Organic Geochemistry 158 (2021) 104270

Contents lists available at ScienceDirect

Organic Geochemistry

journal homepage: www.elsevier.com/locate/orggeochem

Comparison of molecular distributions and carbon and hydrogen isotope compositions of *n*-alkanes from aquatic plants in shallow freshwater lakes along the middle and lower reaches of the Yangtze River, China



Crganic Geochemistry

-

Xiaofang Yu^{a,b}, Xiaoxia Lü^{a,b}, Philip A. Meyers^c, Xianyu Huang^{a,d,*}

^a State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, Wuhan 430078, China

^b Hubei Key Laboratory of Marine Geological Resource, China University of Geosciences, Wuhan 430074, China

^c Department of Earth and Environmental Sciences, The University of Michigan, Ann Arbor, MI48109-1005, USA

^d Hubei Key Laboratory of Critical Zone Evolution, School of Geography and Information Engineering, China University of Geosciences, Wuhan 430078, China

ARTICLE INFO

Article history: Received 28 December 2020 Received in revised form 17 June 2021 Accepted 25 June 2021 Available online 29 June 2021

Keywords: n-Alkanes Aquatic plants Carbon isotope Hydrogen isotope Fractionation

ABSTRACT

Leaf wax *n*-alkanes in lacustrine sequences have been widely applied for reconstructions of Late Quaternary paleoclimate histories. Such applications depend on knowledge of the factors controlling the characterization of the *n*-alkane paleoproxies of aquatic plants. This study analyzed the molecular distributions and carbon and hydrogen isotopic compositions of *n*-alkanes of aquatic plant samples collected from the middle and lower reaches of the Yangtze River (MLYR). In this collection, the *n*-alkane distributions of submersed/floating plants and emersed plants are distinctly different. By combining their P_{aq} and *n*-alkane δ^{13} C values, the *n*-alkane contributions from submersed plants and emersed plants in the MLYR can be differentiated. It is also noteworthy that the hydrogen isotope fractionation ($\varepsilon_{alk/water}$; avg. -166%) between the *n*-C₂₃ alkane of submersed plants in the MLYR and in precipitation agrees well with previous studies, suggesting a nearly constant $\varepsilon_{alk/water}$ for the submersed plants. Moreover, compared to C₃ dicots growing in a similar climate, submersed plants in the humid MLYR have lower *n*-alkane δ^2 H values. This feature may arise from isotopic differences in the evapotranspiration of lake water versus leaf water and in the biosynthetic fractionations of leaf waxes of these two plant groups. These results aid interpretations of the hydrogen isotope offset of the δ^2 H values of *n*-alkanes between aquatic and terrestrial plants in humid climates.

© 2021 Elsevier Ltd. All rights reserved.

1. Introduction

n-Alkanes are important components of plant waxes. They tend to be well preserved in diverse geological archives, including lacustrine and marine sediments, peat deposits, and paleosol-loess sequences (e.g., Meyers, 2003; Eglinton and Eglinton, 2008; Naafs et al., 2019). Their molecular distributions and carbon and hydrogen isotope compositions have become important tools for paleoclimate and paleoecology reconstructions in the Quaternary epoch (Castañeda and Schouten, 2011; Sachse et al., 2012; Diefendorf and Freimuth, 2017). In lacustrine environments, many studies have explored the paleoenvironmental significance of leaf wax *n*-alkanes (e.g., Huang et al., 2004; Sachse et al., 2004; Mügler et al., 2008; Aichner et al., 2010a; Garcin et al., 2012; Rao et al., 2014; Liu et al., 2015). In such settings, sources of *n*-

E-mail address: xyhuang@cug.edu.cn (X. Huang).

alkanes include not only autochthonous contributions of aquatic plants, bacteria, and algae, but also allochthonous contributions of terrestrial plants brought in by incoming runoff (Meyers, 2003; Castañeda and Schouten, 2011). Due to diverse sources of *n*-alkanes in lake sediments, the environmental significance of *n*-alkanes is not well understood.

Previous studies have revealed distinctive patterns of *n*-alkanes from different sources. Short-chain *n*-alkanes (<C₂₁) mainly derive from bacteria and algae (Cranwell et al., 1987; Meyers, 2003), midchain *n*-alkanes (C₂₁-C₂₅) are mainly produced by floating and submersed plants (plants growing or adapted to grow underwater), and long-chain *n*-alkanes (C₂₇-C₃₃) are usually interpreted to be terrestrial in origin (Ficken et al., 2000; Aichner et al., 2010a). The contributions of aquatic plants (submersed versus floating) relative to emersed and terrestrial plants are captured by the P_{aq} ratio [$P_{aq} = (C_{23} + C_{25})/(C_{23} + C_{25} + C_{29} + C_{31})$] (Ficken et al., 2000). This proxy has then been widely utilized for paleoenvironmental



^{*} Corresponding author at: School of Geography and Information Engineering, China University of Geosciences, Wuhan 430078, China.

https://doi.org/10.1016/j.orggeochem.2021.104270 0146-6380/© 2021 Elsevier Ltd. All rights reserved.

reconstructions based on lacustrine sequences (e.g., Das et al., 2009; Aichner et al., 2010a; Oritz et al., 2013; Sun et al., 2018).

Recently, some studies have argued that submersed plants can also contribute a moderate proportion of long-chain *n*-alkanes, which would complicate the application of the P_{aq} ratio in lacustrine sequences (Aichner et al., 2010a; Liu and Liu, 2016; Liu et al., 2016). In addition, some terrestrial plants contain significant amounts of mid-chain n-alkanes, adding complexity to the application of the P_{aq} ratio (e.g., Ladd et al., 2018; Berke et al., 2019; Dion-Kirschner et al., 2020; He et al., 2020). Furthermore, due to the similar *n*-alkane compositions of floating and submersed plants, the $P_{\rm aq}$ ratio does not have the ability to differentiate contributions from these two types of aquatic plants. In many shallow lakes, submersed plants have important ecological functions that are distinct from those of floating and emersed plants. Submersed plants are often major contributors to primary productivity and have important influences on water guality and other biogeochemical processes in lakes (e.g., Carpenter, 1981; Bayley and Prather, 2003). Thus, it would be valuable to develop proxies to track the dynamics of different components of aquatic plants in shallow lakes.

To distinguish the sources of *n*-alkanes in lake sediments, the carbon isotope compositions of *n*-alkanes ($\delta^{13}C_{alk}$) have been investigated in previous studies (e.g., Meyers, 2003; Castañeda and Schouten, 2011; Holtvoeth et al., 2019). Some of these studies commonly observed higher $\delta^{13}C_{alk}$ values in submersed plants than in terrestrial plants (e.g., Chikaraishi and Naraoka, 2003; Aichner et al., 2010a; Liu et al., 2015, 2018). These studies indicate that $\delta^{13}-C_{alk}$ values could act as an effective proxy to distinguish the sources of *n*-alkanes in lake sediments. Combining the P_{aq} and $\delta^{13}C_{alk}$ values has the potential to improve the source interpretation of *n*-alkanes in lacustrine sediments; however, this potential has not yet been tested in shallow lake settings.

In addition to the molecular distributions and carbon isotope compositions, the hydrogen isotope compositions of *n*-alkanes (δ^2 -H_{alk}) have been widely applied to hydroclimate reconstructions in lacustrine sequences (e.g., Castañeda and Schouten, 2011; Sachse et al., 2012). The $\delta^2 H_{alk}$ signals of individual compounds preserved in lake sediments could vield information about the evolution of the isotope composition of precipitation and its associated paleoclimate changes (e.g., Huang et al., 2004; Sachse et al., 2004). In addition, the $\delta^2 H_{alk}$ differences between terrestrial and aquatic plants ($\varepsilon_{terr-aq}$) have been proposed as an effective indicator of paleohumidity (e.g., Mügler et al., 2008; Rach et al., 2014, 2017; Arnold et al., 2018). Interpretation of $\varepsilon_{terr-aq}$ is based on the phenomenon that the isotope composition of the source water of aquatic plants is relatively unaffected by evaporation, whereas the isotope composition of the source water used for wax production in terrestrial leaves is sensitive to evapotranspiration (Mügler et al., 2008; Rach et al., 2014). However, downcore results from lake sediments from the Tibetan Plateau did not reveal a direct relation between $\varepsilon_{terr-aq}$ and paleohumidity (Rao et al., 2014). In addition, the efficacy of this proxy might be limited by the source complexity of *n*-alkanes in lake sediments (Rao et al., 2014). To date, knowledge of $\delta^2 H_{alk}$ variations of *n*-alkanes > C₂₁ is limited for contemporary aquatic plants, particularly in regions with a humid monsoon climate (Chikaraishi and Naraoka, 2003; Aichner et al., 2010b; Liu et al., 2019).

