
141 as concentration per dry weight of extracted material. The concentration from replicate
142 samples was averaged in the statistical analysis.

143

144 2.4. *Statistical analysis*

145 We applied multivariate analysis to study the variation within the biomarker data. To test
146 whether or not the variation was related to the specific plant groups or their component parts,
147 we applied redundancy analysis (RDA) with three plant groups: mosses, above ground and
148 below ground vascular plant parts. We conducted a series of RDA determinations. First, we
149 analyzed the data and tested the significance separately for different variables: *n*-alkanes, *n*-
150 alkane ratios, *n*-alcohols and sterols; we then applied RDA for different compound
151 combinations to find the solution best explained by the three plant group variables. To link
152 biomarker composition to plant species we included the latter as a passive variable into the
153 analysis. A Monte Carlo permutation test was used to test the significance of the RDA
154 solutions in all of the analyses. To make the analyses robust, compounds detected in fewer
155 than four samples were excluded, because they might skew the result in favor of those
156 samples in which they existed. This means that some species-specific markers were not
157 included in the statistical analysis, but they are mentioned when individual plant analyses are
158 discussed. The statistical analyses were conducted using Canoco for Windows 4.52 (ter Braak
159 and Smilauer, 2002).

160

161 **3. Results**

162 We found *n*-alkanes within range of *n*-C₁₇ to *n*-C₃₅ in the apolar fraction. A few samples
163 contained taraxer-14-ene, taraxast-20-ene, and an unidentified triterpanoid and taraxeroid. In
164 the polar fraction we found sterols and *n*-alcohols. The absolute concentrations of compounds
165 did differ between sample sets (original, repeated and replicate), but the dominance order of
166 compounds was maintained (the full data set can be downloaded from www.pangaea.de,
167 reference PANGAEA PDI-4071). The samples contained no evidence of bacterial activity as
168 no traces of hopanoids or archaeol were found. A few replicate samples contained stanols and

169 ketones. This suggests a low level of degradation and that the samples contained compounds
170 solely from the plants under study (e.g. Nishimura 1977, Lehtonen and Ketola 1993, Jiao et
171 al. 2008). There was a linear correlation between concentration calculated as mass per dry
172 weight ($\mu\text{g g}^{-1}$) and as mass per total organic carbon ($\mu\text{g TOC}$), indicating no bias due to
173 selective preservation of OM between plant species or plant groups (Fig. 2).

174

175 3.1. Apolar fraction

176 The total concentration of *n*-alkanes ($\text{C}_{17}\text{-C}_{35}$) in moss species varied between $86.4 \mu\text{g g}^{-1}$
177 (*Sphagnum fimbriatum*) and $9.2 \mu\text{g g}^{-1}$ (SE 3.8) (*W. exannulata*). The distribution of *n*-
178 alkanes of *Sphagnum* species showed an odd/even preference (Fig. 3). The C_{23} *n*-alkane
179 dominated in *S. subsecundum* ($23.6 \mu\text{g g}^{-1}$), *S. riparium* ($12.4 \mu\text{g g}^{-1}$), and *S. papillosum*
180 ($32.6 \mu\text{g g}^{-1}$), whereas *n*- C_{25} dominated in *S. fimbriatum* ($21.3 \mu\text{g g}^{-1}$). In *W. exannulata*, C_{27}
181 and C_{25} *n*-alkanes dominated (2.3 and $1.5 \mu\text{g g}^{-1}$, respectively; Fig. 3). *W. exannulata* was the
182 only moss species where taraxast-20-ene was detected ($4.7 \mu\text{g g}^{-1}$).

183 In above ground sedge parts an odd predominance was also present. Total *n*-alkane
184 concentration was highest in *Carex rostrata* ($332.7 \mu\text{g g}^{-1}$, SE 44.5) and lowest in *C. nigra*
185 ($21.7 \mu\text{g g}^{-1}$). The C_{27} *n*-alkane dominated in above ground parts of *C. livida* ($7.1 \mu\text{g g}^{-1}$), *C.*
186 *nigra* ($12.3 \mu\text{g g}^{-1}$) and *Eriophorum angustifolium* ($10.2 \mu\text{g g}^{-1}$) and C_{29} in *C. rostrata* (188.8
187 $\mu\text{g g}^{-1}$, SE 30.8) and *C. lasiocarpa* ($52.8 \mu\text{g g}^{-1}$) (Fig. 3).

188 The *n*-alkane distributions in the below ground sedge parts were more complex. The short
189 chain *n*-alkanes (C_{19} , C_{21} and C_{23}) were more abundant (Fig. 3) than the long chain *n*-alkanes
190 (C_{27} , C_{29} and C_{31}). However, for instance, *C. nigra* had the highest concentration of *n*- C_{27}
191 ($5.2 \mu\text{g g}^{-1}$) but the short chain *n*-alkanes were also present. In *C. lasiocarpa*, C_{23} had the
192 highest concentration ($15.7 \mu\text{g g}^{-1}$); in *C. rostrata* the most abundant alkane was also *n*- C_{23}

193 (24.7 $\mu\text{g g}^{-1}$; SE 10.2). *C. livida* was dominated by *n*-C₂₁ (5.7 $\mu\text{g g}^{-1}$). The below ground parts
194 *C. lasiocarpa* was the only sample where small amounts of taraxer-14-ene (4.4 $\mu\text{g g}^{-1}$), an
195 unidentified triterpenoid (1.5 $\mu\text{g g}^{-1}$) and taraxast-20-ene (0.6 $\mu\text{g g}^{-1}$) were found. *E.*
196 *angustifolium* was dominated by *n*-C₂₇ (14.7 $\mu\text{g g}^{-1}$; Fig. 3).

197 *Menyanthes trifoliata* above ground parts had the lowest total *n*-alkane concentration (5.6 μg
198 g^{-1} ; SE 0.3). In contrast, below ground plant parts had a much higher total concentration of *n*-
199 alkanes (89.7 $\mu\text{g g}^{-1}$) than the above ground parts. Short chain *n*-C₂₁ and *n*-C₂₃ alkanes
200 dominated below ground plant parts (38.0 and 25.5 $\mu\text{g g}^{-1}$, respectively), while long chain *n*-
201 alkanes were present in small amount (Fig. 3).

202 In *Potentilla palustris*, the above ground and below ground parts were dominated by the
203 long- chain *n*-alkanes and *n*-C₃₁ had the highest concentration in both (497.6 $\mu\text{g g}^{-1}$, SE 182.6
204 and 14.7 $\mu\text{g g}^{-1}$, respectively). *Potentilla palustris* above ground parts had the highest total
205 concentration of *n*-alkanes (985.6 $\mu\text{g g}^{-1}$; SE 350.4; Fig. 3).

206

207 3.2. *n*-Alkane ratios

208 Ratios between different *n*-alkanes have been found to be useful markers for distinguishing
209 species in bog environments (Nott et al., 2000, Ishiwatari et al., 2005, Jansen et al., 2006,
210 Nichols et al. 2006, Zheng et al., 2007, Vonk and Gustafsson, 2009, Bingham et al., 2010,
211 Andersson et al., 2011). The ratios calculated here were used in these studies.

