Comparison of free lipid compositions between roots and leaves of plants in the Dajiuhu Peatland, central China

Xianyu Huang,[1] Canfa Wang,[2] Jinxiang Zhang,[1] Guido L. B. Wiesenberg,[3]
Zhiqi Zhang[4] and Shucheng Xie[2]*

[1]State Key Laboratory of Geological Processes and Mineral Resources, China University of Geosciences, Wuhan 430074, China
[2]Key Laboratory of Biogeology and Environmental Geology, Ministry of Education, China University of Geosciences,
Wuhan 430074, China
[3]Department of Agroecosystem Research, University of Bayreuth, Bayreuth 95440, Germany
[4]Administration of Dajiuhu National Wetland Park of Shennongjia, Shennongjia 442417, China

The main aim of this study was to assess the free lipid composition of plant community in a Chinese peatland. Twelve plant species from the Dajiuhu Peatland were analyzed for the compositions of *n*-alkanes, *n*-fatty alcohols and sterols in leaves and roots. The lipid compositions are significantly different between root and leaf for most plants. In some cases, the roots yield more long chain *n*-alkanes and *n*-alkanols than the leaves of the corresponding plant. The long chain *n*-alkanes of the roots in half of the plant species are characterized by a higher C_{max} (homologue with the maximum concentration) relative to the leaves. The root-derived sterols and steroidal ketones may be important sources for corresponding compounds within the peat. Because of the different lipid compositions of roots and leaves, more attention should be paid to root-derived lipids for investigations of the lipid composition and their source assessment in soils and terrestrial sediments, where root-derived organic matter can be an important source. The contribution of root-derived lipids may be especially important in peatlands, where acidic and/or anoxic conditions in the subsoil limit the degradation of organic matter and the contribution of leaf litter.

INTRODUCTION

The free and bound lipids (*n*-alkanes, *n*-fatty alcohols and steroids) contribute a great part of organic matter preserved in soils and sediments, and these can be used as biomarkers for their source plants and the associated environmental conditions (e.g., Rieley *et al.*, 1991; Meyers and Ishiwatari, 1993; Schwark *et al.*, 2002; Xie *et al.*, 2004; Wiesenberg and Schwark, 2006; Huang *et al.*, 2008). To effectively interpret the origin of the lipids in soils and sediments, it is crucial to investigate how and where lipids are distributed in modern plants. However, most studies have focused on leaf waxes (e.g., Rieley *et al.*, 1991; Ficken *et al.*, 1998, 2000; Schwark *et al.*, 2002; Bi *et al.*, 2005; Jansen *et al.*, 2006; Rommerskirchen *et al.*, 2006; Cui *et al.*, 2008; Grice *et al.*, 2008; Huang *et al.*, 2011). There have been fewer reports of root-derived lipids and their significance for sedimentary organic matter (Otto *et al.*, 1994; Pancost *et al.*, 2002; Wiesenberg *et al.*, 2004, 2010; Jansen *et al.*, 2006; Kuhn *et al.*, 2010; Mendez-Millan *et al.*, 2010). In order to acclimatize to the specific conditions of peatlands (waterlogged, nutrient poor and partly anoxic and acidic), plants develop shallow but flourishing root systems (Rydin and Jeglum, 2006), which can contribute high amount of lipids to peat deposits. To date, only Pancost *et al.* (2002) noted the root-derived lipids from peat-forming plants.

A number of studies have reported the lipid compositions of peatland plant communities (Ficken *et al.*, 1998; Nott *et al.*, 2000; Pancost *et al.*, 2002; Nichols *et al.*, 2006). These communities were distributed in Europe and North America, while the results in other regions were not available in the literature to the best of our knowledge. In addition, except for the peat moss (*Sphagnum* species) shrubs are abundant in the above bogs. In contrast, the Dajiuhu Peatland is characterized by *Sphagnum palustre* and various herbaceous plants. It is, therefore, worth assessing the lipid composition of the plant community in such an herb peatland. The *n*-alkane distributions of several moss species (*S. palustre*, *Aulacomnium palustre*, *Polytrichum commune* and *Hypnum revolutum*) from the area have been previously reported by Huang *et al.* (2010).

*Corresponding author (e-mail: xiecug@163.com)

In this study, we compared the free lipid distributions in roots and leaves of plant species collected from the Dajiuhu Peatland. The aim of this study was to evaluate the composition of root lipids relative to leaf lipids in the plant community of this peatland in central China.