There are many shallow lakes in the middle and lower reaches of the Yangtze River (MLYR), and aquatic plants flourish in many of them (Wang and Dou, 1998; Fang et al., 2006), providing an excellent setting to study the differences of *n*-alkane distributions and compound-specific isotope values in different types of aquatic plants. In this study, we collected aquatic plants from five representative lakes in the MLYR to establish a more effective method to evaluate the autochthonous sources of plant waxes in lake sediments and to interpret any variations of *n*-alkane δ^2 H values in the aquatic plants from these shallow lakes.

2. Materials and methods

2.1. Study area and sampling

The MLYR region in eastern China has a humid subtropical monsoon climate. In this region, there are 108 lakes with surface areas>10 km², including the three largest freshwater lakes in China, Lake Poyang, Lake Dongting and Lake Taihu (Wang and Dou, 1998). The warm and humid climate of the early- and mid-Holocene increased the water volume of the Yangtze River, which combined with the postglacial rise in sea-level, led to the development of lakes in the MLYR (Fang, 1991; Xu et al., 2019). Because the terrain around the lakes has low relief, the lake basins are shallow and their water depths are commonly < 5 m (Wang and Dou, 1998; Wu et al., 2012).

In this study, samples of aquatic plants were collected along an east-west transect of the MLYR in May and June of 2019 (Fig. 1). Twelve species of common aquatic macrophytes were collected from Lake Chang (JZCH), Lake Hong (HH), Lake Longgan (LGH), Lake Shijiu (SJH) and Lake Yangcheng (YCH) in this transect (Table 1). The common aquatic plant species are *Najas marina*, *Myriophyllum spicatum*, *Potamogeton wrightii*, *Potamogeton pectinatus*, *Vallisneria asiatica*, *Hydrilla verticillata*, *Trapa* sp., *Nymphyoides peltatum*, *Phragmites communis*, *Zizania caduciflora*, *Jussiaea repens* and *Alternanthera philoxeroides*. In total, 11 submersed, 6 floating and 6 emersed plant samples were collected for leaf wax *n*-alkane analysis (Table 1).

Immediately after collection in the field, plant samples were individually wrapped in aluminum foil and preserved in a sealed bag. After transport to the laboratory, all samples were rinsed with distilled water and stored in a refrigerator at -20 °C until further analysis.

2.2. Lipid extraction

Plant samples were washed with deionized water, freeze-dried, and cut into small pieces. The homogenized plant samples (ca.1 g) were ultrasonically extracted 15 min \times 4 in CH₂Cl₂/MeOH (9:1, v/ v). Before extraction, cholane was added as an internal standard. The total lipid extract was separated into aliphatic and polar fractions using silica gel chromatography. The aliphatic fraction containing *n*-alkanes was eluted with *n*-hexane.



Fig. 1. Map of shallow lakes investigated in this study.

Table 1

Information of shallow freshwater lakes investigated in this study.

name	abbreviations	latitude (°N)	longitude (°E)	altitude(m)	depth ^a (m)	area ^a (km ²)	рН ^ь
L. Chang	JZCH	30.43	112.46	4	3.5	131	8.5
L. Hong	HH	29.90	113.42	19	2.0	344	8.6
L. Longgan	LGH	29.91	116.11	27	3.2	316	8.6
L. Shijiu	SJH	31.50	118.94	9	3.3	210	8.8
L. Yangcheng	YCH	31.44	120.79	16	1.9	119	8.4

a: data from Wu et al. (2012) and Hao et al. (2015).

b: data from Dang et al. (2016).

2.3. Instrumental analyses

n-Alkanes were analyzed using a Shimadzu GC-2010 gas chromatograph (GC) equipped with a flame ionization detector (FID) and a DB-5 column (30 m \times 0.25 mm \times 0.25 µm film thickness). The sample was injected in splitless mode (1 µl) with the injector at 300 °C. The GC oven temperature was initiated at 70 °C, then ramped to 210 °C at 10 °C/min, and finally raised to 300 °C at 3 °C/min (held 25 min). Compounds were identified by comparison of sample peaks with the retention times of a standard mixture. Quantification was achieved by comparison of peak areas with the internal standard after adjustment for the relevant response factors.

The δ^{13} C values of *n*-alkanes were measured with Finnigan Trace GC coupled with a Finnigan Delta XP isotope ratio mass spectrometer (GC-IRMS) in the State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences (CUG), Wuhan. Samples were injected in splitless mode $(1 \mu l)$, with the injector at 290 °C, and they were separated with a DB-5MS column $(30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \text{ } \mu\text{m} \text{ film thickness})$. The analytical procedures were the same as Zhao et al. (2018). Instrumental performance was verified by running an inhouse *n*-alkane mixture (containing n-C₂₃, C₂₅, C₂₇, C₂₉, C₃₁ and C₃₃ alkanes) and the Indiana A4 mixture (n-C₁₆₋₃₀ alkanes, obtained from Arndt Schimmelmann, Indiana University). Each sample was run twice; only peak intensities (m/z 44) > 500 mv are reported in this study. The standard deviation for duplicate analyses was better than ± 0.5‰. Squalane (δ^{13} C value –19.8‰) was added as internal standard for both samples and standards. Results were reported in the delta notation (%) relative to Vienna Peedee Belemnite (VPDB).

The δ^2 H analysis of *n*-alkanes was achieved using a Trace GC coupled with a Delta V Advantage isotope ratio mass spectrometer in the State Key Laboratory of Biogeology and Environmental Geology, CUG. Samples were injected in splitless mode $(1 \mu l)$ with the injector temperature at 290 °C, and a DB-5MS column (30 m \times 0. $25 \text{ mm} \times 1.0 \text{ }\mu\text{m}$ film thickness) was employed for compound separation. The analytical procedures were identical to that of Huang et al. (2018). Briefly, the H³⁺ factor was monitored daily, with the daily variation < 0.1. An inhouse *n*-alkane mixture (containing *n*-C₂₃, C₂₅, C₂₇, C₂₉, C₃₁ and C₃₃ alkanes) and the Indiana A4 mixture were run between every two samples to monitor the system stability. Squalane (δ^2 H value –167‰) was added as internal standard for both samples and standards. Each sample was run twice. Reproducibility for *n*-alkanes with a sufficient concentration $(m/z \ 2$ intensity in the range of 1000-4000 mv) was better than 5%, based on at least duplicate analyses. All δ^2 H values were reported in the δ notation (%) relative to the Vienna Standard Mean Ocean Water (VSMOW) standard.

3. Results

3.1. Concentrations and distributions of n-alkanes

In the aquatic plant samples in this study, *n*-alkane chain lengths range from C_{21} to C_{33} (Table 2, Fig. 2). The total *n*-alkane $(C_{21}-C_{33})$ concentrations vary among aquatic plants, with higher values in *M. spicatum* (range from 353.6 to 751.9 µg/g dry weight), *Trapa* sp. (range from 135.9 to 354.9 µg/g dry weight), and *A. philoxeroides* (361.9 µg/g dry weight) (Table 2), which are sub-

Table 2

Concentration of n-alkanes (µg/g dry weight) in aquatic plants in the middle-lower reaches of the Yangtze River.