212 Below ground parts of *Carex nigra*, *C. livida* and *C. lasiocarpa* showed the highest *n*-C₂₃ / *n*-
213 C₂₅ (ca. 5 to 7) ratio, whereas *Sphagnum* spp. had lower values (ca. 0.6 to 3). The ratios *n*-
214 C₂₃/*n*-C₂₇, *n*-C₂₃/*n*-C₂₉ and *n*-C₂₃/*n*-C₃₁ were all low for the above ground plant parts (around
215 0) and high in *Sphagnum* species (> 10), especially *S. papillosum*. However, *n*-C₂₃/*n*-C₂₇ for

216 *C. lasiocarpa* below ground parts (ca. 20) and $n\text{-C}_{23}/n\text{-C}_{29}$ and $n\text{-C}_{23}/n\text{-C}_{31}$ for *M. trifoliata*
217 (ca. 40 and 70 respectively) were higher than the values in *Sphagnum* spp. The $n\text{-C}_{25}/n\text{-C}_{29}$
218 ratio had a similar distribution pattern to the three above ratios, but with smaller values and
219 more complex distribution in *Sphagnum* spp. (ca. 6 to 15). *Potentilla palustris* above ground
220 and below ground parts were clearly separated from other samples via $n\text{-C}_{31}/n\text{-C}_{27}$ (> 5) and
221 $n\text{-C}_{31}/n\text{-C}_{29}$ (PANGAEA PDI-4071).

222 The $n\text{-C}_{23}/(n\text{-C}_{23} + n\text{-C}_{29})$ and $n\text{-C}_{25}/(n\text{-C}_{25} + n\text{-C}_{29})$ ratios distinguished *Sphagnum* spp. as
223 their own group (ca. 0.9). The pattern was clearest for $n\text{-C}_{25}/(n\text{-C}_{25} + n\text{-C}_{29})$ where *Sphagnum*
224 spp. ratio values (> 0.8) consistently exceed higher plant values, excluding *M. trifoliata*
225 below ground parts, which was equals to moss values (Fig.4). For $n\text{-C}_{23}/(n\text{-C}_{27} + n\text{-C}_{31})$, *M.*
226 *trifoliata* had the highest value (ca. 11), and *S. papillosum* stood out owing to a higher
227 value (ca. 9) than the rest of the mosses and vascular plants. $P_{aq} [(n\text{-C}_{23} + n\text{-C}_{25})/(n\text{-C}_{23} + n\text{-C}_{25} + n\text{-C}_{29} + n\text{-C}_{31})]$
228 did not seem to separate plant species. However, *S. fimbriatum* and *S.*
229 *papillosum* had higher P_{aq} values (ca. 1) than the rest of the samples. $P_{wax} [(n\text{-C}_{27} + n\text{-C}_{29} + n\text{-C}_{31})/(n\text{-C}_{23} + n\text{-C}_{25} + n\text{-C}_{27} + n\text{-C}_{29} + n\text{-C}_{31})]$
230 showed low values for *Sphagnum* spp. and *M.*
231 *trifoliata* and *C. lasiocarpa* below ground parts (max. 0.2) and high values for most of the
232 vascular plant above ground parts, and *W. exannulata* (> 0.8 ; PANGAEA PDI-4071).

233 According to previous studies the average *n*-alkane chain length (ACL) should separate
234 mosses and vascular plant leaves from each other (Zhou et al., 2005). In our samples the ACL
235 of the mosses and below ground plant parts, except *E. angustifolium* and *C. nigra*, was < 26 .
236 Vascular plant above ground parts recorded ACL values > 26 (Fig. 4).

237

238 *3.3.Polar fraction*

239 The *n*-alcohol concentration had only minor differences between different plant types and the
240 dominant compounds often overlapped. Among the *Sphagnum* mosses the total concentration
241 of sterols was 2100 to 2600 $\mu\text{g g}^{-1}$. It seems that *Sphagna* had no dominant sterol, but
242 generally similar contributions from campesterol [campest-5-en-3 β -ol], stigmasterol [(24*E*)-
243 stigmasta-5,22-dien-3 β -ol] and β -sitosterol [(3 β)-stigmast-5-en-3-ol] were observed.
244 Brassicasterol [(22*E*)-ergosta-5,22-dien-3 β -ol], 24-methylcholest-7-en-3 β -ol, obtusifoliol
245 [4 α ,14 α -dimethyl-5 α -ergosta-8,24(24¹)-dien-3 β -ol], ergost-8,24(28)-dien-3 β -ol were typical
246 for *Sphagnum* spp., but were occasionally also detected in small amounts in some of the
247 vascular plants. *W. exannulata* samples were also characterized by high concentrations of
248 campesterol, stigmasterol and β -sitosterol but, in contrast to the *Sphagnum* spp., obtusifoliol
249 was not detected and the concentration of phytol was clearly highest (2035.9 $\mu\text{g g}^{-1}$, SE
250 913.3, but 437.9 $\mu\text{g g}^{-1}_{\text{rep}}$) among all the mosses. Gramisterol [4 α -methyl-5 α -ergosta-
251 7,24(24¹)-dien-3 β -ol], albeit in low concentration (ca. 10-60 $\mu\text{g g}^{-1}$), was detected in all the
252 mosses but not the vascular plants (Table 2).

253 All vascular plant below ground parts, excluding *C. nigra*, had a higher concentration of
254 sterols than above ground parts. Above parts were dominated by β -sitosterol, with the
255 occasional presence of the associated stanol (3-stigmastanol) and cycloartenol (5 α -cycloart-
256 24-en-3 β -ol). Sedge below ground parts were similar to the above ground parts, only with a
257 smaller amount of phytol [(3,7,11,15-tetramethylhexadec-2-en-1-ol; ca. 10-60 $\mu\text{g g}^{-1}$] and
258 higher amount of lupeol [5 α -lup-20(29)-en-3 β -ol; 20-250 $\mu\text{g g}^{-1}$]. *M. trifoliata* above and
259 below ground parts were dominated by stigmasterol (1483.6 $\mu\text{g g}^{-1}$ and 3647.4 $\mu\text{g g}^{-1}$). β -
260 sitosterol and obtusifoliol were absent from all *M. trifoliata* samples, whereas the schottenol
261 [5 α -stigmast-7-en-3 β -ol] was present only in *M. trifoliata* samples (above ground 678.0 $\mu\text{g g}^{-1}$
262 ¹ and below ground 1029.4 $\mu\text{g g}^{-1}$; PANGAEA PDI-4071).

263 Tocopherols- δ and $-\gamma$ [(2*R*)-2,8-dimethyl-2-((4*R*,8*R*)-4,8,12-trimethyltridecyl)-6-chromanol
264 and (2*R*)-2,7,8-trimethyl-2-(4*R*,8*R*)-4,8,12-trimethyltridecyl)-6-chromanol, respectively]
265 were only detected in *M. trifoliata*: in above ground parts tocopherol- δ 44.1 $\mu\text{g g}^{-1}$ (SE 41.8)
266 and tocopherol- γ 20.5 $\mu\text{g g}^{-1}$, and from both original and replicate below ground part samples
267 tocopherol- δ 288.4 (SE 41.8) and 100.4_{rep} μg^{-1} , and tocopherol- γ 40.2 and 225.8_{rep} $\mu\text{g g}^{-1}$.
268 Triterpenoids were present in *M. trifoliata* and the highest concentration was in *M. trifoliata*
269 below ground parts (788.8 $\mu\text{g g}^{-1}$). *Potentilla palustris* above and below ground parts were
270 dominated by β -sitosterol, below ground parts having more than double the concentration as
271 the above ground parts (4767.8 $\mu\text{g g}^{-1}$ and 1945.6, SE 196.4, respectively) (PANGAEA PDI-
272 4071).

273 Phytol was recorded in every sample. Above ground parts of both sedges and *M. trifoliata*
274 were dominated by phytol, the concentration being highest in *C. nigra* above ground parts
275 (7510.9 $\mu\text{g g}^{-1}$), while below ground parts had a lower concentrations (sedges ca. 7 to 35 μg
276 g^{-1} ; *M. trifoliata* 297.4 $\mu\text{g g}^{-1}$).

