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Distributions and hydrogen isotopic compositions of plant leaf wax from *Orinus kokonorica* along a general aridity gradient around Lake Qinghai, China

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Abstract To quantitatively analyze the response of distributions and hydrogen isotopic compositions (δD) of plant leaf wax to moisture, and to better understand their implications for paleoclimatic reconstruction, we measured average chain length (ACL) and δD values of *n*-alkanes and *n*-fatty acids (*n*-FAs) from *Orinus kokonorica*, a typical and representative plant in Lake Qinghai area, along a distance transect extending from lakeshore to wetland to dryland in the arid ecosystem. The results showed that the ACL values of *n*-alkanes and *n*-FAs were negatively correlated with soil water content (SWC) with R^2=0.593 and R^2=0.924, respectively. This is as a result of plant's response to water loss with more abundance in long-chain *n*-alkyl lipids under increasing aridity by analyzing relationships between the molecular ratios of long-chain *n*-alkyl lipids (*n*-alkanes and *n*-FAs) from *O. kokonorica* and SWC. The δD values of C_{29} *n*-alkane and C_{28} *n*-FA were also negatively correlated with SWC with R^2=0.778 and R^2=0.760, respectively, which may due to enhanced D-enrichment in leaf water by evapotranspiration (soil water evaporation and leaf water transpiration) with increasing aridity. Our results demonstrated that moisture exerts a significant control on the ACL and δD values from *O. kokonorica* in an arid ecosystem. This preliminary study on a modern single plant (*O. kokonorica*) sets a foundation for comprehending these values as quantitative proxies for paleo-humidity reconstruction.

Key words average chain length; hydrogen isotope; moisture; *Orinus kokonorica*; Lake Qinghai

1 Introduction

The lipid components (e.g. *n*-alkanes and *n*-fatty acids) of cuticular waxes of higher terrestrial plants have been widely used as excellent biomarkers for paleoenvironmental reconstruction due to their chemical inertness and resistance to biodegradation (Huang et al., 2004; Liu and Huang, 2005; Scuefuβ et al., 2005; Hou et al., 2006; Schouten et al., 2007; Eglinton and Eglinton, 2008; Zhang and Liu, 2011; Sachse et al., 2012; Wang et al., 2013a, b). Since these lipids protect their leaves with longer chain *n*-alkanes or *n*-fatty acids (*n*-FAs) from water loss (Dodd and Afzal-Rafii, 2000; Dodd and Poveda, 2003), the average chain length (ACL) of plant leaf wax in modern plants can respond to changes in environmental parameters such as relative humidity, temperature, and rainfall (Dodd et al., 1998; Dodd and Poveda, 2003; Schefuβ et al., 2003; Sachse et al., 2006; Tipple and Pagani, 2013), and has been an important proxy for paleoclimatic change (Rommerskirchen et al., 2003, 2007; Hughen et al., 2004; Zhou et al., 2005; Schouten et al., 2007).

Numerous previous studies have shown that ACL values of plant leaf wax increases with increasing aridity and/or temperature and decreasing rainfall (Schefuβ et al., 2003; Rommerskirchen et al., 2003, 2007; Hughen et al., 2004; Zhou et al., 2005; Sachse et al., 2006; Schouten et al., 2007; Tipple and Pagani, 2013). The chain-length distributions of *n*-alkanes in aerosols clearly reflect regional changes in aridity, because the dust samples from regions of lower precipitation and higher aridity are dominated by longer chain *n*-alkanes (Schefuβ et al., 2003). The ACL values of *n*-alkanes from deciduous tree leaves increase from the European North to the South along a climatic gradient, and respond to changes in temperature (Sachse et al., 2006). Tipple and Pagani (2013) found that the ACL values of *n*-alkanes from *A. rubrum* and *J. virginiana* leaves and soils show significant positive correlations with mean annual temperature along the East Coast of the USA that covers a 13℃ mean annual temperature gradient with minimal variation in annual or seasonal relative humidity.