No.	Species	Lake	Туре	C ₂₁	C ₂₂	C ₂₃	C ₂₄	C ₂₅	C ₂₆	C ₂₇	C ₂₈	C ₂₉	C ₃₀	C ₃₁	C ₃₂	C ₃₃	Sum ^a	$P_{\rm aq}$ ^b	CPI ^c	ACL^{d}
1	Najas marina	L. Chang	submersed	2.9	0.4	12.2	0.9	9.8	0.7	6.6	0.5	6.7	0.4	1.5			42.5	0.73	13.1	25.3
2	Najas marina	L. Longgan	submersed	4.8	0.4	12.9	0.8	9.4	0.5	5.7	0.4	6.3	0.3	1.7			43.2	0.74	16.1	25.0
3	Potamogeton pectinatus	L. Chang	submersed	11.7	0.6	27.9	1.0	18.3	0.7	8.8	0.5	4.7	0.2	2.9			78.1	0.86	22.0	24.4
4	Potamogeton wrightii	L. Hong	submersed	18.5	0.5	13.6	0.7	12.1	0.6	7.8	0.4	1.8	0.2	0.5			56.8	0.92	18.6	23.6
5	Potamogeton wrightii	L. Shijiu	submersed	14.5	0.4	8.3	0.5	8.3	0.4	5.5	0.2	1.1					39.2	0.94	20.8	23.4
6	Potamogeton wrightii	L. Yangcheng	submersed	38.5	1.5	40.2	1.8	39.0	1.5	25.0	0.6	5.4					153.5	0.94	23.8	23.9
7	Myriophyllum spicatum	L. Hong	submersed	2.2	2.4	80.4	57.0	446.3	53.2	92.8	8.7	9.1					752.0	0.98	5.2	25.1
8	Myriophyllum spicatum	L. Shijiu	submersed	0.5	0.8	35.2	18.6	231.7	16.2	46.4	1.9	2.4	0.2	0.5			354.4	0.99	8.4	25.1
9	Myriophyllum spicatum	L. Yangcheng	submersed	0.4	1.6	27.9	33.6	207.1	28.3	48.0	3.2	2.9	0.2	0.5			353.6	0.99	4.3	25.2
10	Vallisneria asiatica	L. Yangcheng	submersed	4.3	1.0	54.1	3.8	26.4	5.4	24.1	9.2	24.2	4.0	7.6			164.0	0.72	5.9	25.5
11	Hydrilla verticillata	L. Yangcheng	submersed	18.0	6.6	40.7	2.8	5.2	0.6	1.2	0.5	0.6	0.2	1.4			77.8	0.96	5.5	22.9
12	Trapa sp.	L. Chang	floating	1.0	0.4	33.5	4.4	171.9	2.6	53.1	1.1	16.0	2.3	10.5	0.5	2.4	299.9	0.89	25.4	25.6
13	Trapa sp.	L. Hong	floating	1.0	0.3	22.0	3.8	145.3	2.6	42.1	0.9	12.0	0.4	6.1			236.5	0.90	28.4	25.5
14	Trapa sp.	L. Longgan	floating	0.3	0.1	6.6	2.7	207.7	4.3	80.1	1.5	28.2	1.0	18.2	0.4	3.9	354.9	0.82	34.4	26.2
15	Trapa sp.	L. Yangcheng	floating	1.0	0.5	38.3	4.2	151.1	2.4	51.9	1.0	15.3	0.4	7.7	0.2	1.9	275.9	0.89	30.5	25.5
16	Trapa sp.	L. Shijiu	floating	1.1	0.4	17.6	3.4	76.8	1.6	21.5	0.6	6.9	0.4	4.4	0.2	0.8	135.9	0.89	19.2	25.5
17	Nymphyoides peltatum	L. Shijiu	floating	2.4	1.1	27.4	3.3	21.7	1.0	8.7	0.6	4.5	0.3	2.2			73.3	0.88	10.3	24.8
18	Alternanthera philoxeroides	L. Hong	emersed	1.5	1.8	18.2	6.4	26.0	8.9	69.5	13.3	138.6	10.6	57.3	3.0	6.9	362.9	0.18	7.1	28.3
19	Jussiaea repens	L. Yangcheng	emersed	0.5	0.2	1.1	0.7	6.2	1.1	8.8	1.5	8.9	0.9	4.5	0.5	0.7	35.4	0.35	6.2	27.7
20	Phragmites communis	L. Hong	emersed	0.3	0.5	4.1	4.1	15.3	7.9	36.6	7.4	41.9	1.9	7.2	0.4	1.2	128.8	0.28	4.8	27.7
21	Phragmites communis	L. Shijiu	emersed	0.3	0.2	2.0	1.0	4.1	2.6	14.4	2.9	20.3	0.9	3.8	0.2	1.4	54.1	0.20	5.7	28.0
22	Phragmites communis	L. Yangcheng	emersed	0.3	0.4	5.1	1.8	6.8	4.0	17.0	4.8	29.9	1.1	4.8	0.2	1.3	77.6	0.26	5.3	27.8
23	Zizania caduciflora	L. Yangcheng	emersed	0.2	0.2	1.4	1.5	4.5	3.2	9.4	2.8	12.3	1.1	3.4	0.2	0.8	41.0	0.27	3.5	27.9

 $a Sum = \sum C_{21-33}; b P_{aq} = (C_{23} + C_{25})/(C_{23} + C_{25} + C_{29} + C_{31}); c CPI = [\sum_{odd}(C_{21-31}) + \sum_{odd}(C_{23-33})]/[2 \times \sum_{even}(C_{22-32})]; d ACL = \sum_{odd}(n \times C_n)/\sum_{odd}(C_n) + \sum_{odd}(C_{23-33})/(2 \times \sum_{even}(C_{23-32})); d ACL = \sum_{odd}(n \times C_n)/\sum_{odd}(C_n) + \sum_{odd}(C_{23-33})/(2 \times \sum_{even}(C_{23-32})); d ACL = \sum_{odd}(n \times C_n)/\sum_{odd}(C_n) + \sum_{odd}(C_{23-33})/(2 \times \sum_{even}(C_{23-32})); d ACL = \sum_{odd}(n \times C_n)/\sum_{odd}(C_n) + \sum_{odd}(C_{23-33})/(2 \times \sum_{even}(C_{23-32})); d ACL = \sum_{odd}(n \times C_n)/\sum_{odd}(C_n) + \sum_{odd}(C_n)/\sum_{even}(C_n)/\sum_{odd}(C_n) + \sum_{odd}(C_n)/\sum_{odd}(C_n) + \sum_{odd}(C_n)/\sum_{even}(C_n)$



Fig. 2. Histograms of *n*-alkane distributions of the aquatic plant samples collected from the MLYR. (n is the number of samples; the error bar presents the difference in concentration of *n*-alkanes between different samples.)



Fig. 3. Plots of P_{aq} values against the ACL values (a) and CPI values (b) of *n*-alkanes in the aquatic plant samples collected from the MLYR.

mersed, floating, and emersed plants, respectively. In all submersed and floating plant samples, the concentrations of C_{32} and C_{33} *n*-alkanes are below the detection limit (Table 2). In three submersed specimens (*P. wrightii* collected from Lake Shijiu and Yangcheng, *M. spicatum* collected from Lake Hong), the concentrations of C_{30} and C_{31} *n*-alkanes are also nearly absent. The total *n*alkane concentrations in the other aquatic plants vary in the relatively narrow range from 35.4 to 164.0 µg/g dry weight (Table 2). For *n*-alkane distributions, emersed plants have *n*-alkane distributions similar to terrestrial plants, both being dominated by C_{29} or C_{31} (Fig. 2). In contrast, submersed plants and floating plants are dominated by C_{23} or C_{25} . In the submersed plants and floating plants, *N. marina*, *P. wrightii*, *V. asiatica*, *H. verticillata* and *N. peltatumare* are dominated by the C_{23} *n*-alkane, whereas *M. spicatum* and *Trapa* sp. have a predominance of the C_{25} *n*-alkane. In addition, *P. pectinatus* has an especially high proportion of the C_{21} *n*-alkane. In the emersed plants, *J. repens* is dominated by the C_{27} and C_{29} *n*alkanes, whereas *A. philoxeroides*, *P. communis* and *Z. caduciflora* are dominated by the C_{29} *n*-alkane. The distributions of *n*-alkanes in this study are consistent with the results of previous investigations of aquatic plants (Ficken et al., 2000; Chikaraishi and Naraoka, 2003; Aichner et al., 2010a; Liu and Liu, 2016).

In our dataset, the P_{aq} values in submersed plants range from 0.72 to 0.99 (Table 2, Fig. 3). The floating plants in this study also

have relatively higher P_{aq} values (0.82–0.90, Table 2). In contrast, emersed plants are characterized by lower P_{aq} values (range from 0.18 to 0.35), which are significantly different from those from submersed/floating plants (one-way ANOVA test, p < 0.001). To better compare the *n*-alkane distributions among different types of aquatic plants, the averaged chain length (ACL) and carbon preference index (CPI) were calculated (Fig. 3). The ACL values in emersed plants (avg. 27.9, 1 σ 0.2) are significantly higher than in floating plants (avg. 25.5, 1 σ 0.4) and submersed plants (avg. 24.5, 1 σ 0.9; one-way ANOVA test, p < 0.001). The CPI values in both floating plants (avg. 24.7, 1 σ 8.7) and submersed plants (avg. 13.1, 1 σ 7.5) are variable, whereas the CPI values in emersed plants vary in a relatively narrow range (avg. 5.4, 1 σ 1.2; Table 2; Fig. 3).

3.2. Carbon and hydrogen isotope compositions of n-alkanes

In the aquatic plant samples collected from the MLYR, the δ^{13} - C_{alk} values in submersed plants range from -17.1% to -29.0% (Table 3). In contrast, $\delta^{13}C_{alk}$ values vary from -31.8% to -36.9% in floating plants and -29.9% to -38.2% in emersed

plants (Table 3, Fig. 4). The $\delta^{13}C_{alk}$ values of mid-chain *n*-alkanes differ significantly between the submersed plants and floating plants (one-way ANOVA, F = 9.0, *p* < 0.001), and between the submersed plants and emersed plants (one-way ANOVA, F = 11.6, *p* < 0.001). In contrast, the $\delta^{13}C_{alk}$ values of mid-chain *n*-alkanes are not distinct between the floating plants and emersed plants (one-way ANOVA, F = 2.6, *p* = 0.19). For the long-chain *n*-alkane homologs in most of the submersed plant samples, the concentration of *n*-C₂₉ alkane was not adequate to yield accurate $\delta^{13}C$ values (Table 3). However, the $\delta^{13}C_{alk}$ values of the *n*-C₂₇ alkane are similar between the floating plants and emersed plants (one-way ANOVA, *p* > 0.05), whilst the $\delta^{13}C_{alk}$ values of the *n*-C₂₇ alkane are lower in the submersed plants relative to the other two groups (one-way ANOVA, *p* < 0.001).

