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Is average chain length of plant lipids a potential proxy for vegetation, environment and climate changes?

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Abstract

Average chain length (ACL) of leaf wax components preserved in lacustrine sediments and soil profiles has been widely adopted as a proxy indicator for past changes in vegetation, environment and climate during the late Quaternary. The fundamental assumption is that woody plants produce leaf waxes with shorter ACL values than non-woody plants. However, there is a lack of systematic survey of modern plants to justify the assumption. Here, we investigated various types of plants at two lakes, Blood Pond in the northeastern USA and Lake Ranwu on the southeastern Tibetan Plateau, and found that the ACL values were not significantly different between woody and non-woody plants. We also compiled the ACL values of modern plants in the literatures and performed a meta-analysis to determine whether a significant difference exists between woody and non-woody plants at single sites. The results showed that the ACL values of plants at 19 out of 26 sites did not show a significant difference between the two major types of plants. This suggests that extreme caution should be taken in using ACL as proxy for past changes in vegetation, environment and climate.

1 Introduction

The average chain length (ACL) of higher plants describes the average number of carbon atoms based on the abundance of *n*-alkyl compounds (Poynter and Eglinton, 1990; Poynter et al., 1989). The ACL of plant lipids preserved in various sediments (lake, ocean, soil, peat) has been widely adopted to reconstruct past changes in climate and environment during the late Quaternary (Bai et al., 2009; Hughen et al., 2004; Zhang et al., 2006; Zhou et al., 2005, 2010; Castañeda et al., 2009; Yamamoto et al., 2010). The fundamental assumption for using ACL as such a proxy is that leaf lipids derived from grasslands may on average have longer chain lengths than leaf lipids from plants in forests (Cranwell, 1973). If the assumption were correct, ACL variation would reflect changes between forest and grassland, which could be further interpreted as past changes in environment and climate. A few published ACL records correlated well with other lines of evidence for climate changes, such as pollen record (Pu et al., 2010) and stable isotope records (Hughen et al., 2004; Yamamoto et al., 2010). These studies thus further suggest that ACL could reflect past changes in vegetation. The difference in the lipid distribution between woody and non-woody plants was first reported in the 1970s (Cranwell, 1973), although only a few data were presented in that study. However, there is a lack of a systematic survey of modern plants to justify the assumption and positively to support the idea that ACL could act as a reliable proxy indicator for past changes in vegetation, environment and climate.

Recently, a comprehensive review by Bush and McInerney (2013) summarized ACL values for alkanes in modern plants from around the world, and found that ACL was unable to distinguish graminoids (grasses) from woody plants. However, single types of plants (either woody or non-woody) were present at ca. 57 % of the sites in the Bush and McInerney (2013) ranging from tropical to temperate regions. It has been suggested that plants produce longer-chain compounds in warmer climates (Poynter et al., 1989). Simoneit et al. (1991) have analyzed continental aerosols from China and reported that higher molecular weight *n*-alkanes (C_{31}) of the aerosols predominate in the warmer climate of southern China. It has been found that the modal carbon number of a higher plant *n*-alkane distribution is broadly related to latitude (Poynter et al., 1989; Poynter and Eglinton, 1990), with higher modal carbon numbers occurring at lower latitudes. Therefore, comparison of ACLs between woody and non-woody plants at different regions would inevitably be influenced by the climate and environment. It would be preferable to investigate the ACLs of both woody and non-woody plants at single sites in order to assess whether or not ACL can differentiate between different plant types.

Here, we present ACL data for various types of modern plants at two lakes, Blood Pond in the northeastern USA and Lake Ranwu on the southeastern Tibetan Plateau, to determine whether or not ACL values differ between woody and non-woody plants. We then compiled published ACL data for 751 plants at 24 sites from around the world

that contain both woody and non-woody plants to assess whether ACL could be a potential proxy for past changes in vegetation, environment and climate.

2 Materials and methods

2.1 Study sites and sample collection

Lake Ranwu (29.48° N, 96.77° E, 3920 m a.s.l.) is on the southeastern Tibetan Plateau (Fig. 1). The mean annual precipitation is 849.7 mm, and mean annual temperature is 8.5 °C (Guan et al., 1984). The plants around Lake Ranwu include trees, shrubs and herbs. In June 2010, leaf samples were collected from six different locations around the lake. Leaf samples from 13 plants were collected from the western lakeshore (RW-1), including two species of trees, two species of herbs and nine species of shrubs. Leaf samples were also collected from 19 plants at five sites (RW-2, RW-3, RW-4, RW-5 and RW-6) on the eastern lakeshore, including two species of trees, six species of shrubs and eleven species of herbs (Table 1). Over 20 leaves were collected at different heights from a single plant and were combined into a single leaf sample, in order to minimize the complicating factor of possible ACL variation at different plant heights.

