n-Alkan-2-ones in peat-forming plants from the Roñanzas ombrotrophic bog (Asturias, northern Spain)

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A B S T R A C T

We determined the distribution of lipids (*n*-alkanes and *n*-alkan-2-ones) in present-day peat-forming plants in the Roñanzas Bog in northern Spain. Consistent with the observation of others, most *Sphagnum* (moss) species alkanes maximized at C_{23}, whereas the other plants maximized at higher molecular weight (C_{27} to C_{31}). We show for the first time that plants other than seagrass and *Sphagnum* moss contain *n*-alkan-2-ones. Almost all the species analysed showed an *n*-alkan-2-one distribution between C_{21} and C_{31} with an odd/even predominance, maximizing at C_{27} or C_{29}, except ferns, which maximized at lower molecular weight (C_{21}–C_{23}). We also observed that microbial degradation can be a major contributor to the *n*-alkan-2-one distribution in sediments as opposed to a direct input of ketones from plants.

1. Introduction

Biomarkers such as *n*-alkanes and *n*-alkan-2-ones are widely observed in peat samples from ombrotrophic mires. There is extensive literature about the significance of *n*-alkanes and the profile of these lipids has been used to discriminate *Sphagnum* vs. non-*Sphagnum* inputs and, furthermore, to interpret palaeoenvironmental conditions linked to humid or drier climatic phases (Nott et al., 2000; Pancost et al., 2002; Avsejs et al., 2002; McClymont et al., 2005; Nichols et al., 2006; Ortiz et al., 2010).

n-Alkan-2-ones also occur in peat (Morrison and Bick, 1967; Lehtonen and Ketola, 1990,1993 Xie et al., 2004; Nichols and Huang, 2007; Zheng et al., 2007; Ortiz et al., 2010), but less is known about their significance. In addition to their direct provenance from plants (Arpino et al., 1970; Volkman et al., 1981), long chain ketones may have other origins, including microbial oxidation of the corresponding *n*-alkanes (Cranwell et al., 1987; Amblès et al., 1993; Jaffé et al., 1993, 1996; van Bergen et al., 1998) or microbial β-oxidation and decarboxylation of *n*-fatty acids (Volkman et al., 1983; Chaffee et al., 1986; de Leeuw, 1986; Quénéa et al., 2004). These origins are particularly relevant to peat bogs, which can register considerable microbial activity (cf. Lehtonen and Ketola, 1990; Zheng et al., 2007).

Thus, although *n*-alkan-2-ones have been addressed extensively in various kinds of sediments, most studies have focussed mainly on their distribution and origin, and assessments of their use as palaeoclimate proxies in sediments remain scarce (Xie et al., 2003, 2008) in comparison with other *n*-alkyl lipids. Furthermore, only a few studies show the *n*-alkan-2-one distribution in modern plants, with C_{17} to C_{33} components being observed in cyanobacteria and aquatic macrophytes (Wenchuan et al., 1999), as well as seagrass (Hernández et al., 2001) and *Sphagnum* species (Baas et al., 2000; Nichols and Huang, 2007). C_{25} to C_{31} *n*-alkan-2-ones have also been reported in measurable but very low concentrations in forest and *paramo* plant species in the Northern Ecuadorian Andes (Jansen and Nierop, 2009).

Here we address the distribution of *n*-alkanes and *n*-alkan-2-ones in diverse peat-forming plants collected from the Roñanzas ombrotrophic bog (Asturias, northern Spain) and discuss their possible microbial origin.

2. Methodology

Field specimens of the plants (Table 1) were collected in mid September 2009 from the Roñanzas bog (43°20′13″N; 04°51′01″W; 250 m), which covers ca. 80,000 m^2. In most cases leaves were selected, although for others, both leaves and stems or flowers were analysed. Five *Sphagnum* species were sampled (three of them different from those examined by Baas et al. (2000) and Nichols and Huang (2007): *Sphagnum subnitens*, *Sphagnum subsecundum* and *Sphagnum denticulatum*) together with two non-*Sphagnum* mosses (*Campylopus pyriformis and Campylopus*

Table 1
Predominant *n*-alkane, *n*-alkan-2-one and *n*-alkanoic acid carbon numbers (C_{no}) in leaves and stems of present-day plants from Roñanzas peat bog and *n*-alkan-2-one/*n*-alkane ratio.