277

278 3.4. RDA results

279 RDA showed that the variation in each compound type (*n*-alkanes, *n*-alkane ratios, *n*-alcohols
280 and sterols) was related to plant components (Table 2).

281 We found that the best result was achieved by combining *n*-alkanes, *n*-alkane ratios and
282 sterols in one analysis. Monte Carlo permutation test of the RDA solution showed that all
283 canonical axes were significant (T 0.245, F 2.597, *p* 0.0020) and the three variables explained
284 25% of the variance. Analysis separated mosses and vascular plants as their own clusters
285 along the first axis. Vascular plant below ground parts and mosses, however, partly

286 overlapped over axis 1. Mosses formed a more compact cluster than vascular plants that was
287 also distributed along axis 2. The second axis reflected the differences between the below and
288 above ground parts; they were separated to the opposite ends of the axis (Fig. 5b.).

289 We present compounds which explained > 20% of variation detected in plants position in
290 ordination, i.e. 30 compounds (Fig. 5a.). Compounds such as lupeol, 3-stigmastanol and β -
291 sitosterol seemed to be descriptive for vascular plants in general. Vascular plant above
292 ground parts were characterized by *n*-alkanes in the range *n*-C₂₆ to *n*-C₂₉, *n*-C₂₃/*n*-C₂₁ and the
293 phytol concentration (Fig.5a.). The bryophyte cluster seemed to be formed on the basis of
294 ergost-8,24(28)-dien-3 β -ol, obtusifoliol, and *n*-C₂₅/*n*-C₂₉ (Fig.4b.). Some compounds, such as
295 the C₂₃ *n*-alkane, several *n*-alkane ratios and brassicasterol, commonly detected in vascular
296 plant below ground parts and in mosses, plotted mid-way between these two groups (Fig.5a,
297 b). The *n*-C₂₃/*n*-C₂₅ ratio, and lupeol and β -sitosterol concentrations were the main patterns
298 describing, and consequently separating, vascular plant below ground parts from mosses (Fig.
299 5a, b).

300

301 **4. Discussion**

302 Our results support the observation that, by using *n*-alkane ratios, different plant group
303 contributions to peat can be separated (Nott et al. 2000, Ishiwatari et al., 2005, Jansen et al.,
304 2006, Nichols et al., 2006, Zheng et al., 2007, Vonk and Gustafsson, 2009, Bingham et al.,
305 2010, Andersson et al., 2011). However, they also showed that, when a wider combination of
306 plants and peat habitats is included, the absolute values which affect ratios and the
307 relationships between plant types can change. For most of the ratios tested, vascular plant
308 above ground parts and mosses were separated as different groups. When the contribution of
309 the vascular plant below ground parts were taken into consideration, the published *n*-alkane

310 ratios for bog peat plants were less able to separate vascular plants from *Sphagnum* spp., due
311 to overlap in the distribution patterns between *Sphagnum*, sedge and *M. trifoliata* below
312 ground parts. Some ratios, such as $n\text{-C}_{23}/n\text{-C}_{27}$ (Fig. 4) may potentially be used to separate *S.*
313 *papillosum* from other *Sphagnum* spp. but the probable existence of vascular plant below
314 ground parts in peat might lead to false conclusions about the prevailing vegetation
315 assemblage. The $n\text{-C}_{23}/n\text{-C}_{25}$ ratio, which has been applied in previous studies (e.g. Bingham
316 et al., 2010) as a marker for *Sphagnum* spp., seems to be effective for the fen environment for
317 separating below ground parts (< 3) from other plants sampled (>3) (Fig. 4). Based on the
318 P_{wax} ratio, it might be possible to separate vascular plant above ground parts with lower
319 values (< 0.9) from below ground parts and *Sphagnum* spp. (Fig. 4); this agrees with Zheng
320 et al. (2007), who connected high (0.7) P_{wax} values with dry conditions in peat. Thus, where
321 the P_{wax} ratio can be measured, we would predict a high contribution of vascular plant above
322 ground material, which is consistent with a drier environment (Strak et al. 2006).

323 Our P_{aq} results agree with Ficken et al. (2000): we found similar values for most of the higher
324 plant above ground parts (< 0.1 ; PANGAEA PDI-4071), while mosses and below ground parts
325 gave values (0.4-1) close to plants in wet habitats (submerged and floating plants in lake
326 ecosystems) as in Ficken et al. (2000). Due to the high concentration of C_{31} n -alkane, the n -
327 $\text{C}_{31}/n\text{-C}_{29}$ and $n\text{-C}_{31}/n\text{-C}_{27}$ ratios show potential for distinguishing *P. palustris* from other
328 species. This corresponds to some previous studies describing $n\text{-C}_{31}$ as a marker for higher
329 plants (Jansen et al., 2006).

330 Our results agree with previous studies of bog peats which have shown that LMW n -alkanes
331 ($n\text{-C}_{23}$ and $n\text{-C}_{25}$) are important biomarkers for *Sphagnum* spp. and HMW n -alkanes ($n\text{-C}_{27}$ to
332 $n\text{-C}_{31}$) for above ground parts of vascular plants (Ficken et al., 1998; Baas et al., 2000;
333 Pancost et al., 2002; Nichols et al., 2006). Furthermore, they agree with findings that the n -

334 alkane distribution and concentration in vascular plant below ground parts differ from those
335 of above ground parts (Huang et al., 2011, Dawson et al., 2000, Pancost et al., 2002, Jansen et
336 al., 2006). ACL could also be a useful proxy for separating *Sphagnum* spp. from vascular
337 plants in fen environments (Zhou et al., 2005, Andersson et al., 2011). The LMW *n*-alkane
338 distribution in vascular plant roots has been addressed before and, like studies related to
339 *Sphagnum*.spp., the dominance of LMW *n*-alkanes, e.g. *n*-C₂₃, seems to be related to wet
340 environments (Huang et al., 2011, Xie et al., 2004). Huang et al. (2012) concluded that plants
341 growing in water saturated conditions are unlikely to synthesize longer chain *n*-alkanes in
342 order to prevent water loss. Thus, the presence of LMW *n*-alkanes is consistent with the
343 presence of wet conditions. An additional complicating issue in terms of palaeoecological
344 application is that C₂₃ has also been found in significant concentration in *Betula* spp. leaves
345 (Sachse et al., 2006).

346 Non species-specific or group-specific *n*-alcohol markers were detected, and the dominant
347 homologue within one group varied. Although *n*-alcohols can be distinguished they have not
348 been shown to have great potential when compared with other biomarkers (e.g. Xie et al.,
349 2004). Our study revealed that potential plant group-specific markers may be found among
350 sterols such as gramisterol, which was found only in mosses, and tocopherols and schottenol,
351 which were found only in *M. trifoliata*, and lupeol which was not detected in any of the
352 mosses. Otherwise, most of the sterols were commonly present in most of the samples,
353 although concentrations differed considerably, e.g. in the case of β -sitosterol. In agreement
354 with Huang et al. (2011) we detected a higher concentration of sterols in the vascular plant
355 below ground parts than in above ground parts. For sterol distributions to be used as a proxy
356 for past vegetation inputs to a fen environment, it is important that either the original sterol or
357 corresponding degradation product(s) can be identified within core materials. It has been
358 shown that microbial hydrogenation of sterols within peats can lead to the production of

359 stanols from the original Δ^5 -sterols (e.g. Andersson and Meyers, 2012). It might be expected
360 that with greater degradation of organic matter in a fen environment there will be greater
361 transformation of sterols to stanols. However, if both the sterols and their corresponding
362 stanols can be identified and quantified in a fen core, it may be possible to both assess the
363 degree of organic matter degradation and identify the original vegetation contributions to the
364 peatland. This requires further testing, but our data suggest that, if sterols and stanols are
365 present in peat, they may provide additional information about the contributing vegetation
366 (Meyers 2003).