MATERIALS AND METHODS

Sampling

The Dajiuhu Peatland in Shenongjia Forestry Region, Hubei Province, China, has developed in a sub-alpine closed basin (over 1700 m above sea level) and extends over an area of about 16 km^2. The peat sediments reach a thickness of more than 2 m. The pH of the modern surficial peat is *ca*. 4–5, and the water table is normally 10–50 cm below the surficial horizons (Huang *et al*., 2010). The local climate is dominated by the East Asian monsoon, with a mean annual rainfall of 1560 mm and a mean temperature of 7.2°C. Within the peatland, *Sphagnum palustre* is the dominant cover plant, together with herbaceous plants such as *Carex* spp., *Juncus effusus* and *Euphorbia esula*.

The twelve most abundant plant species were collected for root and leaf lipid analysis from the Dajiuhu Peatland in June, 2009. For the samples to be representative, over ten individual plants were collected for each species. All the plants are herbs except the fern *Dryopteris* sp.

Extraction and fractionation

Plant samples were carefully washed with deionized water, divided into roots and leaves, and then cut into small pieces using scissors. The leaf and root parts were immersed in dichloromethane (DCM) for 1 min to extract the epicuticular wax (Jetter *et al*., 2006). The *n*-eicosane-d$_{42}$, *n*-tetracosane-d$_{50}$, and *n*-triacontane-d$_{62}$ (Chiron, Norway) were added as internal standards before the extraction. The extracts were fractionated into aliphatic (including *n*-alkanes), polar-1 (including *n*-alkanols and sterols) and polar-2 fractions using silica column chromatography, with hexane, DCM and methanol (1:1, v/v), and methanol as eluting solvents, respectively.

GC and GC-MS analyses

Polar-1 fractions, after silylation using bis(trifluoroacetyl)-trifluoroacetamide (BSTFA), were analyzed using gas chromatography-mass spectrometry (GC-MS) with a Hewlett Packard 6890 gas chromatograph coupled to a Hewlett Packard 5973 mass selective detector. The oven temperature was ramped from 70°C (held 1 min) to 210°C at 10°C min^{-1} and then to 300°C (held 20 min) at 2°C min^{-1}. Individual components were identified using a combination of GC relative retention times of standards and published mass spectra (Djerassi, 1978;
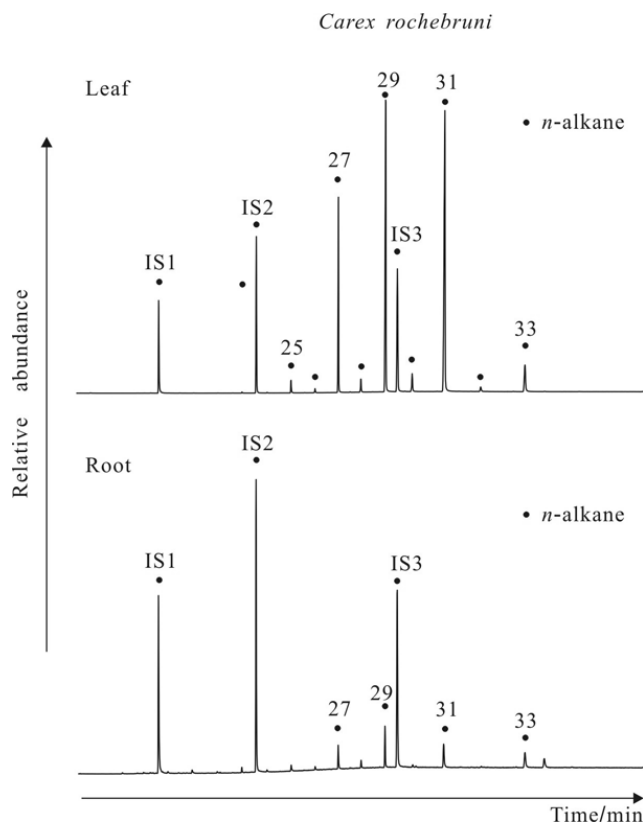

Fig. 1. The total ion chromatograph (TIC) of aliphatic fraction of Carex rochebruni.

Gagosian *et al*., 1982; Duan and Ma, 2001). The C$_{29}$ sterones were tentatively identified as follows: stigmasta-3,5-dien-7-one, characterized by prominent *m/z* 174 from C-ring cleavage and stigmast-4-en-3-one, with a characteristic ion at *m/z* 124 from B-ring cleavage. The root/leaf ratios of *n*-alkanols, sterols and stenones were calculated based on the characteristic ions (*m/z* 103, 129 and 124/174, respectively).