Name	Common name	*n*-Alkane C_{no}	*n*-Alkan-2-one C_{no}	*n*-Alkanoic C_{no}	*n*-Alkan-2-one/*n*-alkane
Calluna vulgaris (leaves)	Scotch heather	31	29	24	0.009
Calluna vulgaris (stem)	Scotch heather	29	27	24	0.200
Campylopus introflexus	Moss	27	27	24	0.078
Campylopus pyriformis	Moss	29	27	24	0.131
Carex canescens	Silvery sedge	27	29	24	0.059
Drosera intermedia	Spoonleaf sundew	27	27	24	0.044
Erica mackaiana (leaves)	Mackay's heather	31	27	28	0.009
Erica mackaiana (stem)	Mackay's heather	31	27	22	0.051
Juncus articulatus	Jointleaf rush	31	31	24	0.008
Juncus effusus (flowers)	Soft rush	23	27	22	0.031
Juncus effusus (stem)	Soft rush	29	29	24	0.075
Molinia caerulea	Purple moor grass	29	27	26	0.037
Molinia sp.	Grass	31	31	24	0.040
Osmunda regalis	Fern	31	–	30	–
Pteridium aquilinum (leaves)	Fern	27	21	24	0.038
Pteridium aquilinum (stem)	Fern	27	23	24	0.016
Rhynchospora alba	White beaksedge	31	29	22	0.019
Sphagnum compactum	*Sphagnum* moss	23	27	24	0.148
Sphagnum cuspidatum	*Sphagnum* moss	31	27	24	0.089
Sphagnum denticulatum	*Sphagnum* moss	23	27	24	0.132
Sphagnum subnitens	*Sphagnum* moss	23	27	24	0.151
Sphagnum subsecundum	*Sphagnum* moss	25	27	24	0.193
Ulex europaeus (leaves)	Gorse	29	27	22	0.017
Ulex europaeus (stem)	Gorse	27	27	22	0.053

introflexus), two Ericaceae species, ferns and some other plant types (Table 1).

In order to avoid degradation and contamination, samples were stored frozen until analysis 2–3 days after collection. They were then washed independently with distilled deionized water, classified and analysed following the same procedure as peat samples taken along the 2 m deep record for palaeoenvironmental reconstruction during the last 8 cal ka B.P. (cf. Ortiz et al., 2010). To prevent contamination between samples (e.g. waxes ejected to the air that might attach to other plants), we worked with only one sample from opening the bag until putting the sample into the cell for extraction.

Samples were extracted using an accelerated solvent extractor (Dionex ASE 200) with dichloromethane (DCM)/MeOH (2:1) at 1500 psi and 175 °C. The heating phase was 8 min and the static extraction time 5 min.

The extract was concentrated using a rotary evaporator. Three fractions, A, B and C respectively, were obtained by passing the extract through a silica/alumina column (14.2 g silica, 7.7 g alumina; 70–230 mm mesh) and washing it using solvents of different polarity (80 ml in all cases): hexane (A), DCM/hexane (4:1, B) and MeOH (C) to afford neutral, polar and acid fractions. Prior to analysis, fractions were methlylated with trimethylsilyldiazomethane and MeOH for 20 min and the solvent evaporated with N_2. Only fractions A and B were analysed. Samples were injected into an HP 6890 gas chromatograph equipped with a selective mass detector (HP 5973) and an ATM-5 column (25 m × 0.25 mm; 0.20 μm). We used He as carrier gas and decafluorobiphenyl as internal standard. The oven temperature programme was: 60–300 °C (held 20 min) at 6 °C/min and the injector was maintained at 275 °C. Components were identified with the Data Analysis programme and the Wiley Library; *n*-alkane distributions were obtained using the base peak chromatograms for fraction A, the *m/z* 59 chromatograms for the *n*-alkan-2-ones in fraction B and the *m/z* 74 chromatograms for the *n*-alkanoic acids in fraction B. For quantification, we used the internal standard for each sample and some *n*-alkane standards at different concentrations, which were analysed before and after the injection of the plant samples.

3. Results and discussion

The leaves and stems of *Sphagnum* and Ericaceae species comprise the main plant input to the Roñanzas peat bog, although other plants also influenced peat composition. For almost all *Sphagnum* spp., the *n*-alkanes maximized at C_{23}, except *S. subsecundum* (bimodal at C_{25} and C_{29}) and *S. cuspidatum*, (bimodal at C_{23} and C_{29}). The other plants exhibited a greater predominance of higher molecular weight (HMW) *n*-alkanes, mainly at C_{29} and C_{31} (Tables 1 and 2). These results coincide with previous studies (Baas et al., 2000; Nott et al., 2000; Pancost et al., 2002; Nichols et al., 2006) showing that the predominant *n*-alkane in *Sphagnum* is C_{23}, while C_{31} is the most abundant in other plants.

In most cases the *n*-alkan-2-ones ranged from C_{21} to C_{31}, with an odd/even predominance (Table 3; Fig. 1). Only for the fern *Osmunda regalis* were no ketones detected. The *n*-alkan-2-ones in *Sphagnum* spp. maximized at C_{27}, like Ericaceae species and other plants, in which the C_{29} homologue was also predominant. Only the fern *Pteridium aquilinum* ketones maximized at C_{21} or C_{23}.