Blood Pond (42.08° N, 71.96° W, 212 m a.s.l.) is a kettle lake in northeastern North America (Fig. 1), and the carbon and hydrogen isotope data have been reported previously (Hou et al., 2007a, b). Here we re-visit the dataset in order to obtain the ACL values of modern plants in this study.

2.2 Analytical methods

All leaf samples were freeze-dried and ultrasonically extracted (3×) with CH_2Cl_2 : MeOH (v/v, 2 : 1) for 15 min. The total lipid extract was fractionated into neutral and acid fractions using solid phase extraction (Aminopropyl Bond Elut®). The neutral fraction was fractionated using silica gel column chromatography into hydrocarbon, ke-

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tone/aldehyde and alcohol fractions using hexane, CH_2Cl_2 and a mixed solvent (hexnae / EtOAc, v/v, 3 : 1), respectively. Acid fractions were methylated with 5 % anhydrous HCl in methanol at 60 °C for 12 h. Hydroxyl acids were removed by eluting the samples through silica gel columns, in order to further purify the fatty acid methyl esters to avoid chromatographic coelution. The hydrocarbon and methylated acid fractions were measured using gas chromatography with flame ionization detector (GC-FID) and GC-mass spectrometry (GC-MS) to quantify and identify the compounds. Samples were passed through the GC (Agilent 6890, with 30 m, 0.25 mm ID Thermo TR-5ms SQC column) for separation and then to a quadruple mass spectrometer for identification (Agilent 5973 MSD). Samples were measured on GC-FID for assessment of relative abundance. The GC oven temperature method is as follows: initial temperature at 80 °C, hold for 2 min, then ramp at 10 °C min^{-1} to 320 °C, and hold at 320 °C for 10 min. All samples were compared to a standard homologous series of *n*-alkanes from C_{21} to C_{40} (Fluka, Sigma-Aldrich).

2.3 Date sources and statistical analysis

We compiled leaf wax ACL values for modern plants from the literatures following two criteria: (i) Investigations of natural plants or plants cultivated in an open field in a botanical garden. Greenhouse plants were excluded as they may suffer from too many human manipulations that would not reflect natural conditions. (ii) Investigations including both woody and non-woody plants at single sites. This would exclude the influences of various climate and environment factors on ACLs at different sites. Because not all original abundance data for plant alkanes or acids are available in literature, we cannot recalculate the ACL values for all plants, then we took the published ACL values in the publication. We intended to compare the ACL values between woody and non-woody plants at single sites. If the ACL values for plants were calculated from alkanes or acids of different carbon number ranges across different sites, the results would not affect the comparison in this study. The compiled ACL dataset included 751 plants at 24 sites,

including 255 woody plants and 496 non-woody plants. Note that most of the ACL data were retrieved from Bush and McInerney (2013).

ACL values of leaf wax components are calculated from the following equations (Poynter et al., 1989)

$$n\text{-alkane ACL,}\ \ ACL_{25-33} = \frac{25 \times C_{25} + 27 \times C_{27} + 29 \times C_{29} + 31 \times C_{31} + 33 \times C_{33}}{C_{25} + C_{27} + C_{29} + C_{31} + C_{33}}$$

$$n\text{-alkanoic acid ACL,}\ \ ACL_{24-32} = \frac{24 \times C_{24} + 26 \times C_{26} + 28 \times C_{28} + 30 \times C_{30} + 32 \times C_{32}}{C_{24} + C_{26} + C_{28} + C_{30} + C_{32}}$$

We adopted an independent samples *t* test to determine whether the difference in ACL between woody and non-woody plant was significant. Prior to the *t* test, all data were tested for homogeneity of variance (Levene's test). The difference is viewed as significant when the error probability is < 0.05. A p value of 0.01 was also used as a critical level of significance in all tests.