367 The differences detected between mosses and vascular plants, as well as the similarities
368 between *Sphagnum* spp. and below ground vascular plant parts could spring from the
369 differences in the surrounding hydrological conditions. Mosses and below ground plant parts
370 are under the influence of stagnant water in fens, where the water table can be close to the
371 mire surface throughout the growing season (Laine et al., 2012). These plants and plant parts
372 in fens might therefore produce wax with a higher abundance of LMW *n*-alkanes for
373 protection against micro-organisms and degradation than in moderately drier habitats, i.e.
374 bogs. In the future, one way to study the source of water and the hydrological environment of
375 different *n*-alkanes in peat is to examine the δD values of different plant *n*-alkanes (e.g. Xie et
376 al., 2004, Nichols et al., 2010, Garcin et al., 2012).

377 The data presented here shows that there are differences in biomarker distributions between
378 fen plants, but also between species which live in fens and bogs. This means that the
379 application of biomarker distributions from plants living in bog environments to cores from
380 fens could give mis-leading information about past vegetation contributions. In order to apply
381 the data presented here to a fen environment may not be straightforward, however. Although
382 some promising individual biomarkers were found, a better way to identify species and plant

383 groups appeared to be to combine the variables and apply constrained multivariate analysis,
384 such as RDA, as applied here. As a result of the similarity in *n*-alkane distributions, and
385 consequently *n*-alkane ratios, similarities between mosses and vascular plant below ground
386 parts remained apparent, but the differences in sterol compositions separated these two
387 groups (Fig. 5b). As a result of our investigations, we would recommend that potential target
388 ratios or markers for down-core analyses might include: (i) lupeol and a high concentration of
389 β -sitosterol, together with $n\text{-C}_{23}/n\text{-C}_{25} > 3$, for prevalence of vascular plant below ground
390 parts; (ii) high $n\text{-C}_{23}/n\text{-C}_{31}$ value, combined with the presence of obtusifoliol and gramisterol
391 to indicate the presence of *Sphagnum* mosses; and (iii) a high concentration of HMW *n*-
392 alkanes, as in previous studies, for a dominance of vascular plant above ground parts. The
393 degradation of the compounds, especially sterols, has to be considered as they might not be
394 preserved in fen environments, due to a high rate of humification or possible transport in the
395 system. A detailed study of this matter is in progress. The effect of peat humification on lipid
396 concentration should also be taken into account by calculating concentration relative to TOC
397 content. This procedure normalizes the results so that different layers with different extent of
398 degradation become more comparable (Meyers 2003; Ortiz et al., 2010). Given the freshness
399 of our samples, this did not impact on our results, but should be considered for palaeo-
400 studies.

401

402 **5. Conclusions**

403 We found no clear difference in the sterol composition of the living fen plants but, when
404 comparing *n*-alkanes and their ratios, vascular plant above ground parts could be separated
405 from mosses. However, due to the similar *n*-alkane composition between most of the vascular
406 plant below ground parts and mosses and consequently similar *n*-alkane ratios, separating

407 these two groups from highly humified peat can be challenging. When *n*-alkanes, their ratios
408 and sterols of the plants were compared, together with redundancy analysis, three groups
409 were formed: mosses, above ground and below ground. Thus RDA, or a comparable
410 approach, has potential for also differentiating plant groups in fossil peats. Our results also
411 show that the existing biomarker proxies for peatlands are challenged when a wider
412 combination of plants and peat environments is taken into account.

413

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417

418 **References**

419 Alm, J., Korhola, A., Turunen, J., Saarnio, S., Jungner, H., Tolonen, K., Silvola, J., 1999.
420 Past and future atmospheric carbon gas (CO₂, CH₄) exchange in boreal peatlands.
421 International Peat Journal 9, 127-135.

422 Andersson, R.A., Meyers, P.A., 2012. Effect of climate change on delivery and degradation
423 of lipid biomarkers in a Holocene peat sequence in the eastern European Russian arctic.
424 Organic Geochemistry 53, 63-72.

425

426 Andersson, R.A., Kuhry, P., Meyers, P., Zebühr, Y., Crill, P., Mörth, M., 2011. Impacts of
427 paleohydrological changes on *n*-alkane biomarker compositions of a Holocene peat sequence
428 in the eastern European Russian Arctic. Organic Geochemistry 42, 1065-1075

429 Avsejs, L.A., Nott, C.J., Xie, S., Maddy, D., Chambers, F.M., Evershed, R.P., 2002. 5-*n*-
430 Alkylresorcinols as biomarkers of sedges in an ombrotrophic peat section. *Organic*
431 *Geochemistry* 33, 861-867.

432 Baas M., Pancost R., Van Geel B., Sinninghe Damsté J.S. 2000. A comparative study of
433 lipids in *Sphagnum* species. *Organic Geochemistry* 31: 535-541.

434 Barber, K., Dumayne-Peaty, L., Hughes, P., Mauquoy, D., Scaife, R. 1998. Replicability and
435 variability of the recent macrofossil and proxy-climate record from raised bogs: Field
436 stratigraphy and macrofossil data from Bolton fell moss and Walton moss, Cumbria,
437 England. *Journal of Quaternary Science* 13: 515-528.

438 Bingham, E.M., McClymont, E.L., Väiliranta, M., Mauquoy, D., Roberts, Z., Chambers, F.M.,
439 Pancost, R.D., Evershed, R.P. 2010. Conservative composition of *n*-alkane biomarkers in
440 *Sphagnum* species: Implications for palaeoclimate reconstruction in ombrotrophic peat bogs.
441 *Organic Geochemistry* 41: 214-220.

442 Dawson, L.A., Mayes, R.W., Elston, D.A., Smart, T.S. 2000. Root hydrocarbons as potential
443 markers for determining species composition. *Plant, Cell and Environment* 23: 743-750.

444 Eurola S., Bendiksen K., Rönkä A. 1992. Suokasviopas. Oulanka Biological Station
445 University of Oulu. Oulu. Oulanka reports, ISSN 0358-3651; 11.

446 Ficken K.J., Barber K.E., Eglinton G. 1998. Lipid biomarker, $\delta^{13}\text{C}$ and plant macrofossil
447 stratigraphy of a Scottish montane peat bog over the last two millennia. *Organic*
448 *Geochemistry* 28: 217-237.

449 Ficken K.J., Li B., Swain D.L., Eglinton G. 2000. An *n*-alkane proxy for the sedimentary
450 input of submerged/floating freshwater aquatic macrophytes. *Organic Geochemistry* 31: 745-
451 749.

452 Garcin Y., Schwab V.F., Gleixner G., Kahmen A., Todou G., Séné O., Onana J.-.,
453 Achoundong G., Sachse D. 2012. Hydrogen isotope ratios of lacustrine sedimentary *n*-
454 alkanes as proxies of tropical African hydrology: Insights from a calibration transect across
455 Cameroon. *Geochimica et Cosmochimica Acta* 79: 106-126.