These results coincide with the findings of previous studies (Baas et al., 2000; Nichols and Huang, 2007), in which long chain odd *n*-alkan-2-ones between C_{23} and C_{31} were observed in all species of *Sphagnum*, maximizing at C_{27}. However, other common plant species in bogs also contain *n*-alkan-2-ones differing from those in *Sphagnum* spp. (Nichols and Huang, 2007), so these compounds cannot be considered exclusive biomarkers for the genus *Sphagnum* in freshwater peatlands. Our study included other non-*Sphagnum* species than those examined by Nichols and Huang (2007) in North America; only one genus (*Carex*) is common to both studies. In our view, the different species addressed in these two studies would explain why we observed the presence of *n*-alkan-2-ones in species other than *Sphagnum* and they did not. In fact, it is noticeable that we did not detect *n*-alkan-2-ones in one fern (*O. regalis*), whereas in leaves and stems of the other fern species (*P. aquilinum*) they were present (Table 2). Another explanation would be because the North American plants analysed in Nichols and Huang (2007) had ketones, but not in detectable amounts.

Table 2
Concentration (n.d. – not detected) of *n*-alkanes in leaves and stems of plants from Roñanzas peat bog (highest values in bold).

Species	Alkane C_{no} (μg/g dry plant matter)																	
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	Sum
Calluna vulgaris (leaves)	0.00	0.39	4.00	0.91	38.98	1.65	8.51	1.42	16.87	4.72	84.06	9.64	51.85	10.90	**186.53**	13.59	99.36	533.38
Calluna vulgaris (stem)	0.19	0.18	0.47	0.46	2.18	1.16	3.95	1.03	4.12	1.33	11.32	1.92	**6.84**	0.88	5.23	0.14	0.83	42.23
Campylopus introflexus	0.42	0.07	0.52	0.19	0.93	1.37	0.29	0.20	0.61	0.22	**4.75**	0.63	4.28	0.46	4.96	0.31	0.81	21.02
Campylopus pyriformis	0.16	0.11	0.23	0.19	1.37	1.12	0.65	0.32	1.16	0.31	2.38	0.76	**4.16**	0.36	3.30	0.22	0.34	17.14
Carex Canescens	0.45	0.20	0.91	0.22	1.71	0.70	3.72	0.99	6.37	1.27	**9.29**	1.17	6.98	0.59	3.07	0.10	0.76	38.50
Drosera intermedia	0.59	0.36	3.57	0.98	2.80	0.73	5.18	1.42	11.95	1.86	**22.15**	4.70	20.73	1.84	6.12	0.46	0.74	86.18
Erica mackaiana (leaves)	0.00	0.00	0.88	28.01	0.64	1.03	11.59	1.20	15.34	1.10	23.15	2.11	22.29	2.48	**41.61**	2.07	13.59	167.09
Erica mackaiana (stem)	0.00	0.00	3.02	1.38	6.95	2.46	3.10	2.13	6.18	2.37	27.89	4.52	17.23	2.51	**29.24**	0.79	3.90	113.67
Juncus articulatus	0.07	0.26	0.44	0.13	1.66	0.24	1.09	0.26	1.52	0.26	2.77	0.55	13.58	1.21	**31.77**	1.64	32.40	89.85
Juncus effusus (flowers)	0.24	0.14	1.89	0.31	3.68	1.00	**13.04**	2.66	14.29	2.20	13.03	2.50	12.07	0.98	2.66	0.11	1.84	72.64
Juncus effusus (stem)	0.00	0.06	0.58	0.05	1.05	0.19	0.29	0.07	0.43	0.13	0.63	1.52	**3.38**	0.61	0.92	0.04	1.00	10.95
Molinia caerulea	0.47	0.66	1.27	0.49	2.67	1.26	1.46	1.02	4.79	1.29	9.79	2.56	**94.67**	0.77	3.70	0.45	0.48	127.80
Molinia sp.	0.07	0.09	0.19	0.14	0.48	0.21	1.04	0.40	2.52	0.88	3.81	3.38	11.68	1.19	**13.73**	0.18	0.74	40.73
Osmunda regalis	0.58	1.83	1.91	0.25	3.07	0.69	1.77	0.64	3.01	0.61	32.16	2.17	28.70	1.49	**91.91**	3.92	4.39	179.10
Pterudium aquilinum	0.00	0.00	0.00	0.00	2.37	0.19	0.68	0.13	1.43	0.20	**3.54**	0.31	2.21	0.13	0.61	0.00	0.00	11.80
Pterudium aquilinum	0.00	0.00	4.92	0.34	1.26	0.14	0.38	0.02	0.76	0.11	**1.76**	0.36	1.06	0.09	0.29	0.03	1.13	12.65
Rhynchospora alba	0.17	0.69	1.44	0.59	4.26	0.77	5.22	1.09	5.30	0.67	6.85	1.29	**19.62**	1.35	15.84	0.24	1.05	66.44
Sphagnum compactum	0.00	0.00	0.22	0.24	5.45	1.35	**21.04**	1.22	10.58	0.52	12.19	1.59	8.09	0.83	7.37	1.04	0.63	72.36
Sphagnum cuspidatum	2.51	0.31	0.99	0.95	0.81	0.98	6.68	1.70	3.93	0.59	6.50	1.61	6.84	2.45	**17.45**	1.45	0.88	56.63
Sphagnum denticulatum	0.00	0.28	0.76	0.26	5.82	1.14	**12.09**	1.34	4.90	0.45	3.09	1.09	3.59	0.58	1.88	0.27	0.34	37.88
Sphagnum subnitens	0.52	0.12	0.32	0.29	3.39	1.09	**11.84**	1.21	4.16	0.49	4.56	1.27	5.53	0.51	4.14	0.59	0.12	40.15
Sphagnum subsecundum	0.00	0.20	0.26	19.26	2.12	0.75	7.39	1.14	**9.19**	0.69	2.46	1.96	7.40	0.94	2.26	0.36	0.44	56.82
Ulex europaeus (leaves)	0.00	0.00	0.00	0.14	3.23	0.84	7.51	1.25	5.31	1.14	12.35	3.59	**21.31**	3.94	19.80	0.23	1.23	81.87
Ulex europaeus (stem)	0.03	0.04	0.17	2.25	0.30	0.06	0.28	0.06	1.25	0.23	**1.65**	0.29	0.85	0.15	0.83	0.10	0.21	8.75