The $\delta^2 H_{alk}$ values of submersed plants range from -177% to -233%, floating plants range from -162% to -203%, and emersed plants are between -166% to -204% (Table 3, Fig. 4). Due to the presence of C₂₅ across sample types, the $\delta^2 H_{alk}$ values of n-C₂₅ alkane ($\delta^2 H_{C25}$) can be used as representative of the mid-chain n-alkanes in the plants. The one-way ANOVA analysis indicates no

Table 3

Carbon and hydrogen isotope compositions of *n*-alkanes in the aquatic plant samples.

				•										
No	Species	Lake	Туре	δ ¹³ C(% <i>c</i> , VPDB)					δ ² H (‰, VSMOW)					
				C ₂₃	C ₂₅	C ₂₇	C ₂₉	C ₃₁	C ₂₃	C ₂₅	C ₂₇	C ₂₉	C ₃₁	
1	Najas marina	L. Chang	submersed	-22.1	-22.2	-22.7	-23.0	n.a.	-232	-233	n.a.	-237	n.a.	
2	Najas marina	L. Longgan	submersed	-22.9	-23.5	-24.4	-24.9	n.a.	-195	-199	-212	-215	n.a.	
3	Potamogeton pectinatus	L. Chang	submersed	-20.4	-20.3	-20.8	n.a.	n.a.	-208	-208	n.a.	n.a.	n.a.	
4	Potamogeton wrightii	L. Hong	submersed	-17.1	-18.2	-19.4	n.a.	n.a.	-202	-200	-203	n.a.	n.a.	
5	Potamogeton wrightii	L. Shijiu	submersed	-21.0	-21.7	-22.5	n.a.	n.a.	-202	-203	-198	n.a.	n.a.	
6	Potamogeton wrightii	L. Yangcheng	submersed	-26.5	-26.7	-27.2	n.a.	n.a.	-202	-200	-203	n.a.	n.a.	
7	Myriophyllum spicatum	L. Hong	submersed	-25.8	-26.1	-26.4	n.a.	n.a.	n.a.	-192	-179	n.a.	n.a.	
8	Myriophyllum spicatum	L. Shijiu	submersed	n.a.	-24.6	-24.3	n.a.	n.a.	n.a.	-214	n.a.	n.a.	n.a.	
9	Myriophyllum spicatum	L. Yangcheng	submersed	n.a.	-27.8	n.a.	n.a.	n.a.	-220	-215	n.a.	n.a.	n.a.	
10	Vallisneria asiatica	L. Yangcheng	submersed	-28.0	-27.6	-28.5	-29.0	n.a.	-188	-177	-180	n.a.	n.a.	
11	Hydrilla verticillata	L. Yangcheng	submersed	-25.7	n.a.	n.a.	n.a.	n.a.	n.a.	-219	n.a.	n.a.	n.a.	
12	Trapa sp.	L. Chang	floating	-32.9	-31.8	-31.8	n.a.	n.a.	n.a.	-197	-187	n.a.	n.a.	
13	Trapa sp.	L. Hong	floating	-33.6	-32.8	-33.2	n.a.	n.a.	n.a.	-188	-180	n.a.	n.a.	
14	Trapa sp.	L. Longgan	floating	n.a.	-32.8	-32.5	n.a.	n.a.	n.a.	-187	-185	n.a.	n.a.	
15	Trapa sp.	L. Yangcheng	floating	-36.9	-34.5	-35.2	n.a.	n.a.	-162	-171	-169	n.a.	n.a.	
16	Trapa sp.	L. Shijiu	floating	-35.9	-32.2	-32.5	n.a.	n.a.	-185	-203	-201	n.a.	n.a.	
17	Nymphyoides peltatum	L. Shijiu	floating	-33.6	-33.4	-33.3	n.a.	n.a.	-188	-190	n.a.	n.a.	n.a.	
18	Alternanthera philoxeroides	L. Hong	emersed	n.a.	-34.1	-34.6	-35.7	-36.9	n.a.	n.a.	-178	-166	n.a.	
19	Jussiaea repens	L. Yangcheng	emersed	n.a.	-34.4	-34.4	-33.7	-34.9	n.a.	-178	-190	-193	n.a.	
20	Phragmites communis	L. Hong	emersed	n.a.	-37.4	-38.4	-38.2	n.a.	n.a.	-195	-192	-204	n.a.	
21	Phragmites communis	L. Shijiu	emersed	n.a.	n.a.	-35.5	-35.3	n.a.	n.a.	-195	-192	-204	n.a.	
22	Phragmites communis	L. Yangcheng	emersed	n.a.	n.a.	-33.9	-33.7	n.a.	n.a.	n.a.	-187	-199	n.a.	
23	Zizania caduciflora	L. Yangcheng	emersed	n.a.	n.a.	-36.0	-34.9	n.a.	n.a.	-183	-202	-194	n.a.	

n.a.: not available.



Fig. 4. Plots of the carbon isotope compositions against the hydrogen isotope compositions of mid-chain (a) and long-chain (b) *n*-alkanes in the aquatic plant samples collected from the MLYR.

significant difference among the $\delta^2 H_{C25}$ values in the three types of aquatic plants (p > 0.05; Fig. 4). Although the average $\delta^2 H_{C25}$ value in the submersed plants (-206%c, $1\sigma 15\%c$) is lower than that in the floating plant (-189%c, $1\sigma 11\%c$), this difference does not pass the significance test (p > 0.05). In the long-chain *n*-alkanes, the *n*-C₂₇ alkane yielded higher $\delta^2 H$ values than its counterparts (Table 3). The of $\delta^2 H$ values of *n*-C₂₇ alkane ($\delta^2 H_{C27}$) are not distinct among the three types of aquatic plants (one-way ANOVA, p > 0.05). The mean $\delta^2 H_{C27}$ values are similar across the three types (submersed -196%c, $1\sigma 13\%c$; floating -184%c, $1\sigma 12\%c$; emersed -190%c, $1\sigma 8\%c$).

4. Discussion

4.1. Distinguishing submersed and floating aquatic plants by combining P_{aq} values and $\delta^{13}C_{alk}$ values

For investigation of lake organic matter sources, the P_{aq} ratio has been an effective indicator to distinguish contributions from submersed/floating aquatic macrophytes relative to subaerial emersed and terrestrial plant inputs (Ficken et al., 2000). As initially proposed by Ficken et al. (2000) and found in later studies, the P_{aq} index can be used to distinguish emersed plant sources from the submersed/floating plant sources in shallow lakes in MLYR. However, P_{aq} values alone cannot effectively distinguish between submersed and floating plant sources due to similar *n*-alkane molecular compositions. To distinguish between the multiple potential aquatic plant sources of *n*-alkanes, additional indicators are required.

For the batch of aquatic plant samples collected from the MLYR, the combination of P_{aq} and $\delta^{13}C_{alk}$ values can effectively distinguish the three types of aquatic plants (Fig. 5). *n*-Alkanes from submersed plants have higher P_{aq} values and higher $\delta^{13}C$ values compared to floating plants and emersed plants (Fig. 5). Although

 P_{aq} values from floating plants are also higher, their $\delta^{13}C_{alk}$ values are relatively lower (Fig. 5).

Carbon isotope compositions of *n*-alkanes in plants closely relate to the carbon fixation pathway, carbon source, and environmental factors (Farquhar et al., 1989; Aichner et al., 2010a; Diefendorf and Freimuth, 2017). Higher plants can be divided by their photosynthetic pathways into C₃ plants, C₄ plants, and crassulacean acid metabolism (CAM) plants (Ting, 1985; Farquhar et al., 1982; Farquhar et al., 1989). The $\delta^{13}C_{alk}$ values of C₃ plants are more negative, whereas those of C₄ plants are more positive, and the $\delta^{13}C_{alk}$ values of CAM plants fall between C_3 and C_4 plants (Collister et al., 1994; Chikaraishi and Naraoka, 2003; Bi et al., 2005; Diefendorf and Freimuth, 2017). Although the C₃, C₄ and CAM pathways can all exist in aquatic plants, most aquatic plants use the C_3 carbon fixation pathway (Keeley, 1998). The aquatic plant species investigated in this study belong to C₃ plants, except for *V. asiatica* and *H. verticillata*. The CAM photosynthetic pathway is used by V. asiatica, and C₄ photosynthetic pathway is used by H. verticillata (Holaday and Bowea, 1980; Keeley, 1998; Yin et al., 2017).