456 Goad L.J., Akihisa T. 1997. *Analysis of sterols*. Blackie Academic and Professional, London.

457 Huang X., Wang C., Zhang J., Wiesenberg G.L.B., Zhang Z., Xie S. 2011. Comparison of
458 free lipid compositions between roots and leaves of plants in the Dajiuhu Peatland, Central
459 China. *Geochemical Journal* 45: 365-373,

460 Huang X., Xue J., Zhang J., Qin Y., Meyers P.A., Wang H. 2012. Effect of different wetness
461 conditions on Sphagnum lipid composition in the Erxianyan peatland, Central China. *Organic*
462 *Geochemistry* 44: 1-7.

463 Ishiwatari R., Yamamoto S, Uemura H. 2005. Lipid and lignin/cutin compounds in Lake
464 Baikal sediments over the last 37 kyr: Implications for glacial-interglacial
465 palaeoenvironmental change. *Organic Geochemistry* 36: 327-347.

466 Jansen B., Nierop K.G.J., Hageman J.A., Cleef A.M., Verstraten J.M. 2006. The straight-
467 chain lipid biomarker composition of plant species responsible for the dominant biomass
468 production along two altitudinal transects in the Ecuadorian Andes. *Organic Geochemistry*
469 37: 1514-1536.

470 Jia, G., Dungait, J.A.J., Bingham, E.M., Valiranta, M., Korhola, A., Evershed, R.P. 2008.
471 Neutral monosaccharides as biomarker proxies for bog-forming plants for application to
472 palaeovegetation reconstruction in ombrotrophic peat deposits. *Organic Geochemistry* 39:
473 1790-1799.

474 Jiao, D.; Perry, R. S.; Engel, M. H.; Sephton, M. A. Biomarker indicators of bacterial activity
475 and organic fluxes during end Triassic mass extinction event. *Instruments, Methods, and*
476 *Missions for Astrobiology XI*; 2008; Vol. 7097, ISBN: 978-081947317-2.

477 Killops S.D., Frewin N.L. 1994. Triterpenoid diagenesis and cuticular preservation. *Organic*
478 *Geochemistry* 21: 1193-1209.

479 Laine, A., Byrne, K.A., Kiely, G., Tuittila, E.-. 2007. Patterns in vegetation and CO₂
480 dynamics along a water level gradient in a lowland blanket bog. *Ecosystems* 10: 890-905.

481 Laine A.M., Juurola E., Hájek T., Tuittila E.-. 2011. *Sphagnum* growth and ecophysiology
482 during mire succession. *Oecologia* 167: 1115-1125.

483 Laine A.M., Bubier J., Riutta T., Nilsson M.B., Moore T.R., Vasander H., Tuittila E.-. 2012.
484 Abundance and composition of plant biomass as potential controls for mire net ecosystem CO₂
485 exchange. *Botany* 90: 63-74.

486 Laine, J., Harju, P., Timonen, T., Laine, A., Tuittila, E., Minkkinen, K., Vasander, H. 2009.
487 *The Intricate Beauty of Sphagnum Mosses: A Finnish Guide for Identification*. University of
488 Helsinki Department of Forest Ecology, Helsinki.

489 Lehtonen, K., Ketola, M. 1993. Solvent-extractable lipids of *Sphagnum*, *Carex*, *Bryales* and
490 *Carex-Bryales* peats: Content and compositional features vs peat humification. *Organic*
491 *Geochemistry* 20: 363-380.

492 Leppälä M., Laine A.M., Seväkivi M.-L., Tuittila E.-T. 2011. Differences in CO₂ dynamics
493 between successional mire plant communities during wet and dry summers. *Journal of*
494 *Vegetation Science* 22: 357-366.

495 Levy P.E., Burden A., Cooper M.D.A., Dinsmore K.J., Drewer J., Evans C., Fowler D.,
496 Gaiawyn J., Gray A., Jones S.K., Jones T., Mcnamara N.P., Mills R., Ostle N., Sheppard L.J.,
497 Skiba U., Sowerby A., Ward S.E., Zieliński P., 2012. Methane emissions from soils:
498 Synthesis and analysis of a large UK data set. *Global Change Biology* 18: 1657-1669.

499 López-Días, V., Borrego, T., Blanco, C.G., Arboleya, M., López-Sáez, J.A., López-Merino,
500 L. 2010. Biomarkers in a peat deposit in northern Spain (Huelga de Bayas, Asturias) as proxy
501 for climate variation. *Journal of Chromatography A* 1217: 3538-3546.

502 Mauquoy, D., Engelkes, T., Groot, M.H.M., Markesteijn, F., Oudejans, M.G., Van Der
503 Plicht, J., Van Geel, B. 2002. High-resolution records of late-Holocene climate change and
504 carbon accumulation in two North-West European ombrotrophic peat bogs. *Palaeogeography,*
505 *Palaeoclimatology and Palaeoecology* 186: 275-310.

506 McClymont, E.L., Mauquoy, D., Yeloff, D., Broekens, P., Van Geel, B., Charman, D.J.,
507 Pancost, R.D., Chambers, F.M., Evershed, R.P. 2008. The disappearance of *Sphagnum*
508 *imbricatum* from Butterburn flow, UK. *Holocene* 18: 991-1002.

509 Meyers P.A. 2003. Applications of organic geochemistry to paleolimnological
510 reconstructions: A summary of examples from the Laurentian Great Lakes. *Organic*
511 *Geochemistry* 34: 261-289.

512 Moore P.D. 2002. The future of cool temperate bogs. *Environment Conservation* 29: 3-20.

513 Moore, T.R., Bubier, J.L., Bledzki, L. 2007. Litter decomposition in temperate peatland
514 ecosystems: The effect of substrate and site. *Ecosystems* 10: 949-963.

515 Nichols, J.E., Booth, R.K., Jackson, S.T., Pendall, E.G., Huang, Y. 2006. Paleohydrologic
516 reconstruction based on *n*-alkane distributions in ombrotrophic peat. *Organic Geochemistry*
517 37: 1505-1513.

518 Nichols J., Booth R.K., Jackson S.T., Pendall E.G., Huang Y. 2010. Differential hydrogen
519 isotopic ratios of *Sphagnum* and vascular plant biomarkers in ombrotrophic peatlands as a
520 quantitative proxy for precipitation-evaporation balance. *Geochimica et Cosmochimica Acta*
521 74: 1407-1416.

522 Nishimura, M. 1977. Origin of stanols in young lacustrine sediments. *Nature* 270(5639): 711-
523 712.

524 Nott C.J., Xie S., Avsejs L.A., Maddy D., Chambers F.M., Evershed R.P. 2000. *n*-Alkane
525 distributions in ombrotrophic mires as indicators of vegetation change related to climatic
526 variation. *Organic Geochemistry* 31: 231-235.

527 Ortiz J.E., Gallego J.L.R., Torres T., Díaz-Bautista A. Sierra C. 2010. Palaeoenvironmental
528 reconstruction of Northern Spain during the last 8000 cal yr BP based on the biomarker
529 content of the Roñanzas peat bog (Asturias). *Organic Geochemistry* 41: 454-466.

530 Ortiz J.E., Díaz-Bautista A., Aldasoro J.J., Torres T., Gallego J.L.R., Moreno L., Estébanez
531 B. 2011. *n*-Alkan-2-ones in peat-forming plants from the Roñanzas ombrotrophic bog
532 (Asturias, Northern Spain). *Organic Geochemistry* 42: 586-592.

533 Pancost, R.D., Baas, M., Van Geel, B., Sinninghe Damsté, J.S. 2003. Response of an
534 ombrotrophic bog to a regional climate event revealed by macrofossil, molecular and carbon
535 isotopic data. *Holocene* 13: 921-932.