Table 3
Concentration (n.d. – not detected) of *n*-alkan-2-ones in leaves and stems of plants from Roñanzas peat bog (highest values in bold).

Name	Alkan-2-one C_{no} (μg/g dry plant matter)											
	21	22	23	24	25	26	27	28	29	30	31	Sum
Calluna vulgaris (leaves)	0.40	0.54	0.58	0.30	0.53	0.28	0.76	0.11	**0.80**	0.06	0.30	4.66
Calluna vulgaris (stem)	0.25	0.13	0.55	0.24	2.02	0.29	**2.37**	0.30	1.99	0.10	0.18	8.42
Campylopus introflexus	0.04	0.08	0.13	0.04	0.31	0.07	**0.53**	0.06	0.27	0.05	0.07	1.65
Campylopus pyriformis	0.11	0.03	0.14	0.05	0.63	0.13	**0.90**	0.05	0.20	0.02	0.05	2.31
Carex canescens	0.11	0.06	0.25	0.20	0.34	0.06	0.41	0.07	**0.42**	0.06	0.32	2.30
Drosera intermedia	0.24	0.05	0.38	0.18	0.83	0.22	**0.87**	0.11	0.38	0.33	0.18	3.77
Erica mackaiana (leaves)	0.34	0.09	0.15	0.02	0.24	0.04	**0.36**	0.04	0.21	0.02	0.01	1.52
Erica mackaiana (stem)	0.11	0.01	0.14	0.05	0.48	0.15	**2.27**	0.15	2.02	0.15	0.30	5.83
Juncus articulatus	0.01	0.00	0.06	0.02	0.07	0.01	0.12	0.01	0.15	0.02	**0.28**	0.75
Juncus effusus (flowers)	0.05	0.03	0.17	0.03	0.16	0.04	**0.37**	0.09	0.84	0.09	0.37	2.24
Juncus effusus (stem)	0.05	0.02	0.04	0.01	0.10	0.02	0.13	0.04	**0.21**	0.02	0.17	0.81
Molinia caerulea	0.12	0.11	0.34	0.26	0.65	0.57	**1.62**	0.07	0.59	0.150	0.19	4.67
Molinia sp.	0.03	0.02	0.10	0.04	0.15	0.04	0.21	0.06	0.33	0.03	**0.61**	1.62
Osmunda regalis	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.00
Pteridium aquilinum (leaves)	**0.13**	0.00	0.11	0.03	0.10	0.01	0.03	0.01	0.02	n.d.	n.d.	0.44
Pteridium aquilinum (stem)	0.02	0.01	**0.05**	0.01	0.04	0.01	0.03	0.01	0.03	n.d.	n.d.	0.21
Rhynchospora alba	0.04	0.03	0.13	0.04	0.12	0.02	0.21	0.02	**0.36**	0.02	0.28	1.27
Sphagnum compactum	0.04	0.31	0.26	0.12	2.71	0.66	**5.59**	0.23	0.57	0.05	0.20	10.74
Sphagnum cuspidatum	0.57	0.16	0.32	0.18	0.92	0.44	**2.72**	0.64	0.77	0.11	0.23	7.06
Sphagnum denticulatum	0.05	0.07	0.14	0.07	0.63	0.41	**2.87**	0.26	0.36	0.03	0.09	4.98
Sphagnum subnitens	0.08	0.13	0.25	0.12	0.85	0.53	**3.12**	0.35	0.46	0.04	0.14	6.07
Sphagnum subsecundum	0.08	0.05	0.19	0.02	1.72	0.61	**6.89**	0.38	0.73	0.05	0.17	10.89
Ulex europaeus (leaves)	0.07	0.05	0.13	0.02	0.05	0.05	**0.31**	0.06	0.26	0.09	0.29	1.38
Ulex europaeus (stem)	0.07	0.01	0.03	0.01	0.06	0.02	**0.12**	0.01	0.06	0.01	0.04	0.44