3 Results

3.1 ACL at Lake Ranwu and Blood Pond

We divided the plants into three types according to growth habit, including herbs, shrubs and trees. The average values for herbs, shrubs and trees were 29.72, 29.36 and 26.93, respectively (Table 1, Fig. 2a). The highest value was 32.10 for *Juniperus squamata* (sample RW1-7, shrub), and the smallest 25.55 for *Salix dalungensis* (RW1-4, tree).

The average acid ACL values for the various plant types at Blood Pond are plotted in Fig. 2b. We follow the classification for plants by Hou et al. (2007a, b) (Table 2). The plants included six types: ferns (2 samples), trees (16), shrubs (8), vines (3), herbs (9) and grasses (7). The average ACL values were 27.56, 27.71, 27.54, 27.24, 28.07

and 27.59, respectively. The highest was 29.40 (*Quercus velutina*, tree) and the lowest 25.68 (*Smilax rotundifolia*, vine).

3.2 Leaf wax ACL values in the literature

We obtained ACL values for major types of plants in the literatures in order to examine the difference between woody and non-woody plants. Non-woody included, grasses and forbs, while woody plants included shrubs and trees. 751 plants from 24 sites from all continents except Antarctica met the criteria above (Table 3). Plant samples included 91 from Asia (Chikaraishi and Naraoka, 2003; Bi et al., 2005; Cui et al., 2008; Lei et al., 2010; Duan and Xu, 2012; Wilkie et al., 2013; Sarkar et al., 2014), 154 from Africa (Ali et al., 2005a; Vogts et al., 2009; Carr et al., 2014), 423 from Europe (Lütz and Gülz, 1985; Maffei, 1994, 1996a, b; Mayes et al., 1994; Ficken et al., 1998; Ali et al., 2005b; Nott et al., 2000), 9 from Oceania (Eglinton et al., 1962) and 74 from America (Salasoo, 1988; Nichols et al., 2006; Feakins and Sessions, 2010; Douglas et al., 2012) (see Supplement for details).

We performed meta-analysis on the 823 plants (529 herbs and 294 woody plants) from 26 sites (including the two case studies used in this study). The results showed that ACL values are statistically different between herbs and woody plants at 4 out of 26 sites (Table 3), including Sudan (Site J and O) (Ali et al., 2005a; Vogts et al., 2009), South China (Site L) (Bi et al., 2005) and Lake Qinghai, China (Site U) (Duan and Xu, 2012). As a meta-analysis cannot be performed on a dataset containing only one sample, three sites including Sudan (Site F) (Ficken et al., 1998), the USA (Site M) (Nichols et al., 2006) and China (Site S) (Lei et al., 2010) are different on the basis of visual observation. All other sites do not show a statistically significant difference in ACL values between woody and non-woody plants (Table 3).

4 Discussion

4.1 Modern plant leaf wax ACL

It is surprising to observe that at the majority of the sites (~ 73 %, 19 out of 26 sites) there is no significant difference in ACL values between woody and non-woody plants. The compiled sites range from a tropical steppe climate (Vogts et al., 2009) to a high-latitude tundra climate (Mayes et al., 1994), according to the Köppen climate classification (Kottek et al., 2006; Peel et al., 2007). Statistical analysis cannot be performed on ACL data at 7 sites because of the limited number of plants (F, I, M, P, R, S, V in Fig. 3 and Table 3). Nevertheless, on the basis of visual observation the ACL values overlapped between the two types of plants at 4 out of 7 sites, namely the UK (Ficken et al., 1998), Thailand (Chikaraishi and Naraoka, 2003), the USA (Feakins and Sessions, 2010), and Russia (Wilkie et al., 2013) (I, P, R, V in Fig. 3 and Table 3). The statistical results from the modern plant samples around the world suggest that there is no significant different between woody and non-woody plants. Therefore the assumption of using ACL as a proxy indicator for changes in vegetation could be problematic. Any inference of changes in climate and environment from the ACL variation should be cautious.

However, around 27 % of the surveyed sites (7 out of 26) do show a significant difference in ACL values between woody and non-woody plants by statistical analysis or visual observation. Examples include the Sudan (Ali et al., 2005a; Vogts et al., 2009), the USA (Nichols et al., 2006), the UK (Ficken et al., 1998) and China (Duan and Xu, 2012; Lei et al., 2010; Bi et al., 2005) (F, J, L, M, O, S, U in Fig. 3 and Table 3). It unclear why the plants at the seven sites show a difference in ACL values between plants, which probably implies that ACL could be used to differentiate plant types and vegetation dynamics in a specific area. However, ACL values behave differently for plants collected in botanical gardens. At a botanical garden in Italy (Site D), ACL is not statistically different between woody plants and non-woody plants (Maffei, 1994, 1996a, b), while ACL values are statistically different in the South China Botanical Garden (Site L) (Bi et al., 2005). This may be the result of a significant difference in the irrigation regime in botanical gardens. In addition, visual observation revealed that the ACL values of non-woody plants are lower than those of woody plants in the UK (Site F) (Ficken et al., 1998), in contrast to the assumption that woody plants have lower ACL values than non-woody plants.