536 Pancost, R.D., Baas, M., Van Geel, B., Sinninghe Damsté, J.S. 2002. Biomarkers as proxies
537 for plant inputs to peats: An example from a sub-boreal ombrotrophic bog. *Organic*
538 *Geochemistry* 33: 675-690.

539 Riutta, T., Laine, J., Aurela, M., Rinne, J., Vesala, T., Laurila, T., Haapanala, S., Pihlatie, M.,
540 Tuittila, E.-. 2007. Spatial variation in plant community functions regulates carbon gas
541 dynamics in a boreal fen ecosystem. *Tellus Series B Chemical and Physical Meteorology*
542 59: 838-852.

543 Rydin H., Jeglum J.K., Hooijer A. 2006. *The biology of peatlands*. Oxford University Press,
544 Oxford.

545 Saarinen T. 1996. Biomass and production of two vascular plants in a boreal mesotrophic fen.
546 *Canadian Journal of Botany* 74: 934-938.

547 Sachse D., Radke J., Gleixner G. 2006. δD values of individual n-alkanes from terrestrial
548 plants along a climatic gradient - Implications for the sedimentary biomarker record. *Organic*
549 *Geochemistry* 37: 469-483.

550

551 Strack M., Waller M.F., Waddington J.M. 2006. Sedge succession and peatland methane
552 dynamics: A potential feedback to climate change. *Ecosystems* 9: 278-287

553 ter Braak, C. J. F., P. Šmilauer. 2002. CANOCO reference manual and CanoDraw for
554 Windows user's guide: Software for canonical community ordination (version 4.5).
555 Microcomputer Power, Ithaca, NY.

556 Tuittila, E-S., Välranta, M., Laine, A., Korhola, A. 2007. Controls of mire vegetation
557 succession in a southern boreal bog. *Journal of Vegetation Science* 18: 891-902.

558 Waddington, J.M., Roulet, N.T. 2000. Carbon balance of a boreal patterned peatland. *Global*
559 *Change Biology* 6: 87-97.

560 Wheeler B.D., Proctor M.C.F. 2000. Ecological gradients, subdivisions and terminology of
561 north-west European mires. *Journal of Ecology* 88: 187-203.

562 Vonk, J.E., Gustafsson, O. 2009. Calibrating n-alkane sphagnum proxies in sub-arctic
563 Scandinavia. *Organic Geochemistry* 40: 1085-1090.

564 Välranta, M., Korhola, A., Seppä, H., Tuittila, E.-., Sarmaja-Korjonen, K., Laine, J., Alm, J.
565 2007. High-resolution reconstruction of wetness dynamics in a southern boreal raised bog,
566 Finland, during the late Holocene: A quantitative approach. *Holocene* 17: 1093-1107.

567 Yu, Z., Loisel, J., Brosseau, D. P., Beilman, D. W., Hunt, S. J. 2010. Global peatland
568 dynamics since the Last Glacial Maximum. *Geophysical Research Letters* 37: L13402

569 Xie S., Nott C.J., Avsejs L.A., Volders F., Maddy D., Chambers F.M., Gledhill A., Carter
570 J.F., Evershed R.P. 2000. Palaeoclimate records in compound-specific δD values of a lipid
571 biomarker in ombrotrophic peat. *Organic Geochemistry* 31: 1053-1057.

572 Xie, S., Nott, C.J., Avsejs, L.A., Maddy, D., Chambers, F.M., Evershed, R.P. 2004.
573 Molecular and isotopic stratigraphy in an ombrotrophic mire for paleoclimate reconstruction.
574 *Geochimica et Cosmochimica Acta* 68: 2849-2862.

575 Zheng Y., Zhou W., Meyers P.A., Xie S. 2007. Lipid biomarkers in the Zoigê-Hongyuan peat
576 deposit: Indicators of Holocene climate changes in West China. *Organic Geochemistry* 38:
577 1927-1940.

578 Zhou W., Xie S., Meyers P.A., Zheng Y. 2005. Reconstruction of late glacial and Holocene
579 climate evolution in Southern China from geolipids and pollen in the Dingnan peat sequence.
580 *Organic Geochemistry* 36: 1272-1284.

581 Økland R.H., Okland T., Rydgren K. 2001. A Scandinavian perspective on ecological
582 gradients in north-west European mires: Reply to Wheeler and Proctor. *Journal of Ecology*
583 89: 481-486.

584

585 **Captions:**

586 **Fig. 1.** Map of study site. Samples were collected from the fens of the Siikajoki commune
587 (64°45'N, 24°42'E), Finland, Northern-Europe.

588

589 **Fig. 2.** Correlation between concentration $\mu\text{g g}^{-1}$ dry wt and $\mu\text{g g}^{-1}$ TOC. R^2 values of
590 compared variables: *n*-C₂₃ 0.99, *n*-C₃₁ 1, β -sitosterol 0.99, campesterol 0.99.

591

592 **Fig.3.** Concentration ($\mu\text{g g}^{-1}$ dry wt.) for C₁₇-C₃₅ *n*-alkanes of plants. Mosses (top), above
593 ground and below ground parts of sedges (middle) and forbes (bottom) are shown. Black bars

594 represent original samples, samples which were re-analyzed have error bars and samples
595 which were replicated are represented with white bars.

596

597 **Fig. 4.** Ratios of *n*-alkanes for mosses and (A) above ground and (B) below ground parts of
598 sedges and forbes: $n\text{-C}_{23}/n\text{-C}_{25}$ (Bingham et al., 2010), $C_{23}/n\text{-C}_{27}$, $n\text{-C}_{23}/n\text{-C}_{31}$ (Nott et al.,
599 2000), $n\text{-}n\text{-C}_{23}/(n\text{-C}_{23} + n\text{-C}_{29})$ (Nichols et al., 2006), $n\text{-C}_{25}/n\text{-C}_{29}$ (Jansen et al., 2006), $n\text{-}$
600 $C_{31}/n\text{-C}_{27}$ (Janssen et al., 2006) and P_{wax} (Zheng et al., 2007) and ACL of the plant
601 components. Values of $n\text{-C}_{23}/n\text{-C}_{31}$ for *Warn. exannulta*, *Carex nigra* and *C. lasiocarpa*
602 below ground parts are 0. For ACL, *Sphagnum papillosum*, *Carex rostrata* above ground and
603 below ground parts and *P. palustris* and *M. trifoliata* below ground parts standard error of
604 mean is < 0.5 .

605

606 **Fig. 5.** RDA (F-ratio 2.597, *p*-value 0.002) shows the distribution of *n*-alkanes, *n*-alkane
607 ratios, sterols and sampled plants (A, above-ground; B, below-ground). Groups: moss, above
608 ground and below ground plant parts, were used as environmental variables. Only compounds
609 with a fit of $> 20\%$ are shown (altogether 30 compounds).

610

611 **Table 1**

612 Studied plants and their status along the nutrient gradient from poor to rich: ombro-, oligo-,
613 meso-, minero-, eutrophic, and their typical location in microhabitats from dry to wet:
614 hummock, lawn, flark.

615

616 **Table 2**

- 617 Results RDA with Monte Carlo permutation test to test the significance of plant components:
- 618 mosses, leaves and roots for the variation in compounds ^a.



Fig 1.