The different methodology used in Nichols and Huang (2007) for the lipid extraction and/or the sensitivity of the gas chromatography–mass spectrometry (GC–MS) method employed might be another reason to explain these differences, but the *n*-alkan-2-one distributions and abundances of *Sphagnum* mosses in North America (Nichols and Huang, 2007) coincide with our results. In our view, the fact that both studies were performed in different areas is not a major reason as the *n*-alkane patterns in *Sphagnum* and non-*Sphagnum* species found in Roñanzas are similar to those in several peat bogs in The Netherlands and Ireland (Baas et al., 2000; Pancost et al., 2002), United Kingdom (Nott et al., 2000) and USA (Nichols et al., 2006).

The concentration of *n*-alkan-2-ones was not very high when compared with *n*-alkanes (Tables 2 and 3). In fact, the *n*-alkan-2-one/*n*-alkane ratio (calculated as the sum of the concentration of *n*-alkan-2-ones relative to the sum of the concentration of *n*-alkanes) in presentday plants from Roñanzas ranged between 0.009 and 0.200 (Table 1). Notwithstanding, Baas et al. (2000) also observed that *Sphagnum* spp. contained only relatively small amounts of C_{25} and C_{27} *n*-alkan-2-ones. Similarly this, in a number of forest and paramo species, Jansen and Nierop (2009) found small concentrations of *n*-alkan-2-ones in the same range as roots of several species and leaves from one species as those detected in the plants addressed in our study. Also, the abundances of *n*-alkan-2-ones in