The abundances of long chain *n*-alkanes were measured using a Shimadzu GC 2010 equipped with a flame ionization detector (FID). The oven temperature was ramped from 70°C (held 1 min) to 210°C at 10°C min^{-1} and then to 300°C (held 25 min) at 3°C min^{-1}. Components were identified by comparison with the retention times of *n*-alkane standards (Supelco, USA). The absolute abundances were calculated by comparison of peak areas with those of known quantities of standards and adjusted with the relevant FID response factors.

RESULTS AND DISCUSSION

n-Alkanes

The aliphatic fractions of all the samples are domi-

Table 1. The quantitative results of long chain n-alkanes in leaves and roots of the plant species collected from Dajiuhu Peatland, central China

	Sanguisorba officinalis	Menyantehes trifoliata	Juncus effusus	Carex argyi	Carex doniana	Carex dimorpholepi	Dryopteris sp.	Polygonum paleaceum*	Carex rochebruni	Euphorbia esula	Geranium wilfordii	Veratrum nigrum
Leaf												
C_{23}	0.0	0.3	0.3	0.3	0.0	1.5	0.5	0.1	0.1	3.8	1.8	0.3
C_{24}	0.0	0.1	0.1	0.0	0.1	0.3	0.0	0.1	0.1	1.4	0.3	0.1
C_{25}	5.2	0.6	1.4	0.5	0.6	1.5	3.0	0.3	1.2	21.1	4.4	0.4
C_{26}	1.5	0.2	0.2	0.0	0.2	0.9	0.1	0.1	0.4	15.1	0.5	1.5
C_{27}	26.3	0.8	2.7	1.1	2.0	6.8	3.0	2.0	14.2	130.1	13.7	3.6
C_{28}	5.8	0.2	0.6	0.0	0.8	0.3	0.0	0.2	1.4	8.9	1.1	0.8
C_{29}	72.5	0.7	22.8	5.6	19.5	1.1	0.3	1.3	30.9	45.0	30.5	14.7
C_{30}	8.2	0.0	2.6	0.0	1.0	0.2	0.0	0.0	2.1	4.8	3.1	2.0
C_{31}	876.4	0.3	134.2	4.0	13.6	0.7	0.0	0.0	38.0	155.3	86.8	22.1
C_{32}	35.9	0.0	5.1	0.0	0.0	0.2	1.0	0.0	0.8	2.1	4.7	1.3
C_{33}	2636.0	0.2	90.5	1.2	4.1	0.4	0.0	0.0	4.7	81.1	80.0	4.7
C_{34}	14.3	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.7	2.1	0.0
C_{35}	58.8	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0	20.3	39.3	0.0
Root												
C_{23}	0.0	1.8	1.4	0.4	0.4	8.1	0.1	0.0	0.4	0.0	0.0	0.3
C_{24}	0.0	0.7	0.3	0.2	0.1	0.6	0.0	0.0	0.1	0.0	0.0	0.0
C_{25}	0.0	2.5	2.5	0.4	0.9	1.8	0.1	0.2	0.4	0.0	0.1	0.0
C_{26}	0.2	0.4	0.3	0.2	0.1	0.4	0.0	0.0	0.2	0.0	0.3	0.3
C_{27}	0.3	2.2	3.9	0.3	0.5	2.9	0.2	0.5	1.4	0.6	0.3	0.3
C_{28}	0.0	0.3	0.7	0.2	0.0	0.8	0.1	0.0	0.6	0.0	0.0	0.0
C_{29}	0.2	1.0	16.4	1.0	1.8	12.1	0.2	0.7	2.3	0.4	0.6	0.7
C_{30}	0.0	0.0	2.2	0.2	0.1	0.8	0.0	0.0	0.3	0.1	0.1	0.1
C_{31}	0.0	0.0	67.1	1.7	2.5	28.5	0.2	0.6	2.4	2.1	3.3	1.4
C_{32}	0.3	0.0	3.5	0.2	0.0	2.1	0.0	0.0	0.2	0.0	0.5	0.0
C_{33}	0.6	0.0	48.1	1.2	1.5	29.9	0.1	0.7	2.1	0.0	7.9	0.7
C_{34}	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0
C_{35}	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.4	0.0