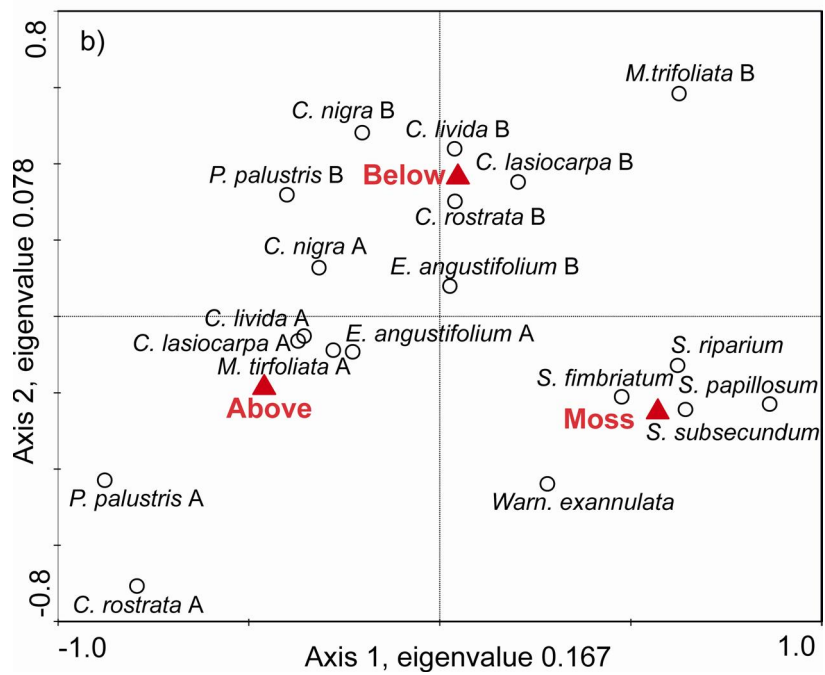
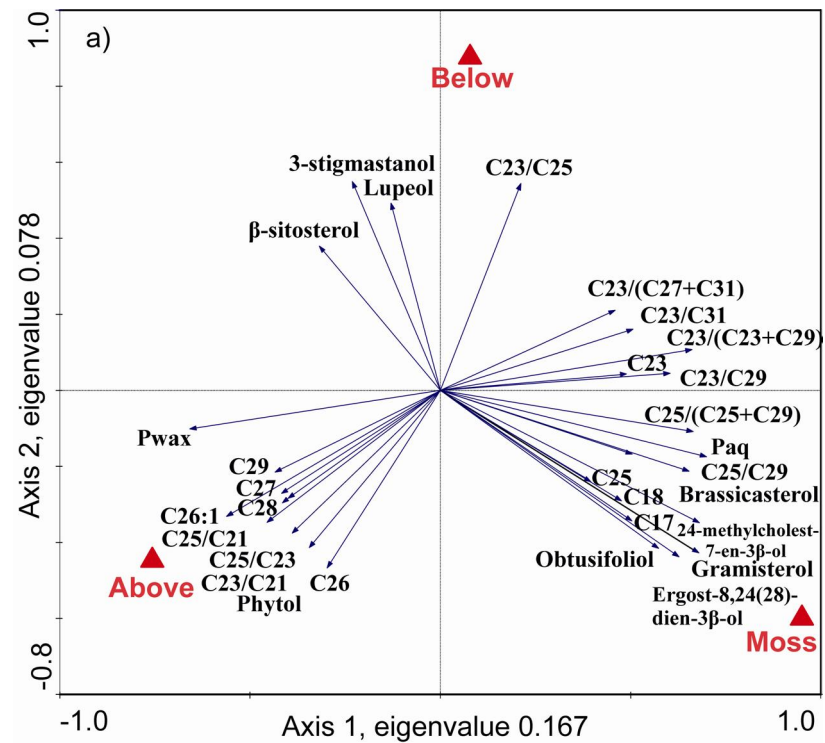


Fig 4.