Recent analytical improvements in gas chromatography/thermal conversion/ isotope ratio mass spectrometry (GC/TC/IRMS) spur a surge of interest in hydrogen isotopic composition of plant leaf wax (δD_{wax}) as a promising paleoclimatic proxy (Sauer et al., 2001; Huang et al., 2004; Sachse et al., 2004; Liu and Huang, 2005; Hou et al., 2006; Garcin et al., 2012; Wang et al., 2013b). Environmental water, as the ultimate hydrogen source of plant leaf wax, is thought to be the fundamental control on δD_{wax} values at large spatial scales (Liu and Yang, 2008; Hou et al., 2008; Sachse et al., 2012; Tipple and Pagani, 2013). Given that δD values of plant's source water are modified by environmental factors (e.g. relative humidity and temperature) through D-enrichment by evapotranspiration (soil water evaporation and leaf water transpiration) with a small effect of exchange with atmospheric vapor (Riley et al., 2002), environmental information can be recorded by δD_{wax} values. Relative humidity, particularly in arid ecosystems, can drive well D-enrichment in leaf water by evapotranspiration, and has an important role in determining δD_{wax} values (Smith and Freeman, 2006). Liu and Huang (2005) showed that the changes in δD_{wax} values in a paleosol profile are consistent with those in aridity on the Chinese Loess Plateau. Model evidence suggests that evaporation and transpiration are highly sensitive to relative humidity, and thus significant information on relative humidity is recorded by δD_{wax} values (Smith and Freeman, 2006). Both climate chamber experiment and observational study along climate gradient in Northern Australia also provided strong evidence on the significant effect of D-enrichment by evapotranspiration on δD_{wax} values (Kahmen et al., 2013a, b). Wang et al. (2013b) developed a record of δD_{wax} values from Lake Sugan in the arid area of the northern Tibetan Plateau to reconstruct the regional changes in relative humidity for the last 1700 years.

While ACL and δD_{wax} values can respond to environmental change, few studies quantitatively analyze the relationship between these values from modern plants and environmental conditions. Here we investigated the ACL and δD values of *n*-alkanes and *n*-fatty acids (*n*-FAs) *Orinus kokonorica* along a distance transect extending from lakeshore to wetland to dryland around Lake Qinghai. The selected transect with significant differences in soil water content (SWC) allows us to the assessment of the effect of moisture on the ACL and δD values and better understanding of these values as quantitative proxies for paleo-humidity reconstruction.

2 Material and methods

2.1 Study location and samples

Lake Qinghai (36°32'–37°15' N, 99°36'–100°47' E), the largest inland brackish lake in China, is in an arid and semi-arid, cold and high altitude (ca. 3200 m) climate zone on the NE Qinghai-Tibetan Plateau. The mean annual precipitation and mean summer air temperature are 400 mm and 11.4℃, respectively (Henderson et al., 2003; Liu et al., 2008). The climate is influenced by the East Asian summer monsoon, the Indian summer monsoon, the winter monsoon, and the westerly jet stream, making the region highly sensitive to global climate change (An et al., 2000). Summer has the highest precipitation, whereas potential evaporation (800–1200 mm) greatly exceeds precipitation in the lake (Liu et al., 2006b).

Around Lake Qinghai, the typical plant species are *Orinus kokonorica, Stipa breviflora, Achnatherum splendens, Carex ivanoviae, S. Krylovii, Stipa purpurea, Kobresia pygmaea, Festuna ovina L., Elymus nutans* and few shrubs including *Potentilla fruticosa* and *Salix oritrepha* (Wang and Liu, 2012). Here we collected typical *Orinus kokonorica* as representative plant in Lake Qinghai area. Plant and surface soil samples (upper 5 cm) along a distance transect extending from the lakeshore to wetland to dryland around Lake Erhai were collected in the summer of 2012 (Table 1, Fig. 1). The Erhai (EH) transect (36°32'43.2"–36°32'46.5" N, 100°43'13.8"–100°43'15.7" E) was in the southeast of Lake Qinghai, and eight plant and surface soil samples from EH transect were collected at a distance of ca. 0, 10, 20, 40, 50, 50, 60, and 80 m from the lakeshore, respectively. All plant samples were frozen and stored on dry ice in the field and kept at -20℃ until analysis.