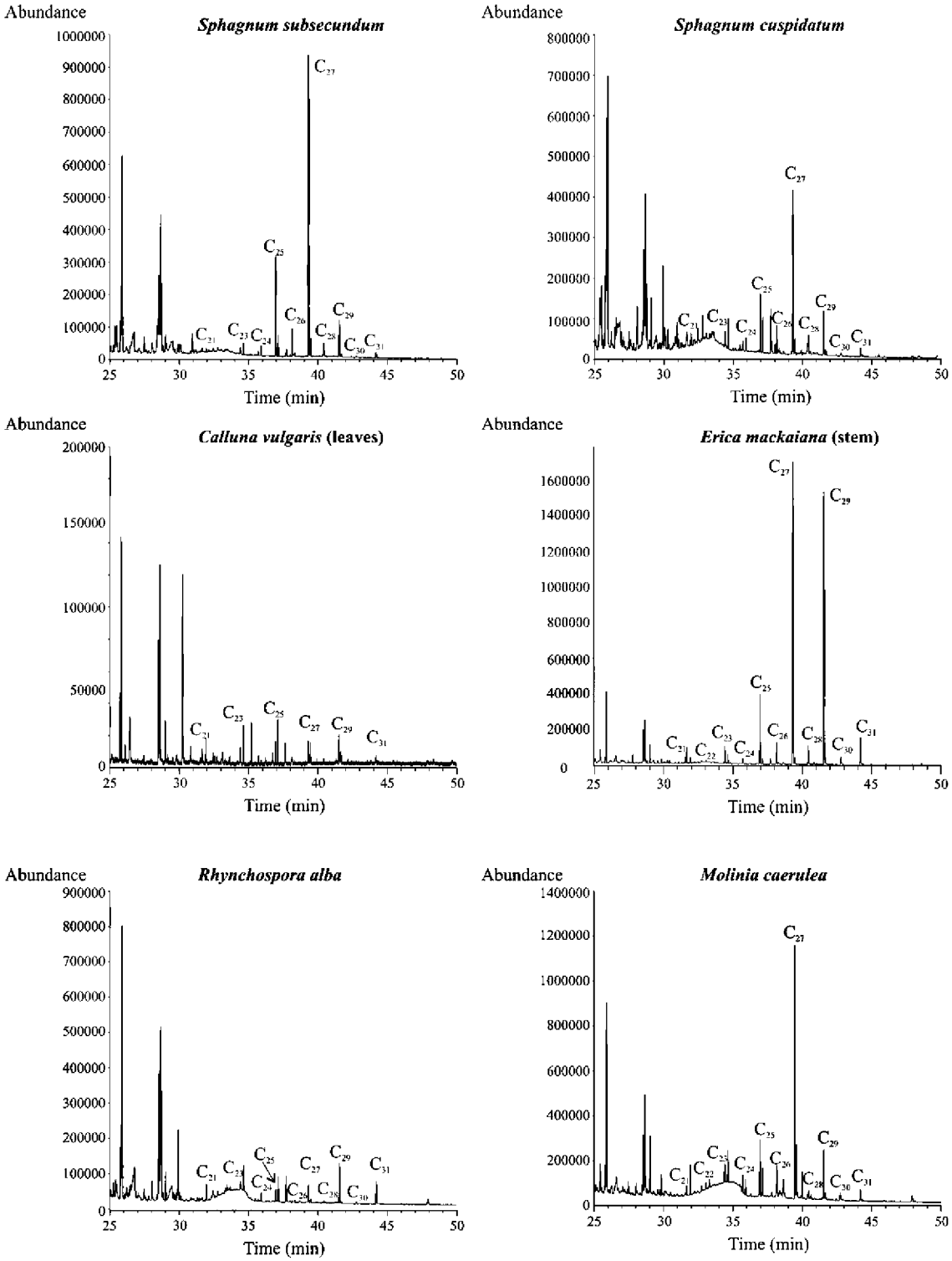

Fig. 1. Distribution of *n*-alkan-2-ones in (a) *Sphagnum subsecundum* (moss), (b) *Sphagnum cuspidatum* (moss), (c) *Calluna vulgaris* (Scotch heather), (d) *Erica mackaiana* (Mackay's heather), (e) *Rhynchospora alba* (white beaksedge) and (f) *Molinia caerulea* (purple moor grass).

the *Sphagnum* spp. (Table 3) do not differ to those reported by Nichols and Huang (2007) for other species of this genus.

In general, *Sphagnum* spp. showed a higher concentration of *n*-alkan-2-ones than other plants (Table 3), except the Ericaceae species (*Calluna vulgaris* and *Erica mackaiana*). These represent the other main components of ombrotrophic bogs and are commonly used for discriminating between more humid episodes (in which *Sphagnum* spp. dominate) and drier periods (with predominating Ericaceae spp.; cf. Nott et al., 2000; Avsejs et al., 2002; Pancost et al., 2002; McClymont et al., 2005; Nichols et al., 2006; Ortiz et al., 2010). Of note, the stems of the heathers *C. vulgaris* and *E. mackaiana* contained higher concentrations of *n*-alkan-2-ones than their leaves, whereas, for *Ulex europaeus*, the opposite was apparent (Table 3).

3.1. Possible microbial origin of the n-alkan-2-ones?

We discard any microbial origin of the *n*-alkan-2-ones from the point of plant collection until analysis because the samples we worked with remained frozen. Moreover, in the Roñanzas record the most abundant *n*-alkan-2-one in almost all the peat samples was C_{19} (Fig. 2; cf. Ortiz et al., 2010), whereas in most present-day plants the C_{27}, C_{29} and C_{31} homologues are dominant (Table 3), except in the fern *P. aquilinum*, whose ketones maximized at C_{21} or C_{23}. A similar *n*-alkan-2-one profile to the Roñanzas peat record was found by Lehtonen and Ketola (1990). These authors reported that the *n*-alkan-2-ones maximized at C_{17}, with the shorter chain C_{17}–C_{23} homologues greatly increasing in abundance with increasing humification. Furthermore, some bacterial taxa that produce *n*-alkan-2-ones via the metabolism of fatty acids (Lawrence, 1966), fatty alcohols (Hou et al., 1983) and alkanes (Forney and Markovets, 1971), or many fungal taxa, which also produce ketones via an abortive β-oxidation sequence (Forney and Markovets, 1971), are responsible for the predominance of short chain (C_{19}) ketones. Thus, the biodegradation of *n*-alkanes is the most plausible explanation for the predominance of the C_{19} ketone throughout the Roñanzas record (cf. Ortiz et al., 2010), especially given that the C_{23} isomer is the most abundant in most *Sphagnum* species. In fact, according to Zheng et al. (2007), microbial activity can be considerable in peat, especially during warm and humid episodes. In our view, these processes may occur in the Roñanzas bog, in which desiccation produced during peat extraction during 1970s and 1980s would have accelerated biodegradation.

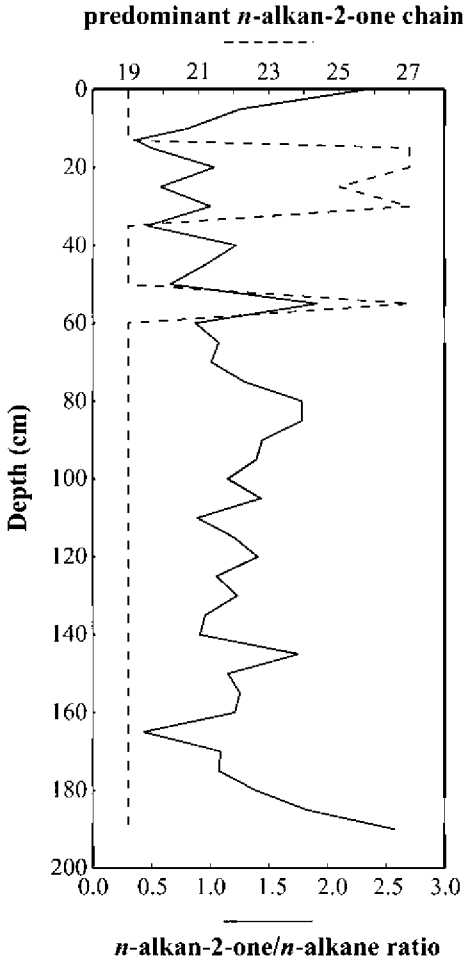

Fig. 2. Predominant *n*-alkan-2-one chain and *n*-alkan-2-one/*n*-alkane ratio in peat samples along the 2.0-m deep Roñanzas record (cf. Ortiz et al., 2010).