4.2 Why are ACL values not significantly different between woody and non-woody plants?

ACL values of terrestrial plant leaf waxes could be affected by many environmental and plant physiological factors that would make them indistinguishable between woody and non-woody plants. Temperature and precipitation and their combinations have been found to affect the lipid distributions of terrestrial plants. Plants tend to biosynthesize longer chain lipid compounds for waxy coatings in warm regions and shorter chain lipids in cool regions (Castañeda et al., 2009). Plants in warmer climates have been suggested to produce longer-chain compounds (Poynter et al., 1989). Simoneit et al. (1991) found that the plants in warmer climate of southern China produced higher molecular weight *n*-alkanes by analyzing continental aerosols.

Regional precipitation could also influence the chain length distribution of leaf wax lipids. Under water stress, plants tend to synthesize longer carbon chain alkanes in order to provide a more efficient waxy coating (Calvo et al., 2004). For instance, atmospheric dust samples collected in transects along the west African coast reveal changes in the chain length distributions of *n*-alkanes responding to aridity in the source regions (Huang et al., 2000; Schefuss et al., 2003). ACLs for alkanes in Oregon conifers decrease with increasing distance away from the Coastal Range, which suggests an adaptation by conifers to humid climate conditions (Oros et al., 1999). It has been found that the carbon number of a higher plant *n*-alkane distribution is broadly related to latitude (Poynter et al., 1989; Poynter and Eglinton, 1990), with higher carbon numbers occurring at lower latitudes. Therefore, under different climatic regimes, particular combinations of temperature and precipitation would complicate the lipid dis-

tribution of plants. As climate changed in the past, various combinations of temperature and precipitation, such as humid/warm or humid/dry would occur, and thus the pattern of changes in the ACL of the plants would be very complex. Furthermore, the effect of lipid degradation on the ACL values remains unclear.

For those plants with the same growth habit, the lipid distribution may be distinct. For example, coniferous trees tend to produce longer carbon chain lipids than broad-leaf plants (Zhang et al., 2008). The ACL could vary significantly even in leaves from individual plants of the same genus. Hoffmann et al. (2013) found that ACL varied from 26.87 to 28.99 for *Eucalyptus*. Schwark et al. (2002) also observed significant differences in ACL values for *Betula*. Furthermore, data from the case studies presented here also indicate that leaf samples obtained from different heights of a single plant show different ACL values. For example, at Blood Pond, leaves at different heights (2, 4 and 6 m) from Black Cherry yield ACL values ranging from 25.06 to 27.56 (Table 2). In addition, woody plants and non-woody plants could produce lipids with similar ACL values (Gao et al., 2011; Carr et al., 2014). Carr et al. (2014) found that both herbs and woody shrubs in South African yielded lower ACL values. Furthermore, aquatic plants could produce high molecular weight *n*-alkyl lipids (Lichtfouse et al., 1994; Ficken et al., 2000; Mead et al., 2005; Aichner et al., 2010; Duan and Xu, 2012) that would be mixed in the sedimentary lipids and be indistinguishable during analysis.

5 Conclusions

The average chain length of plant lipids that are preserved in various sediments has been widely used as a proxy indicator for past changes in vegetation, environment and climate. However, the fundamental assumption of using ACL as a proxy indicator seems problematic based on the meta-analysis of two case studies and a data set compiled from around the world. About 73 % of the surveyed sites (19 out of 26 sites) and over 90 % of plants (741 out of 823 plants) do not show a statistical difference in ACL values between woody plants and non-woody plants. Nevertheless, about 27 %

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of the surveyed sites and less than 10 % of plants do show a difference on a global basis. The results suggest that considerable caution is necessary in using ACL values as a proxy indicator for vegetation dynamics, and for interpreting ACL variation in terms of past changes in environment and climate.

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Table 1. ACL values for modern plants at Lake Ranwu on the southeastern Tibetan Plateau, China.