*The roots and rhizome of Polygonum paleaceum was treated as the root part.
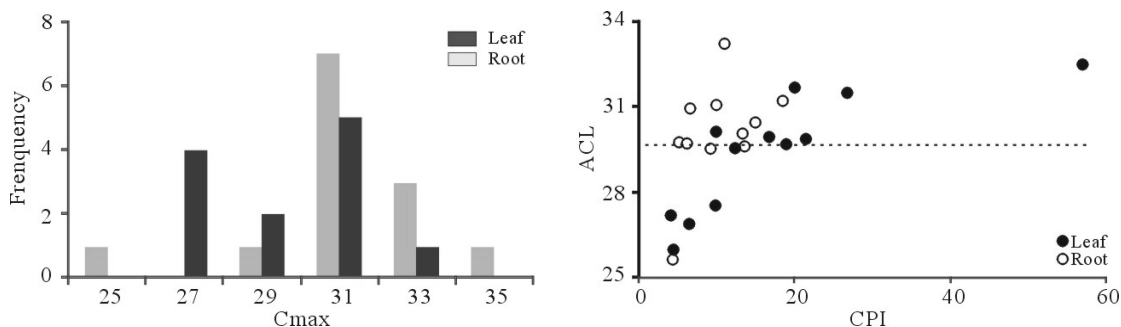
Fig. 2. (a) Frequency of C_{max} of long chain n-alkanes in the roots and leaves of plant species from the Dajiuhu Peatland. In the example (root of C. rochebruni) where the pattern has two dominant homologues, both are treated as C_{max}. (b) Plot of ACL against CPI of n-alkanes. Dashed horizontal line refers to mean value of ACL of all plant samples (29.7).

$$ACL = \sum_{i}^{n}(C_i \times i) \Big/ \sum_{i}^{n} C_i \ \ (i = 23 - 35, \ odd).$$

$$CPI = \frac{(C_{23} + C_{25} + C_{27} + C_{29} + C_{31} + C_{33}) + (C_{25} + C_{27} + C_{29} + C_{31} + C_{33} + C_{35})}{2(C_{24} + C_{26} + C_{28} + C_{30} + C_{32} + C_{34})}.$$

Table 2. Plant species, type and root/leaf ratio of lipid contents normalized to 1 g biomass

Species	Type	n-Alkane	n-Alcohol	Sterol	Stenone
Sanguisorba officinalis	Herb	0.00	0.00	3.09	9.45
Menyantehes trifoliata	Herb	2.93	0.43	4.88	
Juncus effusus	Herb	0.57	2.61	146.65	8.92
Carex argyi	Herb	0.30	0.21	21.92	95.27
Carex doniana	Herb	0.10	0.28	9.53	128.76
Carex dimorpholepis	Herb	6.72	13.94	16.84	7.10
Carex rochebruni	Herb	0.11	41.88	222.69	23.86
Dryopteris sp.	Fern	0.07	0.01	1.99	45.10
Polygonum paleaceum	Herb	0.59	0.10	10.56	385.29
Euphorbia esula	Herb	0.01	1.53	27.94	41.62
Geranium wilfordii	Herb	0.09	1.92	242.47	348.46
Veratrum nigrum	Herb	0.08	0.42	7.10	1.37

nated by long chain n-alkanes (C_{21}–C_{35}; Fig. 1, Table 1). All the patterns of carbon distribution show an obvious odd/even predominance, with carbon preference index (CPI, see Fig. 2 for formula) values ranging from 4.5 to 57.2 (avg. 14.1; Fig. 2b). These CPI signatures are similar with the previous results of leaf cuticle waxes (e.g., Rieley *et al.*, 1991; Ficken *et al.*, 2000; Cui *et al.*, 2008).

The concentrations of the n-alkanes in the leaves vary greatly, ranging from less than 1 to over 2500 μg/g. *Sanguisorba officinalis*, *J. effuses* and *E. esula* contain relatively high amounts of n-alkanes (>100 μg/g). The C_{max} of these species maximizes at a concentration of >100 μg/g (relative to dry wt; Table 1). The leaves of five other species including *Menyantehes trifoliata*, *Carex*

argyi, *Carex dimorpholepis*, *Dryopteris* sp. and *Polygonum paleaceum* contain relatively low amounts of n-alkanes, with a concentration of each compound being <10 μg/g (Table 1).

The roots generally have quite low amounts (normally <10 μg/g) of long chain n-alkanes (Table 1). Only the roots of *J. effusus* and *C. dimorpholepis* show comparatively high concentrations of the dominant long chain n-alkanes (>10 μg/g; Table 1).

For all the Dajiuhu plant species, the leaves generally contain significantly more n-alkanes than the roots (Table 2). For example, the concentrations of the n-alkanes in *S. officinalis* leaves are *ca*. 100 times as high as those in the roots (Table 2). *E. tetralix* and *C. vulgaris* in Pancost

et al. (2002) also showed an enrichment of the *n*-alkanes in leaves compared to roots. However, the roots of *M. trifoliata* and *C. dimorpholepis* contained more long chain *n*-alkanes than their leaves (Table 2).