Furthermore, the abundance of *n*-alkan-2-ones in the peat samples from the Roñanzas profile in most cases was higher than the *n*-alkane content, with *n*-alkan-2-one/*n*-alkane ratio varying between 0.499 and 2.579 (Fig. 2). This observation implies that, in most cases, the presence of *n*-alkan-2-ones was not due only to direct input from plant material but also from the alteration of other organic compounds, probably the oxidation of *n*-alkanes, which is thought to be the only significant pathway for the in situ genesis of longer chain *n*-alkan-2-ones in soils and peat (Amblès et al., 1993; Jaffé et al., 1996; van Bergen et al., 1998).

In this regard, the *n*-alkan-2-one/*n*-alkane ratio values calculated from concentrations provided by Jansen et al. (2006) and Jansen and Nierop (2009) for present-day plants range between 0.016 and 0.254, values similar to those found in our study (Table 1). Also, although we cannot calculate this ratio for the *Sphagnum* specimens studied by Nichols and Huang (2007) because they did not provide the total *n*-alkane concentration (only the abundance of tricosane), the values for total *n*-alkan-2-ones and the C_{23} *n*-alkane are the same order of magnitude as ours. We wish to highlight that one of the plants (*Rhynchospora ruiziana*) studied by Jansen and Nierop (2009) contained a similar concentration of *n*-alkan-2-ones in its roots to that of the same genus analysed here, namely *Rhynchospora alba*, a common specimen of bogs and mires.

However, microbial oxidation can take place in the plant itself, especially in the rhizosphere, where roots are accompanied by considerable microbial activity. In fact, such activity may explain why all the samples studied by Jansen and Nierop (2009) containing *n*-alkan-2-ones were mostly roots. This was not our case, as our samples were from leaves and stems. Nevertheless, we also tested the possible in situ microbial contribution to the *n*-alkan-2-ones in the peat-forming plants from Roñanzas, as they appeared in low concentration. Apart from their possible origin as constituents of plants, there would be two major pathways for the presence of *n*-alkan-2-ones, namely β-oxidation and decarboxylation of *n*-alkanoic acids, and microbially mediated oxidation of *n*-alkanes (Volkman et al., 1983; Chaffee et al., 1986; de Leeuw, 1986; Cranwell et al., 1987; Amblès et al., 1993; Jaffé et al., 1993, 1996; van Bergen et al., 1998; Quénéa et al., 2004).

The oxidative decarboxylation of a certain *n*-alkanoic acids would yield a corresponding *n*-alkan-2-one with one carbon atom fewer, e.g. the degradation of C_{24} alkanoic acid would produce the C_{23} ketone. The absence of this correspondence between the predominant of *n*-alkan-2-ones and *n*-alkanoic acids in the present-day plants studied here (Table 1) led us to discard the degradation of fatty acids as a significant route for the in situ genesis of ketones. It should be noted that the most abundant ketone has even more carbons than the alkanoic acid in all cases.

In order to determine the possible microbial oxidation of *n*-alkanes that would produce *n*-alkan-2-ones, we compared their chain length distributions in each sample. In most cases, the ketone profiles appeared not to coincide with the *n*-alkane profiles (Tables 1–3), i.e. four *Sphagnum* species, *Juncus effusus* (flowers) and *Carex canescens* showed a longer chain predominant *n*-alkan-2-one- than the predominant *n*-alkane; in *E. mackaiana* (leaves and stems), *P. aquilinum* (leaves and stems) and *S. cuspidatum* the most abundant *n*-alkane had four carbons more than the predominant ketone, and *C. vulgaris* (leaves and stems), *C. pyriformis*, *Molinia caerulea*, *R. alba* and *U. europaeus* (leaves) maximized at an *n*-alkane chain with two carbons more than the most abundant *n*-alkan-2-one. For a better appreciation of these findings, Fig. 3 shows the *n*-alkane and *n*-alkan-2-one distributions of representative species of these four clusterings.