Sample ID	Latin name	Plant type[a]	ACL[b]
RW1 [14 Jun 2010 West bank of Lake Ranwu, riverside and lakeside; N 29°28′15″, E 96°46′48″, alt 3938 m]			
RW1-1	*Ribes orientale* Desf.		
RW1-2	*Fragaria nubicola* Lindl. ex Lacaita	HB	31.70
RW1-3	*Salix variegata* Franch.	SH	26.50
RW1-4	*Salix dalungensis* C. Wang et P.Y. Fu	TR	25.55
RW1-5	*Lonicera spinosa* Jacquem. ex Walp.	SH	29.67
RW1-6	*Hippophae rhamnoides* L. subsp. *yunnanensis* Rousi	SH	28.73
RW1-7	*Juniperus squamata* Buchanan-Hamilton ex D. Don	SH	32.10
RW1-8	*Myricaria rosea* W.W. Smith	SH	29.56
RW1-9	*Berberis umbratica* Ying	SH	28.38
RW1-10	*Berberis jamesiana* Forrest et W.W. Sm.	SH	29.12
RW1-11	*Adonis brevistyla* Franch.	HB	30.46
RW1-12	*Picea brachytyla* (Franch.) Pritz. var. *complanata* (Mast.) Cheng ex Rehd.	TR	28.46
RW1-13	*Sibiraea angustata* (Rehd.) Hand.-Mazz.	SH	29.66
RW2 [14 Jun 2010 East bank of Lake Ranwu, hillside; N 29°28′40″, E 96°46′59″, alt 3942 m]			
RW2-1	*Juniperus squamata* Buchanan-Hamilton ex D. Don	SH	31.38
RW2-2	*Berberis jamesiana* Forrest et W.W. Sm.	SH	28.90
RW2-3	*Incarvillea younghusbandii* Sprague	HB	30.20
RW2-4	*Draba altaica* (C.A. Mey.) Bunge	HB	30.49
RW2-5	*Astragalus monbeigii* N.D. Simpson	HB	27.93
RW2-6	*Potentilla bifurca* L.	HB	31.19
RW2-7	*Dracocephalum tanguticum* Maxim.	HB	30.90
RW2-8	*Taraxacum brevirostre* Hand.-Mazz.	HB	26.69
RW2-9	*Spiraea alpina* Pall.	SH	29.02
RW2-10	*Ephedra gerardiana* Wall.	SH	28.12
RW2-11	*Rosa omeiensis* Rolfe	SH	28.71
RW2-12	*Lancea tibetica* Hook. f. et Thoms	HB	30.67
RW2-13	*Potentilla anserina* L.	HB	30.99
RW3 [15 Jun 2010 East bank of Lake Ranwu, lakeside; N 29°28′36″, E 96°47′08″, alt 3943 m]			
RW3-1	*Halerpestes tricuspis* (Maxim.) Hand.-Mazz.	HB	27.72
RW3-2	*Cotoneaster rotundifolius* Wall. ex Lindl.	SH	29.91
RW4 [15 Jun 2010 East bank of Lake Ranwu, near camp, lakeside; N 29°28′36″, E 96°47′03″, alt 3927 m]			
RW4-1	*Kobresia macrantha* Boeck.	HB	30.13
RW5 [14 Jun 2010 East bank of Lake Ranwu, lakeside; N 29°28′35″, E 96°47′07″, alt 3943 m]			
RW5-1	*Populus pseudoglauca* C. Wang et P.Y. Fu	TR	27.40
RW5-2	*Populus davidiana* Dode	TR	26.78
RW6 [15 Jun 2010 East bank of Lake Ranwu, arid with sany soils; N 29°28′36″, E 96°47′08″, alt 3943 m]			
RW6-1	*Trisetum clarkei* (Hook. f.) R.R. Stewart	HB	27.33

[a] SH, shrub; HB, herb; TR, tree.
[b] ACL: averaged chain length of odd carbon numbered n-C_{25} to n-C_{33} alkanes.

Table 2. ACL values for modern plants at Blood Pond in the northeastern United States.