Table 1 Soil water content, hydrogen isotopic compositions of C_{29} *n*-alkane and C_{28} *n*-FA, and distributions of *n*-alkanes and *n*-FAs from *Orinus kokonorica* in the EH transect

Sample	Distance	SWC	*n*-alkane					*n*-FA				
	(m)	(%)	C_{29}/C_{27}	C_{31}/C_{29}	C_{33}/C_{27}	$ACL_{(25-33)}$	$\delta D_{C_{29}}$ (‰)	C_{28}/C_{26}	C_{30}/C_{26}	C_{32}/C_{26}	$ACL_{(24-32)}$	$\delta D_{C_{28}}$ (‰)
EHP12-1	0	23.3	2.28	0.95	0.05	28.8	-184	2.22	0.48	0.06	27.0	-190
EHP12-2	10	32.6	2.59	2.69	0.18	29.4	-193	1.68	0.49	0.11	26.5	-194
EHP12-3	20	42.4	2.29	1.61	0.08	28.7	-200	1.39	0.46	0.06	26.5	-195
EHP12-4	40	34.4	2.16	1.23	0.06	28.4	-199	1.56	0.62	0.08	26.8	-201
EHP12-5	50	18.2	1.86	2.86	0.35	29.5	-191	2.06	1.27	0.56	27.1	-182
EHP12-6	50	33.1	2.48	2.64	0.17	29.4	-198	1.80	0.63	0.12	26.6	-193
EHP12-7	60	12.4	2.77	6.32	0.87	30.1	-184	1.84	1.06	0.74	27.5	-182
EHP12-8	80	17.6	2.01	2.77	0.32	29.5	-183	2.06	1.58	0.79	27.2	-186

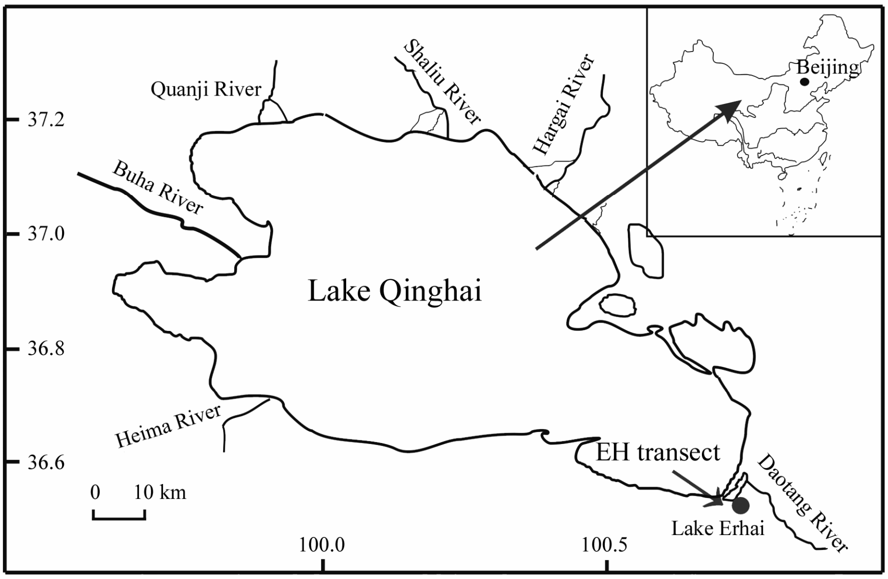

Fig. 1. Map showing location of the Erhai (EH) transect around Lake Qinghai.