The Dajiuhu plant roots are generally dominated by C_{31} and C_{33} *n*-alkanes (Fig. 2a). The roots of both *E. tetralix* and *C. vulgaris* in a Dutch ombrotrophic bog were also dominated by C_{31} and C_{33} (Pancost *et al.*, 2002). An exception exists in *M. trifoliata* roots, which possesses a relatively lower C_{max} (C_{25}) that is similar to those of emersed/floating plants (Ficken *et al.*, 2000) and *Sphagnum fuscum* (Ficken *et al.*, 1998; Vonk and Gustafsson, 2009; Bingham *et al.*, 2010). The C_{31} homologue is also the dominant C_{max} in most of the Dajiuhu plant leaves. However, in four of the twelve plants (*C. dimorpholepis*, *M. trifoliata*, *P. paleaceum* and *Dryopteris* sp.), C_{27} alkane is dominant (Fig. 2a).

Five of the twelve plant species (*S. officinalis*, *J. effusus*, *Carex rochebruni*, *E. esula* and *Veratrum nigrum*) show identical C_{max} for roots and leaves. *M. trifoliata* contains higher C_{max} in the leaves (Table 1). The other six species (*C. argyi*, *Carex doniana*, *C. dimorpholepis*, *Dryopteris* sp., *P. paleaceum* and *G. wilfordii*), however, have a higher C_{max} in the roots than in the corresponding leaves. These differences in distribution patterns between roots and leaves are also clearly revealed by the values of average chain length (ACL; Fig. 2b). Eight of the twelve roots show a higher ACL value than the mean value of all plant samples (29.7).

The distributions of long chain *n*-alkanes also show variations within species of the same genera. Both the content and distribution patterns of the *n*-alkanes vary among the four *Carex* species (Table 1). The concentration of the C_{max} in *C. doniana* and *C. rochebruni* leaves is one order of magnitude higher than that in the leaves of *C. argyi* and *C. dimorpholepis*. In contrast, the roots of *C. dimorpholepis* contain a relatively higher amount of *n*-alkanes than the other three species. Furthermore, the leaves of these four *Carex* species all show quite different C_{max}, while the roots are dominated by C_{31} or C_{33} homologue. Leaves of *C. bigelowii* (Ficken *et al.*, 1998) also showed a C_{max} at C_{27}, similar to *C. argyi* in this study. The four Cyperaceae species analyzed by Nott *et al.* (2000) were all dominated by C_{31} *n*-alkane. Variability in the *n*-alkane compositions within several species of one genus is common and has been described for other peatland plants, such as *Sphagnum* species (Baas *et al.*, 2000).

n-Alkanols

All the samples contain *n*-alkanols in a range of C_{16} to C_{32} with an obvious even/odd predominance (Figs. 3 and 4). The long chain homologues (C_{22}–C_{32}) show a predominance over the short chain homologues (<C_{22}; Fig.
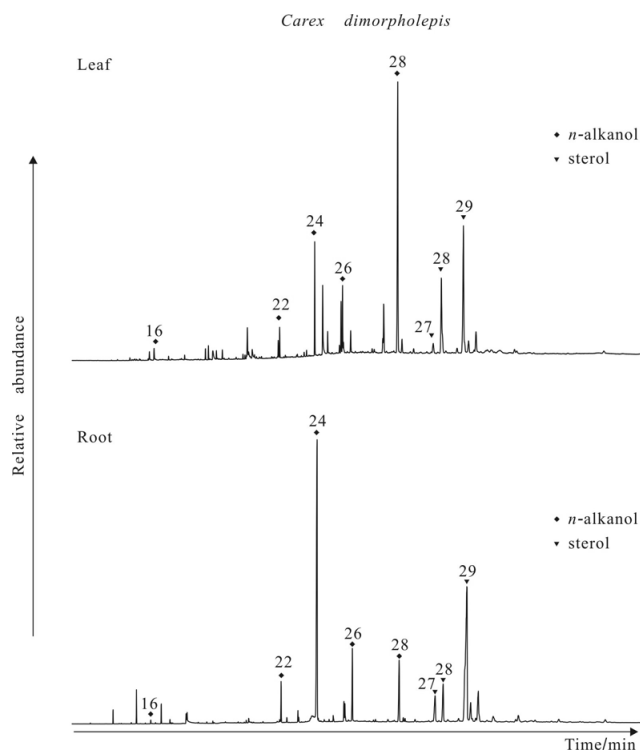

Fig. 3. The total ion chromatograph (TIC) of polar fraction of Carex dimorpholepis.