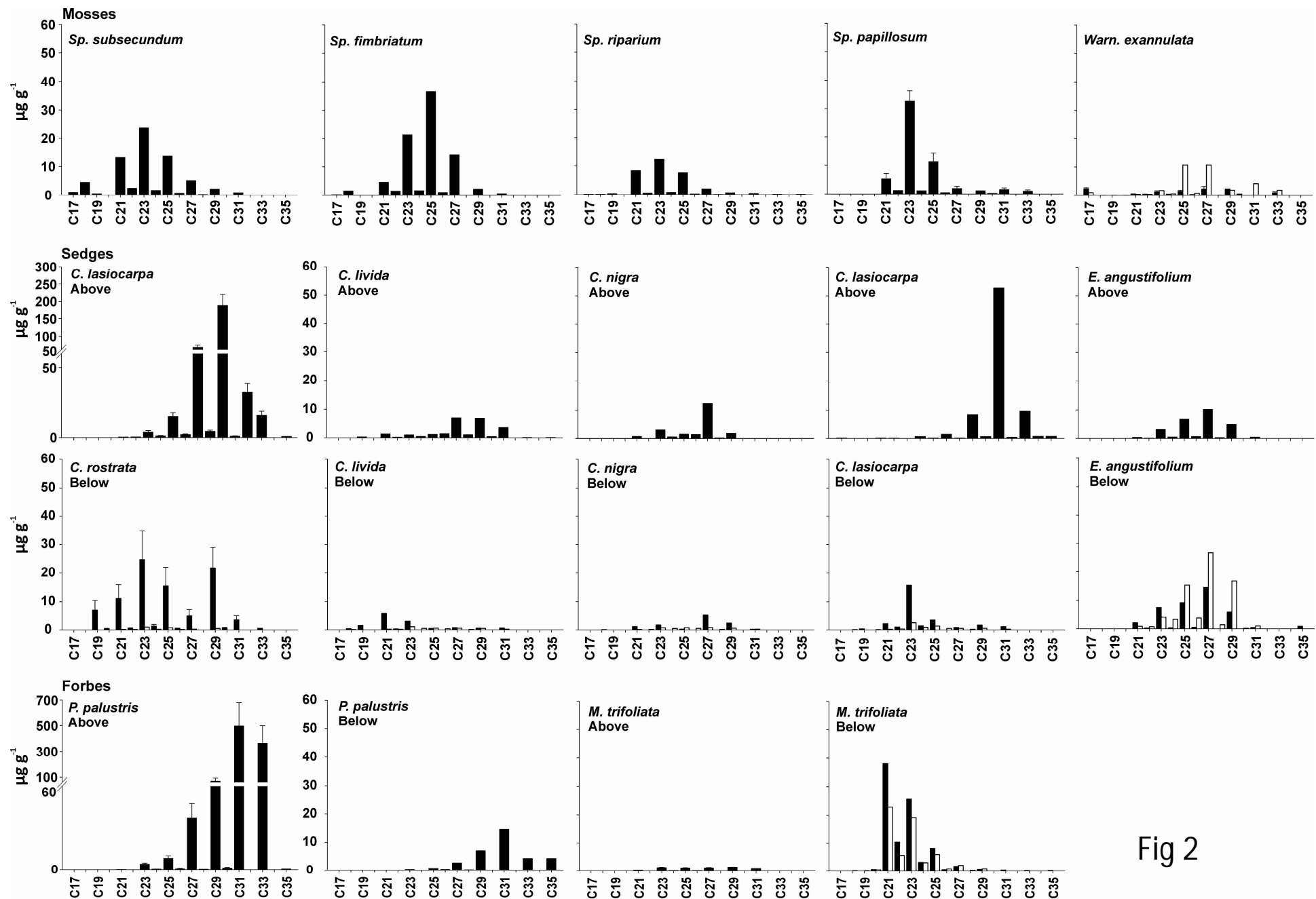


Fig 2

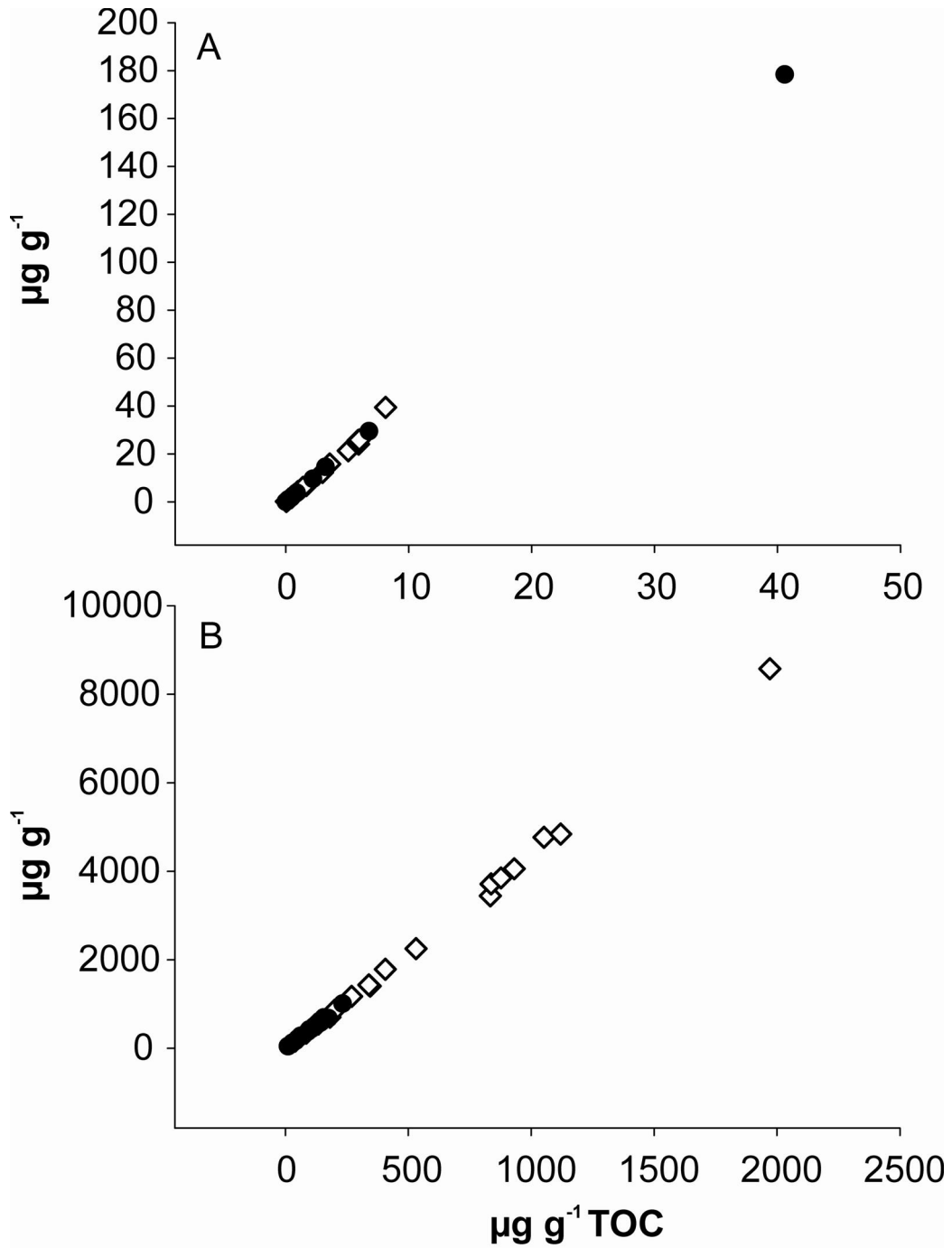


Fig 3

A: \diamond C₂₃ \bullet C₃₁ B: \diamond β-sitosterol \bullet Campesterol

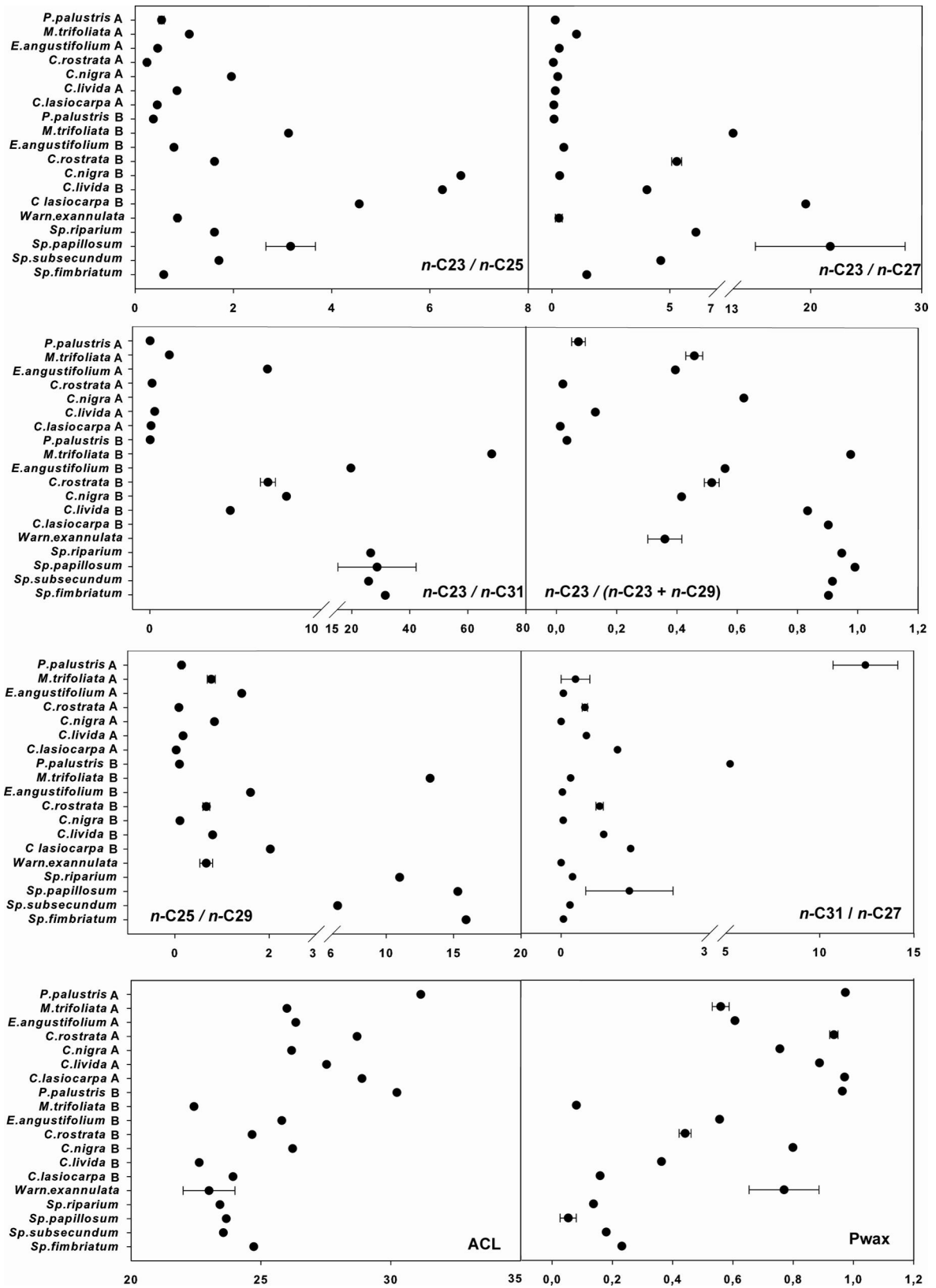


Fig 3.