2.2 Analytical methods

2.2.1 Soil water content (SWC) analysis

SWC was obtained by measuring soil sample weight before and after freeze drying.

2.2.2 Extraction and purification of n-alkanes and n-FAs

Methods for extraction and purification of *n*-alkanes and *n*-FAs were similar to those described previously (Wang and Liu, 2012). All plant samples were firstly freeze dried, and then about 0.5 g plant materials were extracted ultrasonically (3 min×20 min) with dichloromethane (DCM) and methanol (9:1, v/v). The solvent was then removed with a N_2 stream. For methylation, an acetyl chloride in methanol (5%) was added to extracted samples and the mixture was heated at 70℃ for 12 h. After the acid-catalyzed transesterification, NaCl (5% aqueous, 2 mL) was added. The lipids [including alkanes and fatty acid methyl esters (FAMEs)] were extracted with hexane three times. The total extraction was separated by silica gel chromatography using hexane and then DCM as the eluent. The alkanes was collected in the first fraction after elution with hexane and the FAMEs were collected in the second fraction after elution with DCM. The total FAMEs (including saturated and unsaturated FAMEs) were separated by column chromatography with $AgNO_3$ on silica gel (10%, w/w), and the saturated FAMEs were eluted first with hexane/DCM (4:1, v/v).

2.2.3 Gas chromatography analysis

The *n*-alkanes and *n*-FAs were identified by comparing the retention times defined by gas chromatography (GC) analysis using a mixed *n*-alkanes and *n*-FAs standard. GC was performed using an Agilent 6890 GC with a HP1-ms column (60 m, 0.32 mm i.d., and 0.25 µm film thickness) and flame ionization detector. The samples were injected in split mode, with a GC inlet temperature of 310℃ and a flow rate of 1.2 mL/min. The oven temperature program was: 40℃ (1 min) to 150℃ at 10℃/min, then to 310℃ (holding for 20 min) at 6℃/min. GC analysis was carried out at the stable isotope laboratory of the Institute of Earth Environment, Chinese Academy of Sciences.

The average chain length (ACL) of *n*-alkanes and *n*-FAs was calculated by:

$$\text{ACL values of } n\text{-alkanes}= (25A25+27A27+29A29+31A31+33A33)/ (A25+A27+A29+A31+A33)$$

$$\text{ACL values of } n\text{-FAs} =(24A24+26A26+28A28+30A30+32A32)/ (A24+A26+A28+A30+A32)$$

where A is the area of individual *n*-alkanes and *n*-FAs peak.

2.2.4 Hydrogen isotope analysis

Hydrogen isotope ratio of individual *n*-alkanes was analyzed using GC-thermal conversion-isotope ratio mass spectrometry (GC-TC-IRMS). A Thermo Trace Ultra GC was used along with a high temperature H/D pyrolysis reactor connected online to a Thermo Delta V Advantage isotope ratio mass spectrometer. Compounds separated with GC column (identical temperature program and GC column with an Agilent 6890 GC used for GC analysis) were converted to H_2 by a pyrolysis reactor at 1450℃, and then H_2 was introduced into the mass spectrometer. H_3 factors were calculated daily using the same H_2 reference gas. The precision of isotopic measurements of H_2 reference gas after H_3 factor correction was 1‰ or better. Analytical error was <3‰ for samples. Compound-specific hydrogen isotope analysis was carried out at the Stable Isotope Laboratory of the Institute of Earth Environment, Chinese Academy of Sciences.