In our results, the $\delta^{13}C_{alk}$ values of some C₃ aquatic plants (ranging from -17.1% to -27.2% in *P. malaianus*) are higher than found for the C₄ plants (ranging from -27.6% to -29.0% in *H. verticillata*), suggesting that the carbon fixation pathway has little effect on $\delta^{13}C_{alk}$ values of submersed plants (Table 3). In addition to carbon fixation pathway, the source of inorganic carbon is also an important determinant of isotopic composition of organic compounds. CO₂ diffuses more slowly in water than in the atmosphere (Smith and Walker, 1981; Maberly and Madsen, 1998). Thus, the concentration of CO₂ in water is lower than in air and often is a limiting factor for the photosynthesis of aquatic plants (Madsen, 1984). In alkaline conditions (pH ca. 8 or more) like the lakes in this study (Table 1), HCO₃ is the main form of dissolved inorganic carbon. All leaves of submersed plants are under water, and these



Fig. 5. Plots of *P*_{aq} values against the carbon and hydrogen isotope compositions of mid-chain (a, c) and long-chain (b, d) *n*-alkanes in the aquatic plant samples collected from the MLYR.

species utilize HCO₃ for biosynthesis (Madsen, 1984). Because the δ^{13} C value of HCO₃ is higher than that of atmospheric CO₂ (Chikaraishi and Naraoka, 2003), the $\delta^{13}C_{alk}$ values of submersed plants are less negative (Allen and Spence, 1981; Prins and Elzenga, 1989; Aichner et al., 2010c). In contrast, the $\delta^{13}C_{alk}$ values of the C₂₃ and C₂₅ *n*-alkanes in the floating plants are similar to those of the C₂₇ and C₂₉ *n*-alkanes in the emersed plants (Fig. 5) and terrestrial plants growing in a similar climate context (e.g., Chikaraishi and Naraoka, 2003; Bi et al., 2005), suggesting that these plants mainly utilize atmospheric CO₂.

Other than the dominant influence of the carbon source, environmental factors and phylogeny would bring additional effects on the variations of $\delta^{13}\bar{C}_{alk}$ values in aquatic plants. In this study, some samples belong to the same species or the same genera (e.g., *P. wrightii*); however, the $\delta^{13}C_{alk}$ values of the C_{23} and C_{25} *n*alkanes are variable in the specimens collected from different lakes (Table 3). These differences may result from the influence of environmental factors on the growth rates of the aquatic plants by controlling the supply of carbon sources (Keeley and Sandquist, 1992). The spatial pattern of carbon pool in lakes may also affect the carbon isotope compositions of $\delta^{13}C_{alk}$ values in aquatic plants (Keeley and Sandquist, 1992). Wind-induced mixing is common in the shallow lakes of the MLYR (Li et al., 2017). Thus, the dissolved inorganic carbon is nearly homogeneous in these lakes due to weak to absent water stratification. In this case, submersed plants from the same lake have similar $\delta^{13}C_{alk}$ values of the mid-chain *n*-alkanes. Taking Lake Yangcheng as an example, the $\delta^{13}C_{alk}$ values of the C_{23} and C_{25} *n*-alkanes in the four submersed specimens range from -25.7% to -28.0% (P. wrightii, M. spicatum, V. asiatica, and H. verticilata; Table 3).

4.2. Comparison of hydrogen isotope fractionation between aquatic plants and C_3 terrestrial plants

Different from the $\delta^{13}C_{alk}$ values, our results reveal that the δ^2 -H_{alk} values could not distinguish the different aquatic plant types in the batch of samples collected from the MLYR (Fig. 5). In addition, in the same type of aquatic plants, a relatively poor correlation is found between the $\delta^{13}C_{alk}$ and δ^2 H_{alk} values of the same *n*-alkane (Fig. 4). An exception is the *n*-C₂₅ alkane, which shows a moderately negative correlation between its carbon and hydrogen isotope compositions in the floating plants (*r* = -0.90, *p* = 0.014). This difference may result from the different factors controlling carbon and hydrogen isotope compositions of *n*-alkanes in aquatic plants.

C₃ terrestrial plants are the dominant vegetation in the humid regions influenced by the East Asian summer monsoon and are the major contributors of long-chain *n*-alkanes to lacustrine sedimentary sequences (e.g., Rao et al., 2016; Sun et al., 2016; Xue et al., 2017). To improve interpretation of the hydrogen isotope signals of *n*-alkanes in lake sediments in regions dominated by the East Asian summer monsoon, it is valuable to compare the apparent fractionation ($\varepsilon_{alk/water}$) between the hydrogen isotope composition of *n*-alkanes and source water ($\delta^2 H_{water}$) in aquatic plants in the MLYR with those of terrestrial C₃ plants growing under a similar climate context (Chikaraishi and Naraoka, 2003; Bi et al., 2005). The $\varepsilon_{alk/water}$ value (‰) was calculated using the equation of Sachse et al. (2012):

$$\varepsilon_{alk/water} = (\delta^2 H_{alk} + 1000) / (\delta^2 H_{water} + 1000) - 1 \tag{1}$$

The actual δ^2 H values of lake water (δ^2 H_{lw}) are not available for the present study. Instead, we estimate mean annual δ^2 H values of precipitation (δ^2 H_p), from the precipitation isotope calculator (Bowen et al., 2005; https://wateriso.utah.edu/waterisotopes/pages/data_access/oipc.html) to obtain the $\varepsilon_{alk/water}$ values. This approach has been widely applied in previous studies (e.g., Sachse et al., 2004, 2012; Rao et al., 2009; McFarlin et al., 2019). Previous studies note that there is little offset between lake water and precipitation δ^2 H values in the MLYR (Zhang et al., 2020; Wu et al., 2021), providing confidence for using δ^2 H of precipitation as a constraint on water source. The $\varepsilon_{alk/water}$ values of the C₂₃ to C₂₉ *n*-alkanes in terrestrial C₃ plants range from -35% to -176%, in submersed plants from -133% to -202%, in floating plants from -117% to -160%, and in emersed plants from -128% to -169% (Fig. 6). The C₂₃ and C₂₉ *n*-alkanes have been widely selected as representative of aquatic plants and C₃ terrestrial plants, respectively. In our dataset, the $\varepsilon_{alk/water}$ values of the C₂₃ *n*-alkane ($-166 \pm 16\%$) in submersed plants are more negative than those of the C₂₉ *n*-alkane ($-128 \pm 30\%$) in C₃ terrestrial plants (one-way ANOVA F = 11.9, *p* < 0.01).

The $\delta^2 H_{alk}$ values in leaves of higher plants are affected by various factors, particularly the isotope compositions of source water, the influence of evapotranspiration, and biosynthetic fractionation (Sachse et al., 2012). In humid climates, precipitation exceeds evaporation, and previous studies conclude that evaporation has only a minor influence on the hydrogen isotope composition of lake water in these regions (Sachse et al., 2012; Sessions, 2016; Zhang et al., 2020). In contrast, evapotranspiration could enrich the leaf water of terrestrial plants in ²H (Kahmen et al., 2013a). In this way, aquatic plants probably utilize source water with $\delta^2 H$ values close to $\delta^2 H_p$ as discussed above, whilst leaf water used for leaf wax synthesis in terrestrial C₃ plants has higher $\delta^2 H$ values than $\delta^2 H_p$ (Sachse et al., 2004; Mügler et al., 2008).

Biosynthetic fractionation (ε_{bio}) may have an additional role in the relatively negative $\delta^2 H_{alk}$ values of aquatic plants found in this study. The mean $\varepsilon_{alk/water}$ values of the C₂₃ and C₂₅ *n*-alkanes observed in the MLYR are close to published values of hydrogen fractionation (Huang et al., 2004; Sachse et al., 2004; Li et al., 2015). In a batch of surface sediments of 36 lakes in North America, Huang et al. (2004) observed an average $\varepsilon_{alk/water}$ value of -156%for the C₁₇ *n*-alkane sourced from algae or photosynthetic bacteria in the lakes. Across a range of lakes, Sachse et al. (2004) found a similar average $\varepsilon_{alk/water}$ value of -157% for the C₁₇ *n*-alkane. In the transect from Sihailongwan Maar Lake to Lake Baikal, Li et al. (2015) calculated a mean $\varepsilon_{alk/water}$ value of -159% for the C₂₃ nalkane. For algae and submersed plants collected from lakes in the Tibetan Plateau, Liu and Liu (2019) found a mean $\varepsilon_{alk/water}$ value of -162‰ for n-alkanes. In three submersed species collected from Gunma, Japan, Chikaraishi and Naraoka (2003) showed a mean ε_{alkl} water value of $-135 \pm 17\%$. In fact, two of the three species had an $\varepsilon_{alk/water}$ value of -156% and -161% for the *n*-C₂₃ alkane, quite close to the mean value observed in the MLYR. The latest synthesis of global apparent hydrogen isotope fractionation between sedimentary n-C₂₃ alkane and lake waters yielded an intercept of -159% (McFarlin et al., 2019). Because the influence of evaporation on the isotope composition of lake waters is weak in humid climates (Sessions, 2016), the similar $\epsilon_{alk/water}$ values for *n*alkanes in algae and submersed plants from global studies clearly support a nearly constant (~-160%) hydrogen isotope fractionation during lipid production in submersed plants (Liu and Liu, 2019; McFarlin et al., 2019). This finding has important implications for paleoenvironmental applications. With the nearly constant $\epsilon_{alk/water}$ value, the $\delta^2 H_{alk}$ values of submersed plants are effective recorders of the hydrogen isotope composition of the source water, which is precipitation in most cases (Sachse et al., 2004; Liu and Liu, 2019).