Sample ID	Common name	Scientific name	Height (m)	Plant type[a]	ACL[b]
#1-1	Gray Birch,Shady	*Betula populifolia*	6	TR	27.11
#1-2	Gray Birch.Shady	*Betula populifolia*	5	TR	27.02
#1-3	Gray Birch,Shady	*Betula populifolia*	3	TR	27.12
#6-1	Gray Birch,Sunny	*Betula populifolia*	6	TR	27.35
#6-2	Gray Birch,Sunny	*Betula populifolia*	5	TR	27.44
#2-1	Black Birch	*Betula lenta*	6	TR	27.75
#2-2	Black Birch	*Betula lenta*	5	TR	26.82
#2-3	Black Birch	*Betula lenta*	3	TR	27.63
#3-1	Black Birch	*Betula lenta*	6	TR	27.58
#3-2	Black Birch	*Betula lenta*	5	TR	27.70
#3-3	Black Birch	*Betula lenta*	3	TR	27.73
#7-1	White Ash	*Fraxinus americana*	6	TR	28.91
#7-2	White Ash	*Fraxinus americana*	5	TR	29.36
#7-3	White Ash	*Fraxinus americana*	3	TR	29.16
#8-1	Hemlock	*Tsuga* Carr.	5	TR	27.06
#8-2	Hemlock	*Tsuga* Carr.	3	TR	27.07
#11	Red Maple, young, shady	*Acer rubrum* L.	2	TR	27.75
#40	Red Maple	*Acer rubrum* L.	5	TR	27.38
#22-1	Red Maple	*Acer rubrum* L.	6	TR	27.42
#22-2	Red Maple	*Acer rubrum* L.	6	TR	27.86
#33-1	Black Cherry	*Prunus serotina*	6	TR	25.06
#33-2	Black Cherry	*Prunus serotina*	4	TR	26.93
#33-3	Black Cherry	*Prunus serotina*	2	TR	27.56
#30-1	Black Gum	*Nyssa sylvatica*	6	TR	26.49
#30-2	Black Gum	*Nyssa sylvatica*	5	TR	26.63
#30-3	Black Gum	*Nyssa sylvatica*	4	TR	27.07
#15-1	Red Oak	*Quercus rubra* L.	6	TR	27.31
#15-2	Red Oak	*Quercus rubra* L.	3	TR	27.48
#12	Black Oak	*Quercus velutina* Lam.		TR	29.40
#13-1	White Pine	*Pinus strobus* L.	6	TR	26.76
#13-2	White Pine	*Pinus strobus* L.	5	TR	26.95
#13-3	White Pine	*Pinus strobus* L.	2	TR	30.57

[a] SH, shrub; HB, herb; TR, tree; FE, fern; GR, grass.
[b] ACL: averaged chain length of even carbon numbered n-C_{24} to n-C_{32} acids.

Table 2. Continued.

Sample ID	Common name	Scientific name	Height (m)	Plant type[a]	ACL[b]
#14-1	Hickory	*Carya* Nutt.	5	TR	28.18
#14-2	Hickory	*Carya* Nutt.	5	TR	28.05
#14-3	Hickory	*Carya* Nutt.	2	TR	27.62
#31	Maple leaved viburnum	*Viburnum acerifolium*		SH	28.53
#5	Raspberry	*Rubus aliceae* Bailey		SH	27.40
#4	Spice Bush	*Lindera benzoin*		SH	27.66
#10	Honey Suckle	*Lonicera tatarica* L.		SH	28.15
#9-1	Witch Hazel	*Hamamelis virginiana*	5	SH	26.42
#9-2	Witch Hazel,Shady	*Hamamelis virginiana*		SH	26.63
#33	Witch Hazel	*Hamamelis virginiana*		SH	27.12
#21	Winterberry	*Ilex verticillata*		SH	25.99
#16	Hay-scented Fern	*Dennstaedtia punctilobula*		FE	24.00
#25	Christmas Fern	*Polystichum acrostichoides*		FE	25.87
#29	Sensitive Fern	*Onoclea sensibilis*		FE	28.08
#24	Cat Briar	*Smilax rotundifolia* L.		SH	25.68
#18	Cat tail	*Typha latifolia*		HB	28.28
#17	Jewel Weed	*Impatiens capensis* Meerb		HB	28.00
#19	Grass #1 unidentified			GR	27.64
#28	Carrion Flower	*Smilax herbacea* L.		HB	28.94
#32	Sweet Pepperbush	*Clethra alnifolia* L.		SH	27.64
#34	White Swamp Honeysuckle	*Rhododendron viscosum* or *nudiflorum*		SH	28.41
#45	White Wood Aster	*Aster divaricatus*		HB	27.99
#46	Plantago major			HB	29.34
#47	Dandelion-like			HB	28.01
#48		*carya pennsylvania*		HB	27.95
#49	Red Clover	*Trifolium pratense* L.		HB	27.94
#50	hickory	*Carya* sp.		HB	28.35
#51	Monkey flower	*Mimulus ringens*		HB	27.12
#52	Path rush	*Juncus tenuis*		HB	26.13
#53	wheat grass	*Agropyron* sp.		HB	27.31
#55	barnyard grass	*Dactylis glomerata*		HB	27.24
#56	Timothy Weed	*Phleum pratense*		HB	27.83
#57	Queen Annas Lace	*Daucus carota*		HB	28.13
#58	Foxtail	*Alopecurus* L.		HB	28.77