3 Results and discussion

3.1 Distributions of *n*-alkanes and *n*-FAs from *O. kokonorica*

In this study, we selected a natural transect extending from lakeshore to wetland to dryland with a considerable difference in SWC (12.4% to 42.4%; Table 1), which provided an ideal environmental condition for investigating the effect of moisture on the distributions and hydrogen isotopic compositions of plant leaf wax. Theoretically, a site with lower SWC can provide a drier growing environment for plants, and changes in SWC reflect significant changes in moisture around plants growing on the soils in the EH transect. The ACL values of *n*-alkanes and *n*-FAs from *O. kokonorica* were negatively correlated with SWC with R^2=0.593 and R^2=0.924, respectively (Fig. 2a and b), indicating that moisture is a significant driver for chain length distributions of plant leaf wax in our study area. The negative correlations may be the result of plant's response to water loss with longer chain *n*-alkyl lipids (*n*-alkanes and *n*-FAs) under increasing aridity (Sachse et al., 2006). Similarly, a shift in the carbon number maximum from C_{29} to C_{31} *n*-alkane in aerosols has been observed and deemed to be related to increasing aridity along a west coast transect of Africa rather than temperature (Schefuβ et al., 2003). However, some studies have demonstrated that temperature also exerts an important control on distributions of plant leaf wax. For example, terrestrial plants tend to biosynthesize longer chain waxes in warmer climates but shorter chain lipid components in cooler conditions (Gagosian and Peltzer, 1986). The ACL values of *n*-alkanes from *A. rubrum* and *J. virginiana leaves* and soils have significant positive correlations with mean annual temperature along the East Coast of the USA that covers a 13℃ mean annual temperature gradient with minimal variation in annual or seasonal relative humidity (Tipple and Pagani, 2013). These inconsistent results are attributed to differences in dominant climatic factors controlling water loss (transpiration) in these area, which result in response of the ACL values to different environmental conditions (relative humidity or temperature). The Lake Qinghai area is a typical arid and semi-arid inland (Lister et al., 1991), and aridity is an important climatic feature. The EH transect showed a significant variations in SWC, which ranged from 12.4% to 42.4%, with little variation in temperature due to a 80 m small distance range (Table 1). We have observed that moisture exerts a primary control on variations in ACL values of *n*-alkyl lipids from *O. kokonorica* (Fig. 2a and b). Remarkably, the correlation between ACL values of *n*-FAs and SWC has a higher R^2 than that between ACL values of *n*-alkanes and SWC (Fig. 2a and b), suggesting that chain length of *n*-FAs from *O. kokonorica* is more sensitive to moisture than that of *n*-alkanes.

Although ACL values of plant leaf wax have been widely used as an important tool for paleoclimatic reconstrucion (Rommerskirchen et al., 2003, 2007; Hughen et al., 2004; Zhou et al., 2005; Schouten et al., 2007), the biosynthetic mechanism how *n*-alkyl lipids respond to environmental conditions with variations in molecule distributions is still unclear (Tipple and Pagani, 2013). To preliminarily discuss the biosynthetic mechanism of *n*-alkyl lipids with increasing aridity, we analyzed the changes in distribution characteristics of long-chain *n*-alkyl lipids (C_{27}-C_{33} *n*-alkanes and C_{26}-C_{32} *n*-FAs) of *O. kokonorica* in the EH transect. As shown in Figs. 3 and 4, the C_{31}/C_{27} and C_{33}/C_{27} ratios of *n*-alkanes, and C_{28}/C_{26}, C_{30}/C_{26}, and C_{32}/C_{26} ratios of *n*-FAs were negatively correlated with SWC, respectively, whereas the C_{29}/C_{27} ratios of *n*-alkanes were poorly correlated with SWC (Figs. 3 and 4). This indicates that *O. kokonorica* has increasing ratios of longer long-chain n-alkyl lipids for minimizing water loss with increasing aridity except C_{29} *n*-alkane. The slope (-0.019) of the relationship between C_{33}/C_{27} ratios of *n*-alkanes and SWC was obviously higher than that (-0.101) between C_{31}/C_{27} ratios of *n*-alkanes and SWC (Fig. 3), suggesting that C_{31} *n*-alkane of *Orinus kokonorica* is more sensitive to aridity condition than C_{33} *n*-alkane, whereas the slopes of the relationship between SWC vs. C_{28}/C_{26}, C_{30}/C_{26}, and C_{32}/C_{26} ratios of *n*-FAs are not significantly different (Fig. 4). Therefore, *O. kokonorica* may be biased to product more C_{31} *n*-alkane and collectively increase abudance in C_{28}, C_{30}, and C_{32} *n*-FAs for preventing water loss from the leaf with increasing aridity.