In our view, our results indicate that *n*-alkan-2-ones in present-day plants from Roñanzas peat bog are not derived from the

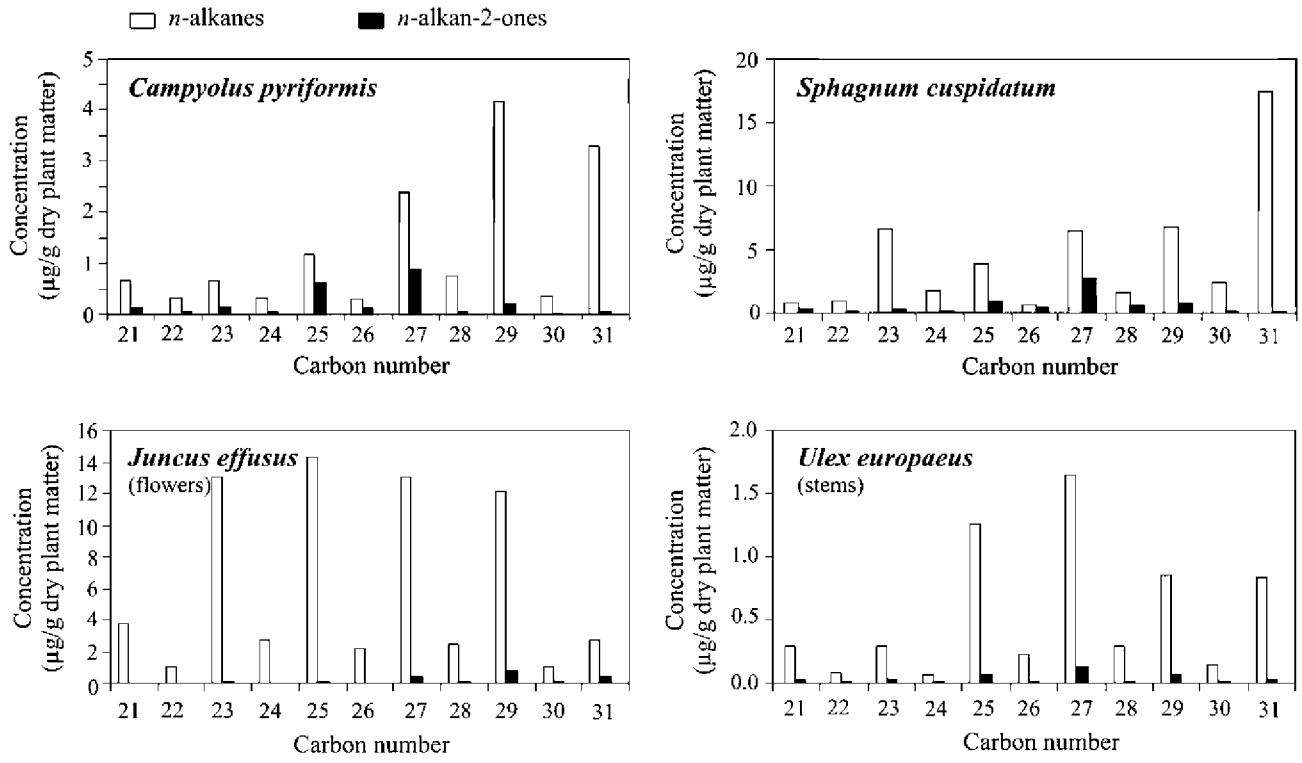

Fig. 3. Distribution of long chain *n*-alkanes and *n*-alkan-2-ones in representative species from the Roñanzas peat bog.

microbial oxidation of *n*-alkanes. Only in six species did the predominant *n*-alkane and *n*-alkan-2-one coincide and we cannot totally discard microbial oxidation in these cases.

Thus, we considered that the concentration and distribution pattern of *n*-alkan-2-ones in the present-day plants studied here were not affected by degradation processes, whereas those from the Roñanzas peat would be derived both from microbial oxidation of the corresponding *n*-alkanes and/or from decarboxylation of the even chain length *n*-alkanoic acids (cf. Arpino et al., 1970; Volkman et al., 1981), as concluded by Ortiz et al. (2010).

4. Conclusions

The lipid distributions in present-day peat-forming plants in the Roñanzas bog (northern Spain) reveal the presence of *n*-alkan-2-ones in almost all the species analysed, both those belonging to the *Sphagnum* genus, as well as non-*Sphagnum* spp., except one fern taxon. In most cases, distributions ranged between C_{21} and C_{31}, with an odd predominance, and maximized at C_{27} or C_{29} (Table 3), except for ferns, which maximized at lower MW (C_{21}–C_{23}). Of note, *Sphagnum* spp. and Ericaceae species (*C. vulgaris* and *E. mackaiana*) showed a similar abundance of *n*-alkan-2-ones (Table 3), and higher than in other plant taxa which are commonly used for discriminating between more humid and drier conditions, respectively. However, they cannot be relied on as biomarkers from these plants due to the generally higher production of *n*-alkan-2-ones in sediments through microbial activity. Thus, following Jansen and Nierop (2009), we also conclude that a direct input of *n*-alkan-2-ones from plants is not the dominant contributor to the *n*-alkan-2-one distribution in certain soils and peat bogs.

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