4), which is typical for higher plants (e.g., Kolattukudy *et al.*, 1976). In all species, the roots normally contain shorter chain *n*-alkanols relative to the leaves (Fig. 4).

In seven of the twelve species, the leaves contain greater amounts of *n*-alkanols than the roots (Table 2). In contrast, *n*-alkanols are significantly enriched in the roots for two *Carex* species (*C. dimorpholepis* and *C. rochebruni*; Table 2). A similar enrichment in roots vs. leaves has also been shown for *E. tetralix* (Pancost *et al.*, 2002).

The C_{max} of *n*-alkanols varies in both roots and leaves (Fig. 4). For example, the leaves of the four *Carex* species show the C_{max} at C_{30}, C_{28} and C_{24} *n*-alkanols, while in the roots it is at C_{30}, C_{28} and C_{26} *n*-alkanols. The *n*-alkanol distribution of *C. bigelowii* given in Ficken *et al.* (1998) was dominated by C_{30} homologue. Plants other than mosses from peatlands normally have C_{max} at C_{28} or C_{30} homologue (Ficken *et al.*, 1998; Pancost *et al.*, 2002).

Steroids

Sterols are obvious in the polar fractions (Fig. 3). In all species, C_{29} sterols (24-ethylcholest-5-en-3β-ol and 24-ethylcholesta-5,22-dien-3β-ol) dominate over C_{28} sterols. The dominant C_{27} sterol is cholest-5-en-3β-ol, present at quite low amounts.

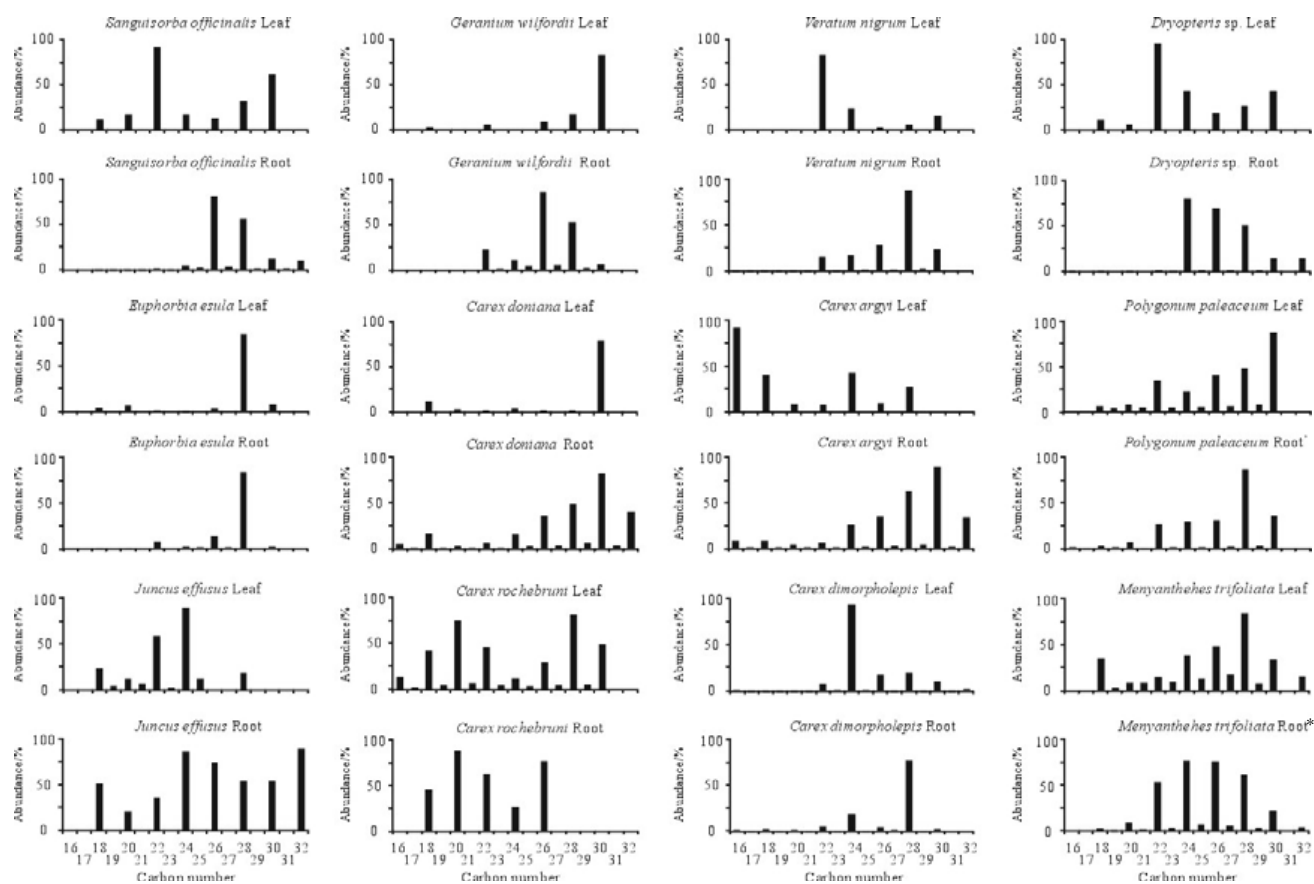Sterols are selectively enriched in the roots of all the