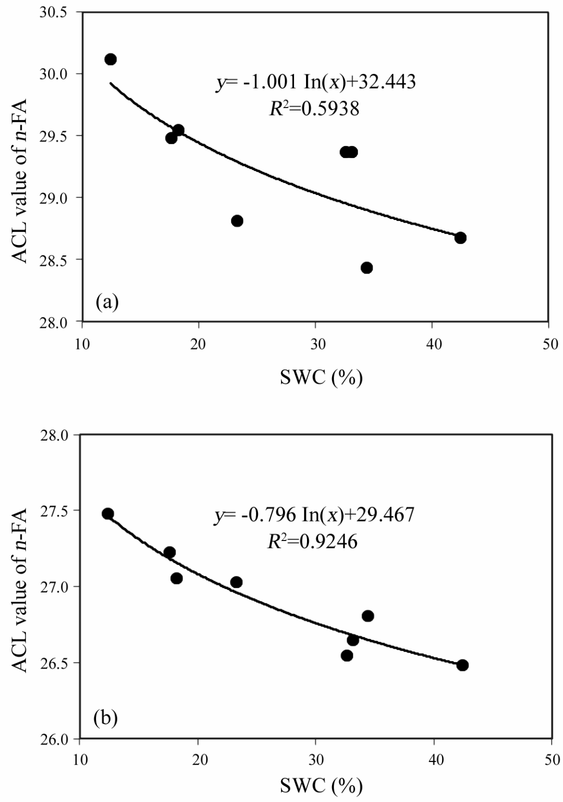

Fig. 2. Correlation between (a) ACL values of *n*-alkanes and SWC, and (b) ACL values of *n*-FAs and SWC.

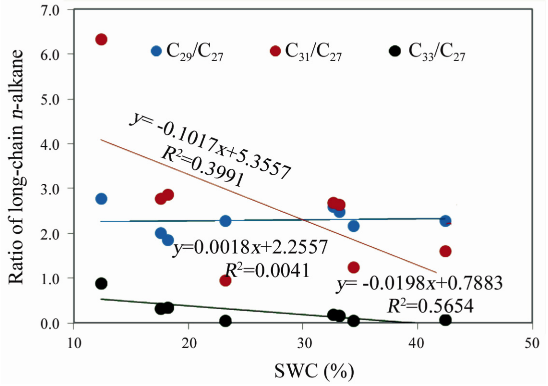

Fig. 3. Correlation between SWC vs. C_{29}/C_{27}, C_{31}/C_{27} and C_{33}/C_{27} ratios of *n*-alkanes.

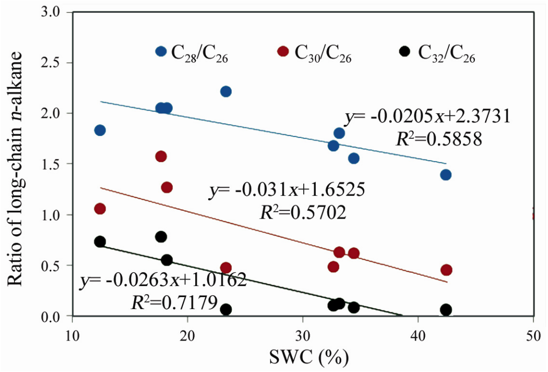

Fig. 4. Correlation between SWC vs. C_{28}/C_{26}, C_{30}/C_{26} and C_{32}/C_{26} ratios of *n*-FAs.