Due to the difficulty in constraining the isotope composition of leaf water used for wax production in terrestrial plants, there is no consensus for their ε_{bio} values. Some studies have argued that the ε_{bio} values are variable among different species or different plant life-form types (e.g., Newberry et al., 2015; Sachse et al., 2015;



Fig. 6. Box-whisker plots of $\varepsilon_{alk/water}$ values in plant samples collected from the East Asian summer monsoon regions. (a) C_{23} , (b) C_{25} , (c) C_{27} and (d) C_{29} *n*-alkanes. Data were synthesized from Chikaraishi and Naraoka (2003), Bi et al. (2005), and this study. Filled symbols refer data from this study, while unfilled symbols refer data from references.

Tipple and Ehleringer, 2018). However, the hydrogen isotope fractionation between leaf water and lipid ($\varepsilon_{l/w}$) is often treated as a reliable representative of ε_{bio} (Berke et al., 2019). The latest synthesis by Liu and Liu (2019) showed mean ε_{bio} values of -162% for grasses and -139% for woody plants. This signature of ε_{bio} in grasses is close to the above $\varepsilon_{alk/water}$ values of submersed plants. If woody plants have an ε_{bio} of -139% (Sachse et al., 2006; Rach et al., 2014), the offset between ε_{bio} and the mean $\varepsilon_{alk/water}$ value of the C_{29} *n*-alkane in Chikaraishi and Naraoka (2003), Bi et al. (2005), and the global synthesis (-113 ± 31‰, Sachse et al., 2012) has an amplitude of about 20‰. This amount matches the variation of deuterium leaf water evaporative enrichment modeled by Kahmen et al. (2013b).

The seasonality of precipitation isotope compositions and the major synthesis time of *n*-alkanes in aquatic plants impose additional constraints on interpretations of their $\delta^2 H$ values. In the MLYR, the δ^2 H values of rain display a large seasonal difference, with more negative values in the summer monsoon dominated seasons (June to September; e.g., Huang et al., 2018; Zhang et al., 2020). Previous studies have demonstrated that the synthesis of leaf wax *n*-alkanes in terrestrial plants is not uniform in the growing season, with some bias towards specific intervals such as the early leaf development stage (e.g., Tipple et al., 2013; Freimuth et al., 2017). Knowledge of the seasonality of *n*-alkane synthesis in freshwater aquatic plants is limited. If the mode of *n*-alkane synthesis in terrestrial C₃ plants is the same as for aquatic plants, the comparison of results in the MLYR with published data would be affected by the sampling time. However, previous studies frequently observed a decreasing trend of $\delta^2 H_{alk}$ values in C_3 terrestrial plants along with leaf growth (e.g., Tipple et al., 2013; Freimuth et al., 2017; Huang et al., 2018). To constrain the $\varepsilon_{alk/water}$ difference between submersed plants and C₃ terrestrial plants, both a larger dataset and better information on the time of *n*-alkane synthesis in aquatic plants would be needed.

The comparison of $\epsilon_{alk/water}$ between submersed plants and C_3 terrestrial plants clearly supports the potential of $\varepsilon_{terr-ag}$ to reflect the degree of evapotranspiration and the associated relative humidity (Rach et al., 2014, 2017). With greater evapotranspiration, $\varepsilon_{terr-aq}$ values will increase, due to the relatively stronger influence of evapotranspiration on terrestrial plants than on aquatic plants. In lake sediment cores from the Tibetan Plateau spanning the Holocene, $\epsilon_{terr\text{-}aq}$ values reach as low as -80% (Rao et al., 2014). In Lake Meerfelder Maar, the $\varepsilon_{terr-aq}$ values were < -30% in the wetter climate interval before the onset of the Younger Dryas cooling event (Rach et al., 2014). However, the $\varepsilon_{terr-aq}$ values are not necessarily < 0 under humid climates with little or weak influence of evaporation. In the humid East Asian summer monsoon dominated regions, the $\varepsilon_{terr-aq}$ value will be as high as 28‰ (Fig. 6). In this case, it is best to focus on the trend rather than the absolute $\varepsilon_{terr-aq}$ values to track humidity-derived evapotranspiration processes.

The C₂₉ *n*-alkane is relatively high in abundance in some submersed and floating plants (Fig. 2, Table 2). Previous studies suggest that this compound comes from both terrestrial and aquatic sources, so it may not confidently be used as a biomarker for terrestrial input into lacustrine environments (e.g., Liu et al., 2016; Liu and Liu, 2019; Andrae et al., 2020). In our sample set, the C₂₉ *n*-alkane from submerged plants has very low hydrogen isotope values that resemble those of the mid-chain *n*-alkanes (Fig. 4). This isotopic feature can aid interpretation of the sources of C₂₉ *n*-alkane in lacustrine sediment sequences.

5. Summary and conclusions

Aquatic plant samples were collected from five shallow lakes in the MLYR to analyze the molecular distributions and carbon and hydrogen isotopic compositions of their n-alkanes to differentiate the n-alkane sources among different plant types in shallow

freshwater lakes and to improve interpretation of their $\delta^2 H_{alk}$ values. The main findings are:

1. In the MLYR, the P_{aq} ratio could distinguish the *n*-alkane contributions of submersed and floating plants from emersed plants. In addition, *n*-alkane δ^{13} C values are distinct between submersed plants and emersed plants, the latter having lower δ^{13} C values. This difference is due to the utilization of different dissolved inorganic carbon sources. Hence, combining P_{aq} and *n*-alkane δ^{13} C values has the potential to differentiate *n*-alkane contributions from different aquatic plant types, particularly the submersed and emersed plants.

2. In the humid MLYR, the apparent fractionation of hydrogen isotopes between mid-chain *n*-alkanes in submersed plants and precipitation (-166 ± 16%*e*) is similar to previous results, confirming a nearly constant $\varepsilon_{alk/water}$ for submersed plants. Thus, the δ^2 -H_{alk} values of submersed plants can be effective indicators of the hydrogen isotope composition of precipitation.

3. Compared to terrestrial C₃ dicots in the East Asian summer monsoon-dominated regions, the mean $\varepsilon_{alk/water}$ value is lower in aquatic plants. A probable explanation is the different influences of evaporation on lake water and evapotranspiration on leaf water. In this situation, it is more reliable to focus on isotopic patterns rather than absolute $\varepsilon_{terr-aq}$ values to track humidity-derived evapotranspiration processes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (U20A2094, 41877317). Jiantao Xue and Meiling Zhao are thanked for helping in the sample collection and lipid analyses. The Associate Editor, Dr. Isla S. Castañeda, and the three anonymous reviewers are thanked for their constructive comments to improve the quality of this manuscript.

Associate Editor-Isla S. Castaneda

References

- Aichner, B., Herzschuh, U., Wilkes, H., 2010a. Influence of aquatic macrophytes on the stable carbon isotopic signatures of sedimentary organic matter in lakes on the Tibetan Plateau. Organic Geochemistry 41, 706–718.
- Aichner, B., Herzschuh, U., Wilkes, H., Vieth, A., Böhner, A., 2010b. öD values of nalkanes in Tibetan lake sediments and aquatic macrophytes – A surface sediment study and application to a 16 ka record from Lake Koucha. Organic Geochemistry 41, 779–790.
- Aichner, B., Herzschuh, U., Wilkes, H., Mischke, S., Zhang, C., 2010c. Biomarker and compound specific carbon isotope evidence for changing environmental and carbon-limiting conditions at Koucha Lake, Eastern Tibetan Plateau. Journal of Paleolimnology 44, 873–899.
- Allen, E.D., Spence, D.H.N., 1981. The differential ability of aquatic plants to utilize the inorganic carbon supply in freshwater. New Phytologist 87, 269–283.
- Arnold, T.E., Diefendorf, A.F., Brenner, M., Freeman, K.H., Baczynski, A.A., 2018. Climate response of the Florida Peninsula to Heinrich events in the North Atlantic. Quaternary Science Reviews 194, 1–11.
- Andrae, J.W., McInerney, F.A., Sniderman, J.M.K., 2020. Carbon isotope systematic of leaf wax n-alkanes in a temperate lacustrine depositional environment. Organic Geochemistry 150, 1–9.
- Bayley, S.E., Prather, C.M., 2003. Do wetland lakes exhibit alternative stable states? Submersed aquatic vegetation and chlorophyll in western boreal shallow lakes. Limnology and Oceanography 48, 2335–2345.
- Berke, M.A., Sierra, A.C., Bush, R., Cheah, D., O'Connor, K., 2019. Controls on leaf wax fractionation and δ²H values in tundra vascular plants from western Greenland. Geochimica et Cosmochimica Acta 244, 565–583.
- Bi, X.H., Sheng, G.Y., Liu, X.H., Li, C., Fu, J.M., 2005. Molecular and carbon and hydrogen isotopic composition of *n*-alkanes in plant leaf waxes. Organic Geochemistry 36, 1405–1417.