*Fig. 4. Histograms showing the distributions of n-alkanols in the roots and leaves of plants from Dajiuhu Peatland (C_{16}–C_{32}; *refers that the root of Menyantehes trifoliata is actually the mixture of roots and rhizome).*

species (Table 2). This pattern is especially obvious for *J. effusus* and *C. rochebruni*, in which the amount of sterols in the roots is two orders of magnitude higher than that in the leaves (Table 2). A similar enrichment of sterols in roots relative to stems was also described for *E. tetralix* (Pancost *et al.*, 2002). In the study of Dutch coastal dune soils, the oak roots were found to be comprised largely of triterpenoids and steroids (Nierop *et al.*, 2005).

Two steroidal ketone compounds, stigmasta-3,5-dien-7-one and stigmast-4-en-3-one, occur in the roots of all species (Fig. 5). Steroidal ketones are presumably intermediates in the oxidation of sterols to stanols (Wakeham, 1987), which has been demonstrated to occur through microbial oxidation by radiolabelling experiments (Gagosian *et al.*, 1982). Thus, the occurrence of steroidal ketones in the roots of peatland plants probably results from microbial activity around the root systems.

Implications for root derived lipids in peat deposits

This study shows that plant roots can contribute relatively high amounts of free lipids to peatlands. In gen-

eral, long chain *n*-alkanes and *n*-alkanols are more abundant in leaves than in roots, but in some species they show the enrichment in roots (e.g., *C. dimorpholepis*; Table 2). A significant contribution from root-derived lipids is confirmed by the steroids (sterols and steroidal ketones; Table 2), which predominantly occur in the roots of all the species. In addition, roots can contribute more short chain *n*-alkyl compounds to peat (e.g., *n*-alkanols) relative to their leaf counterparts.

These preliminary results reveal that more attention should be paid to root-derived lipids when interpreting the sources of lipids in soil and peat samples. This idea is probably more suitable for environments such as peatlands and wetlands, where plants are characterized by well-developed rootlets and shallow root systems that are acclimatized to the waterlogged conditions (Rydin and Jeglum, 2006). The importance of root-derived lipids has been postulated in the study of the Upper Oligocene Thierbach strata, Germany (Otto *et al.*, 1994). The study of an experimental grassland soil also suggested that the root material was the predominant source of aliphatic acids in the soil of temperate grassland biomes (Bull *et*
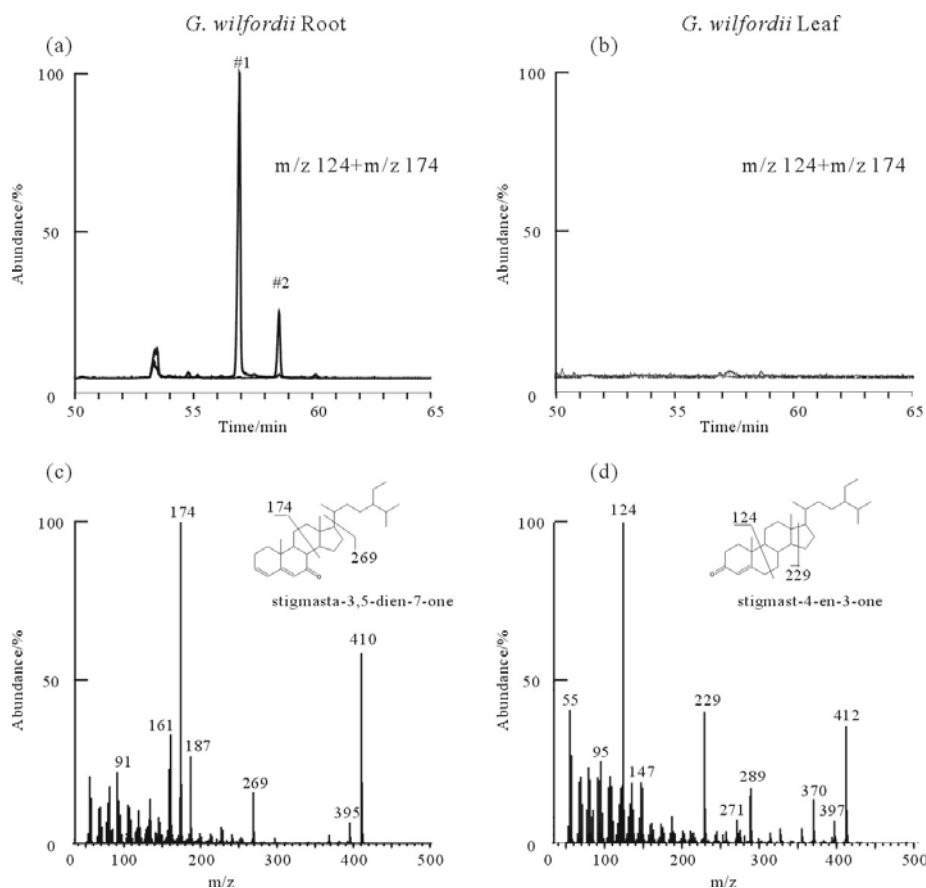
Fig. 5. Steroidal ketones in roots (a) and leaves (b) of G. wilfordii from Dajiuhu Peatland (#1 and #2 in (a) refer to stigmasta-3,5-dien-7-one and stigmast-4-en-3-one, respectively). (c) and (d), mass spectra of stigmasta-3,5-dien-7-one and stigmast-4-en-3-one, respectively.