3.2 Hydrogen isotopic compositions of *n*-alkanes from *O. kokonorica*

Given that environmental and biological factors play significant roles in controlling δD_{wax} values (Chikaraishi and Naraoka, 2003; Chikaraishi et al., 2004; Liu et al., 2006a; Hou et al., 2007; Feakins and Sessions, 2010; Polissar and Freeman, 2010; Duan and He, 2011; McInerney et al., 2011), δD_{wax} values of the single plant (*O. kokonorica*) with same plant physiology in the EH transect were investigated in order to quantify and better understand the effect of moisture on δD_{wax} values. In this paper, we focused on δD values of C_{29} *n*-alkane and C_{28} *n*-FA as representive isotope values of higher terrestrial plants, because C_{29} *n*-alkane and C_{28} *n*-FA have high concentration of the long-chain *n*-alkyl lipids in our samples. The δD values of C_{29} *n*-alkane and C_{28} *n*-FA from *O. kokonorica* were negatively correlated with SWC with R^2=0.778 and R^2=0.760, and ranged from -200‰ to -183‰ and -201‰ to -182‰ with changes in SWC from 12.4% to 42.4%, respectively (Table 1; Fig. 5a and b). This is attributed to enhanced D-enrichment by evapotranspiration (soil water evaporation and leaf water transpiration) with increasing aridity, and indicates that moisture may be the primary control on δD_{wax} values in the arid ecosystem.

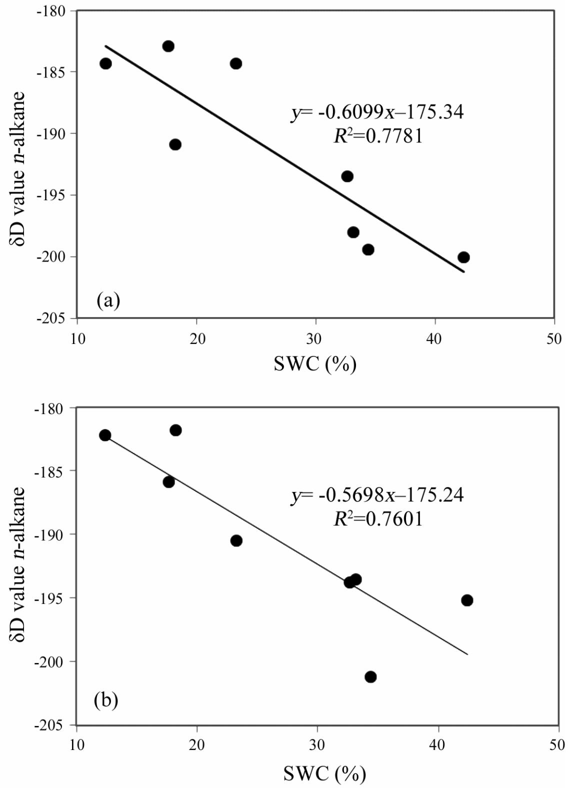

Fig. 5. Correlation between (a) δD values of C_{29} *n*-alkane and SWC, (b) δD values of C_{28} *n*-FA and SWC.

In an arid area where D-enrichment in soil water toward soil surface is significant (Allison, 1982; Barnes and Allison, 1983; Tang and Feng, 2001), moisture markedly affects δD_{wax} values of shallow-rooted grasses through D-enrichment by soil evaporation (Smith and Freeman, 2006; Hou et al., 2008; McInerney et al., 2011). In addition, leaf water transpiration can also drive D-enrichment under arid conditions, and this signal will be recorded by δD_{wax} values as a result of changes in moisture (Feakins and Sessions, 2010; Kahmen et al., 2013a, b). A study on grasses from greenhouses and fields has shown that smaller apparent hydrogen isotopic fractionation between *n*-alkanes and precipitation is due to D-enrichment by evapotranspiration in dryer sites, and model predictions have suggested that leaf water D-enrichment derived primarily from soil water evaporation in an arid area (Smith and Freeman, 2006). Similarly, based on model results using hydrogen isotopic compositions of precipitation and surface sediments spanning large relative humidity gradients, Hou et al. (2008) found that evaporative D-enrichment in soil water makes the largest contribution to δD_{wax} values. Feakins and Freeman (2010) porvided direct evidence on significant effect of transpirational D-enrichment in leaf water on δD_{wax} values in an arid ecosystem by measuring the hydrogen isotopic compositions of plant xylem water and leaf water. Both climate chamber experiment and observational study along a climate gradient in Northern Australia have also provided strong evidence on the significant record of evapotranspirational D-enrichment signals in δD_{wax} values (Kahmen et al., 2013a, b).