- Bowen, G.J., Wassenaar, L.I., Hobson, K.A., 2005. Global application of stable hydrogen and oxygen isotopes to wildlife forensics. Oecologia 143, 337–348.
- Carpenter, S.R., 1981. Submersed vegetation: an internal factor in lake ecosystem succession. The American Naturalist 118, 372–383.Castañeda, I.S., Schouten, S., 2011. A review of molecular organic proxies for
- examining modern and ancient lacustrine environments. Quaternary Science Reviews 30, 2851–2891.
- Chikaraishi, Y., Naraoka, H., 2003. Compound-specific $\delta D \delta^{13}C$ analyses of *n*-alkanes extracted from terrestrial and aquatic plants. Phytochemistry 63, 361–371.
- Cranwell, P.A., Eglinton, G., Robinson, N., 1987. Lipids of aquatic organisms as potential contributors to lacustrine sediments- II. Organic Geochemistry 11, 513-527.
- Collister, J.W., Rieley, G., Stern, B., Eglinton, G., Fry, B., 1994. Compound-specific δ^{13} C analyses of leaf lipids from plants with differing carbon dioxide metabolisms. Organic Geochemistry 21, 619–627.
- Dang, X., Xue, J., Yang, H., Xie, S., 2016. Environmental impacts on the distribution of microbial tetraether lipids in Chinese lakes with contrasting pH: Implications for lacustrine paleoenvironmental reconstructions. Science China Earth Sciences 59, 939–950.
- Das, S.K., Routh, J., Roychoudhury, A.N., 2009. Biomarker evidence of macrophyte and plankton community changes in Zeekoevlei, a shallow lake in South Africa. Journal of Paleolimnology 41, 507–521.
- Diefendorf, A.F., Freimuth, E.J., 2017. Extracting the most from terrestrial plantderived *n*-alkyl lipids and their carbon isotopes from the sedimentary record: A review. Organic Geochemistry 103, 1–21.
- Dion-Kirschner, H., McFarlin, J.M., Masterson, A.L., Axford, Y., Osburn, M.R., 2020. Modern constraints on the sources and climate signals recorded by sedimentary plant waxes in west Greenland. Geochimica et Cosmochimca Acta 286, 336– 354.
- Eglinton, T.I., Eglinton, G., 2008. Molecular proxies for paleoclimatology. Earth and Planetary Science Letters 275, 1–16.
- Fang, J., 1991. Influence of sea level rise on the middle and lower reaches of the Yangtze River since 12100 BP. Quaternary Science Reviews 10, 527–536.
- Fang, J.Y., Wang, Z.H., Zhao, S.Q., Li, Y.K., Tang, Z.Y., Yu, D., Ni, L.Y., Liu, P.X., Da, L.J., Li, Z.Q., Zheng, C.Y., 2006. Biodiversity changes in the lakes of the Central Yangtze. Frontiers in Ecology and the Environment 4, 369–377.
- Farquhar, F.D., O'Leary, M.H., Berry, J.A., 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Australian Journal of Plant Physiology 1 9, 121–137.
- Farquhar, G.D., Ehleringer, J.R.A., Hubick, K.T., 1989. Carbon isotope discrimination and photosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology 40, 503–537.
- Ficken, K.J., Li, B., Swain, D.L., Eglinton, G., 2000. An *n*-alkane proxy for the sedimentary input of submerged/floating freshwater aquatic macrophytes. Organic Geochemistry 31, 745–749.
- Freimuth, E., Diefendorf, A., Lowell, T.V., 2017. Hydrogen isotopes of n-alkanes and n-alkanoic acids as tracers of precipitation in a temperate forest and implications for paleorecords. Geochimica et Cosmochimica Acta 206, 166–183.
- Garcin, Y., Schefuβ, E., Schwab, V.F., Garreta, V., Gleixner, G., Vincerns, A., Todou, G., Séné, O., Onana, J.M., Achoundong, G., Sachse, D., 2012. Reconstructing C₃ and C₄ vegetation cover using n-alkane carbon isotope ratios in recent lake sediments from Cameroon, Western Central Africa. Geochimica et Cosmochimica Acta 142, 482–500.
- Hao, M., Yang, L., Kong, X., Xu, X., Lu, W., Li, Z., 2015. Diversity and community succession of macrophytes in Lake Changhu, Hubei Province. Journal of Lake Sciences 27, 94–102 (in Chinese with English abstract).
- He, D., Ladd, S.N., Saunders, C.J., Mead, R.N., Jaffé, R., 2020. Distribution of *n*-alkanes and their δ²H and δ¹³C values in typical plants along a terrestrial-coastaloceanic gradient. Geochimica et Cosmochimica Acta 281, 31–52.
- Holaday, A.S., Bowea, G., 1980. C₄ acid metabolism and dark CO₂ fixation in a submerged aquatic (*Hydrilla verticillata*). Plant Physiology 65, 331–335.
- Huang, X.Y., Zhao, B.Y., Wang, K., Hu, Y., Meyers, P.A., 2018. Seasonal variations of leaf wax *n*-alkane molecular composition and δD values in two subtropical deciduous tree species: Results from a three-year monitoring program in central China. Organic Geochemistry 118, 15–26.
- Holtvoeth, J., Whiteside, J.H., Engels, S., Freitas, F.S., Grice, K., Greenwood, P., Johnson, S., Kendall, I., Lengger, S.K., Lücke, A., Mayr, C., Naafs, B.D.A., Rohrssen, M., 2019. The paleolimnologist's guide to compound-specific stable isotope analysis e An introduction to principles and applications of CSIA for Quaternary lake sediments. Quaternary Science Reviews 207, 101–133.
- Huang, Y.S., Shuman, B., Wang, Y., Web III, T., 2004. Hydrogen isotope ratios of individual lipids in lake sediments as novel tracers of climate and environmental change: a surface sediment test. Journal of Paleolimnology 31, 363–375.
- Kahmen, A., Schefuβ, E., Sachse, D., 2013a. Leaf water deuterium enrichment shapes leaf wax *n*-alkane δD values of angiosperm plants I: Experimental evidence and mechanistic insights. Geochimica et Cosmochimica Acta 111, 39–49.
- Kahmen, A., Hoffmann, B., Schefuß, E., Arndt, S.K., Cernusak, L.A., West, J., B., Sachse, D.,, 2013b. Leaf water deuterium enrichment shapes leaf wax *n*-alkane δD values of angiosperm plants II: Observational evidence and global implications. Geochimica et Cosmochimica Acta 111, 50–63.
- Keeley, J.E., Sandquist, D.R., 1992. Carbon: freshwater plants. Plant, Cell and Environment 15, 1021–1035.
- Keeley, J.E., 1998. CAM photosynthesis in submerged aquatic plants. The Botanical Review 64 (2), 121–175.