al., 2000). Since this work only focuses on free plant lipids, it is better to further consider the contribution of bound lipids especially wax esters to evaluate the importance of bound lipids. Previous studies have shown that bound lipids can also contribute great amount of fatty acids, alkanols and sterols in sediments (Cranwell and Volkman, 1981; Disnar et al., 2005).

Half of the Dajiuhu plant species show a larger n-alkane C_{max} in the roots than in the leaves (Table 1). In leaf cuticles, main physiological function of long chain n-alkanes is thought to reduce the loss of water (e.g., Eglinton and Hamilton, 1967; Hauke and Scheriber, 1998; Jetter et al., 2006). It was found that plants growing in arid conditions synthesized greater proportions of longer chain homologues relative to their counterparts in humid conditions (Dodd et al., 1999). On the other hand, in peatlands, the surficial peat layers where the plant roots grow are typically wet, so that roots are unlikely to synthesize longer chain n-alkanes to reduce water loss. A more reasonable interpretation is that roots synthesize longer chain n-alkanes to compensate for the higher wa-

ter osmotic pressure in the peatland. The longer C_{max} in roots vs. leaves also argues against the degradation theory recently proposed by authors for terrestrial soil-paleosol sequences (Buggle et al., 2010; Zech et al., 2010). These authors observed that, in deeper parts of paleosoils as well as recent soils, the ACL, C_{max} and CPI values decreased, and they attributed the effect exclusively to degradation. Our results indicate that the contribution of root-derived lipids can also result in similar modifications in molecular parameters. Therefore, especially in well rooted soils and sediments, the degradation effects on root-derived organic components have to be taken into account, rather than just above ground plant tissue.

The comparison between roots and leaves shows that the relative concentrations of short chain n-alkanols ($<C_{22}$) and steroidal ketones are generally enriched in roots (Figs. 4 and 5). Short chain n-alkanols are thought to be biomarkers for microbes (Volkman et al., 1999; Xie et al., 2003), while the origins of steroidal ketones are attributed to microbial oxidation of sterols (Wakeham, 1987). In this study, the relative enrichment of short chain

n-alkanols (<C_{22}) and steroidal ketones in roots may be associated with microorganisms in and around the root systems, which cannot be completely separated from roots. Therefore, the potential contributions of rhizomicrobial remains have to be considered when analyzing root tissues for their lipid composition (Wiesenberg *et al.*, 2004, 2010).

CONCLUSIONS

Analysis of the free lipid compositions of the roots and leaves of plant species collected from the Dajiuhu Peatland reveals that roots may be important sources of lipid biomarkers in peats and especially of sterols and steroidal ketones. In some cases, roots can contribute more long chain *n*-alkanes and *n*-alkanols than their leaf counterparts. By considering both leaf- and root-derived free and bound lipids, we can better understand organic matter production, preservation, and the associated carbon cycle in soils and terrestrial sedimentary environments like peatlands, which are important terrestrial carbon sinks.

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