It is worth considering, however, that the δD_{wax} value from lakeshore in the EH transect is potentially affected by D-enriched lake water as fractional hydrogen sources of *n*-alkanes. In the Lake Qinghai area where evaporation exceeds precipitation (Liu et al., 2006b), lake water is more D-enriched than precipitation due to strong evaporation (Mügler et al., 2008; Xia et al., 2008; Aichner et al., 2010). Thus theoretical more negative δD_{wax} value from the lakeshore due to the higher SWC relative to dryland is countered by the opposing influence of lake water (Table 1). Although the input of lake water in the lakeshore is potentially troublesome for analyzing quantitatively the response of δD_{wax} value to moisture, the δD_{wax} values still showed a good correlation with SWC (Fig. 5a and b). This indicates that moisture weakens the effect of lake water on the δD_{wax} value from lakeshore and exerts primary control on δD_{wax} values in the arid ecosystem.

The δD_{wax} values of plant leaf wax have been used as a paleo-humidity indicator in different sedimentary archives. In a record of δD_{wax} values from a marine sediment core close to the Congo River mouth spanning 20 ka in African, variations in δD_{wax} values were explained to indicate wetter or drier conditions (Schefuβ et al., 2005). A study on a paleosol profile spanning the last 130 ka in the Chinese Loess Plateau found that the changes in δD_{wax} values in the loess were strongly consistent with those in aridity (Liu and Huang, 2005). A recent study has also shown a record of δD_{wax} values from the Lake Sugan in the Qaidam Basin in the arid area of the northern Tibetan Plateau to reconstruct regional changes in humidity in the last 1.7 ka (Wang et al., 2013b). In the present study, our preliminary investigation of δD_{wax} values from the modern single plant (*O. kokonorica*) along the EH transect suggests that δD_{wax} values may be qualitatively used to respond to changes in moisture in an arid ecosystem.

4 Conclusions

Our investigation on ACL and δD values of *n*-alkanes and *n*-FAs from *O. kokonorica* along a distance transect extending from lakeshore to wetland to dryland around Lake Qinghai shows that these values have significant negative correlations with SWC, indicating that moisture exerts a significant control on the distributions and hydrogen isotopic compositions of plant leaf wax in the arid ecosystem. By analyzing the relationships between molecular ratios of long-chain n-alkyl lipids (*n*-alkanes and *n*-FAs) and SWC, we infer that *O. kokonorica* may tend to synthesize more C_{31} *n*-alkane and collectively increase abundance in C_{28}, C_{30}, and C_{32} *n*-FAs for preventing water loss from the leaf, which provides evidence that ACL values of *n*-alkanes and *n*-FAs respond to moisture with the longer long-chain *n*-alkyl lipids. In addition, the negative correlation between δD values of C_{29} *n*-alkane and C_{28} *n*-FA and SWC is attributed to enhanced D-enrichment in leaf water by evapotranspiration with increasing aridity, and this significant signals of soil water evaporation and leaf water transpiration can be recorded by δD_{wax} values.

However, considering interspecies variability in ACL and δD_{wax} values, we need to caution when using these indexes as paleo-humidiy indicators. Since the role of environment and physiology on the effect of ACL and δD_{wax} values still remains controversial, systematic investigations on multiple species and environmental factors are urgently needed to quantify the contributions of environmental and biological factors in determining ACL and δD_{wax} values in future works in order to strengthen their use as paleoenvironmental proxies.

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