X. Yu, X. Lü, P.A. Meyers et al.

- Ladd, S.N., Nelson, D.B., Schubert, C.J., Dubois, N., 2018. Lipid compound classes display diverging hydrogen isotope responses in lakes along a nutrient gradient. Geochimica et Cosmochimica Acta 237, 103–119.
- Li, D.W., Han, J.T., Sun, H.G., Li, D., Pang, Z.H., Cui, L., Wang, X., Cao, Y.N., Liu, W.G., 2015. n-Alkanes and hydrogen isotope fractionations of aquatic plants in lakes on the Changbai Mountains-Lake Baikal transect. Chinese Science Bulletin 60, 2774–2883 (in Chinese with English abstract).
- Li, Y.P., Jalil, A., Du, W., Gao, X.M., Wang, J.W., Luo, L.C., Li, H.Y., Dai, S.J., Hashim, S., Yu, Z.B., Acharya, K., 2017. Wind induced reverse flow and vertical profile characteristics in a semi-enclosed bay of large shallow Lake Taihu, China. Ecological Engineering 102, 224–233.
- Liu, H., Liu, W.G., 2016. n-Alkane distributions and concentrations in algae, submerged plants and terrestrial plants from the Qinghai-Tibetan Plateau. Organic Geochemistry 99, 10–22.
- Liu, H., Yang, H., Cao, Y., Liu, W.G., 2018. Compound-specific δD and its hydrological and environmental implication in the lakes on the Tibetan Plateau. Science China Earth Sciences 61, 765–777.
- Liu, H., Liu, W.G., 2019. Hydrogen isotope fractionation variations of *n*-alkane and fatty acids in algae and submerged plants from Tibetan Plateau lakes: Implications for palaeoclimatic reconstruction. Science of the Total Environment 695, 133925.
- Liu, H., Liu, Z.H., Zhao, C., Liu, W., 2019. *n*-Alkyl lipid concentrations and distributions in aquatic plants and their individual δD variations. Science China Earth Sciences 62, 1193–1206.
- Liu, W.G., Yang, H., Wang, H., An, Z.S., Wang, Z., Leng, Q., 2015. Carbon isotope composition of long chain leaf wax *n*-alkanes in lake sediments: A dual indicator of paleoenvironment in the Qinghai-Tibet Plateau. Organic Geochemistry 83 (84), 190–201.
- Liu, W.G., Yang, H., Wang, H.Y., Yao, Y., Wang, Z., Cao, Y.N., 2016. Influence of aquatic plants on the hydrogen isotope composition of sedimentary long-chain *n*alkanes in the Lake Qinghai region, Qinghai-Tibet Plateau. Science China Earth Sciences 59, 1368–1377.
- Maberly, S.C., Madsen, T.V., 1998. Affinity for CO₂ in relation to the ability of freshwater macrophytes to use HCO₃. Functional Ecology 12, 99–106.
- McFarlin, J.M., Axford, Y., Masterson, A.L., Osburn, M.R., 2019. Calibration of modern sedimentary δ²H plant wax-water relationships in Greenland lakes. Quaternary Science Reviews 225, 105978.
- Madsen, T.V., 1984. Resistance to CO₂ fixation in the submerged aquatic macrophyte *Callitriche stagnalis* Scop. Journal of Experimental Botany 35 (3), 338–347.
- Meyers, P.A., 2003. Applications of organic geochemistry to paleolimnological reconstructions: a summary of examples from the Laurentian Great Lakes. Organic Geochemistry 34, 261–289.
- Mügler, I., Sachse, D., Wener, M., Xu, B., Wu, G., Yao, T., Gleixner, G., 2008. Effect of lake evaporation on δD values of lacustrine *n*-alkanes: A comparison of Nam Co (Tibetan Plateau) and Holzmaar (Germany). Organic Geochemistry 39, 711– 729.
- Naafs, B.D.A., Inglis, G.N., McClymont, E.L., Lauretano, V., Xie, S., Evershed, R.P., 2019. The potential of biomarker proxies to trace climate, vegetation, and biogeochemical processes in peat: A review. Global and Planetary Change 179, 57–79.
- Newberry, S.L., Kahmen, A., Dennis, P., Grant, A., 2015. *n*-Alkane biosynthetic hydrogen isotope fractionation is not constant throughout the growing season in the riparian tree *Salix viminalis*. Geochimica et Cosmochimica Acta 165, 75–85.
- Oritz, J.E., Moreno, L., Torres, T., Vegas, J., Ruiz-Zapata, B., Garcia-Cortrs, A., Ganlan, L., Perez-Gonzalez, A., 2013. A 220 ka palaeoenvironmental reconstruction of the Fuentillejo maar lake record (Central Spain) using biomarker analysis. Organic Geochemistry 55, 85–97.
- Prins, H.B.A., Elzenga, J.T.M., 1989. Bicarbonate utilization: function and mechanism. Aquatic Botany 34, 59–83.
- Rach, O., Brauer, A., Wilkes, H., Sachse, D., 2014. Delayed hydrological response to Greenland cooling at the onset of the Younger Dryas in western Europe. Nature Geoscience 7, 109–112.
- Rach, O., Kahmen, A., Brauer, A., Sachse, D., 2017. A dual-biomarker approach for quantification of changes in relative humidity from sedimentary lipid D/H ratios. Climate of the Past 13, 741–757.
- Rao, Z.G., Zhu, Z.Y., Jia, G.D., Henderson, A.C.G., Xue, Q., Wang, S.P., 2009. Compound specific δD values of long chain *n*-alkanes derived from terrestrial higher plants are indicative of the δD of meteoric waters: Evidence from surface soils in eastern China. Organic Geochemistry 40, 922–930.

- Rao, Z.G., Jia, G.D., Qiang, M.R., Zhao, Y., 2014. Assessment of the difference between mid and long chain compound specific δD *n*-alkanes values in lacustrine sediments as a paleoclimatic indicator. Organic Geochemistry 76, 104–117.
- Rao, Z., Jia, G., Li, Y., Chen, J., Xu, Q., Chen, F., 2016. Asynchronous evolution of the isotopic composition and amount of precipitation in North China during the Holocene revealed by a record of compound-specific carbon and hydrogen isotopes of long chain n-alkanes from an alpine lake. Earth and Planetary Science Letters 446, 68–76.
- Sachse, D., Radke, J., Gleixner, G., 2004. Hydrogen isotope ratios of recent lacustrine sedimentary *n*-alkanes record modern climate variability. Geochimica et Cosmochimica Acta 68, 4877–4889.
- Sachse, D., Radke, J., Gleixner, G., 2006. δD values of individual *n*-alkanes from terrestrial plants along a climatic gradient—implications for the sedimentary biomarker record. Organic Geochemistry 37, 469–483.
- Sachse, D., Billault, I., Bowen, G.J., Chikaraishi, Y., Dawson, T.E., Feakins, S.J., Freeman, K.H., Magill, C.R., McInerney, F.A., van der Meer, M.T.J., Polissar, P., Robins, R.J., Sachs, J.P., Schmidt, H.L., Sessions, A.L., White, J.W.C., West, J.B., Kahmen, A., 2012. Molecular paleohydrology: interpreting the hydrogen isotopic composition of lipid biomarkers from photosynthesizing organisms. Annual Review of Earth and Planetary Sciences 40, 221–249.
- Sachse, D., Dawson, T.E., Kahmen, A., 2015. Seasonal variation of leaf wax *n*-alkane production and δ^2 H values from the evergreen oak tree, *Quercus agrifolia*. Isotopes in Environmental and Health Studies 51, 124–142.
- Sessions, A.L., 2016. Factors controlling the deuterium contents of sedimentary hydrocarbons. Organic Geochemistry 96, 43–64.
- Smith, F.A., Walker, N.A., 1981. Photosynthesis by aquatic plants: effects of unstirred layers in relation to assimilation of CO_2 and HCO_3 and to isotopic discrimination. New Phytologist 86, 245–259.
- Sun, H.L., Bendle, J., Seki, O., Zhou, A.F., 2018. Mid- to- late Holocene hydroclimatic changes on the Chinese Loess Plateau: evidence from n-alkanes from the sediments of Tianchi Lake. Journal of Paleolimnology 60, 511–523.
- Sun, Q., Xie, M.M., Lin, Y., Shan, Y.B., Zhu, Q.Z., Xu, D., Su, Y.L., Rioual, P., Chu, G.Q., 2016. An *n*-alkane and carbon isotope record during the last deglaciation from annually laminated sediment in Lake Xiaolongwan, Northeastern China. Journal of Paleolimnology 56, 189–203.
- Ting, I.P., 1985. Crassulacean acid metabolism. Annual Review of Plant Physiology and Molecular Biology 36, 595–622.
- Tipple, B.J., Berke, M.A., Doman, C.E., Khachaturyan, S., Ehleringer, J.R., 2013. Leafwax n-alkanes record the plant-water environment at leaf flush. Proceedings of the National Academy of Sciences of the United States of America 110, 2659– 2664.
- Tipple, B.J., Ehleringer, J.R., 2018. Distinctions in heterotrophic and autotrophicbased metabolism as recorded in the hydrogen and carbon isotope ratios of normal alkanes. Oecologia 187, 1053–1075.
- Wang, S.M., Dou, H.S., 1998. Lakes in China. Science Press, Beijing (in Chinese).
- Wu, H., Huang, Q., Fu, C., Song, F., Liu, J., Li, J., 2021. Stable isotope signatures of river and lake water from Poyang Lake, China: implications for river-lake interactions. Journal of Hydrology 592, 125619.
- Wu, J., Zeng, H., Yu, H., Ma, L., Xu, L., Qin, B., 2012. Water and sediment quality in lakes along the middle and lower reaches of the Yangtze River, China. Water Resources and Management 26, 3601–3618.
- Xu, Y.T., Lai, Z.P., Li, C.A., 2019. Sea-level change as the driver for lake formation in the Yangtze Plain-A review. Global and Planetary Change 181, 102980.
- Xue, J., Li, J., Dang, X., Meyers, P.A., Huang, X., 2017. Paleohydrological changes over the last 4000 years in the middle and lower reaches of the Yangtze River: Evidence from particle size and *n*-alkanes from Longgan Lake. The Holocene 27, 1318–1324.
- Yin, L.Y., Li, W., Madsen, T.V., Maberly, S.C., Bowes, G., 2017. Photosynthetic inorganic carbon acquisition in 30 freshwater macrophytes. Aquatic Botany 140, 48–54.
- Zhao, B.Y., Zhang, Y.M., Huang, X.Y., Qiu, R.Y., Zhang, Z.Q., Meyers, P.A., 2018. Composition of *n*-alkane molecular, carbon and hydrogen isotope compositions of different types of plants in the Dajiuhu peatland, central China. Organic Geochemistry 124, 1–11.
- Zhang, Z.P., Liu, J.B., Liu, X.K., Chen, S.Q., Yu, A.L., Zhang, X.S., Chen, J., Shen, Z.W., Chen, J., Zhang, S.J., Xia, H., Chen, F.H., 2020. Seasonal variations in the lakewater oxygen isotope composition of four lakes in the East Asian summer monsoon region: Implications for the interpretation of paleo-isotope records. Progress Physical Geography: Earth and Environment 44, 572–588.