[a] SH, shrub; HB, herb; TR, tree; FE, fern; GR, grass.
[b] ACL: averaged chain length of even carbon numbered n-C_{24} to n-C_{32} acids.

Discussion Paper | Discussion Paper | Discussion Paper | Discussion Paper |

Table 3. *T* test results for modern plants at Lake Ranwu, Blood Pond and complied sites around the world.

No.	Study area	HB	Woody	*t* test Significance	ACL	References	Climate
A	New Zealand	3	6	0.205	C_{23}-C_{35}	Eglinton et al. (1962)	Cfb
B	Austria	6	4	0.689	C_{23}-C_{35}	Lütz and Gülz (1985)	Dfc
C	Oregon, USA	2	13	0.762	C_{21}-C_{35}	Salasoo (1988)	Csb
D	BGUT, Italy	363	16	0.086	C_{19}-C_{35}	Maffei (1994,1996a,1996b)	Cfb
E	Griningsdalen, Norway	6	4	0.330	C_{21}-C_{35}	Mayes et al. (1994)	ET
F	Cairngorms, UK	1	2	[a2]	C_{19}-C_{35}	Ficken et al. (1998)	Cfc
G	Cumbria, UK	4	5	0.312	C_{25}-C_{33}	Nott et al. (2000)	Cfb
H	Japan	10	16	0.578	C_{25}-C_{33}	Chikaraishi and Naraoka (2003)	Cfa
I	Thailand	3	1	–	C_{25}-C_{33}	Chikaraishi and Naraoka (2003)	Aw
J	Sudan	21	4	0.015[a1]	C_{21}-C_{35}	Ali et al. (2005a)	BSh
K	Aberdeenshire, UK	5	7	0.720	C_{21}-C_{35}	Ali et al. (2005b)	Cfb
L	SCBG, China	10	12	0.013[a1]	C_{24}-C_{35}	Bi et al. (2005)	Cwa
M	MI and NY, USA	1	3	[a2]	C_{21}-C_{33}	Nichols et al. (2006)	Dfa
N	Hubei, China	2	3	0.563	C_{23}-C_{37}	Cui et al. (2008)	Cfa
O	Sudan	8	3	0.028[a1]	C_{25}-C_{33}	Vogts et al. (2009)	BSh
P	Jacinto, USA	1	11	–	C_{27}-C_{33}	Feakins and Sessions (2010)	Csa
Q	Mojave, USA	2	9	0.633	C_{27}-C_{33}	Feakins and Sessions (2010)	BWh
R	Topanga, USA	1	16	–	C_{27}-C_{33}	Feakins and Sessions (2010)	Csb
S	Xinglong Mt., China	1	3	[a2]	C_{15}-C_{34}	Lei et al. (2010)	BSk
T	Mexico, Guatemala, Honduras	2	13	0.754	C_{24}-C_{34}	Douglas et al. (2012)	Am and Aw
U	Lake Qinghai, China	9	4	0.000[a1]	C_{25}-C_{33}	Duan and Xu (2012)	Dwc
V	Lake El'gygytgyn, Russia	4	1	–	C_{20}-C_{30}	Wilkie et al. (2013)	ET
W	South Africa	25	93	0.594	C_{21}-C_{35}	Carr et al. (2014)	BWk and BWh
X	Lonar Lake, India	6	6	0.881	C_{23}-C_{35}	Sarkar et al. (2014)	Aw
Y	Lake Ranwu, China	13	19	0.151	C_{25}-C_{33}	This study	ET
Z	Blood Pond, USA	16	24	0.394	C_{24}-C_{32}	This study	Dfb

[a] Indicates there is significant differences between woody and non-woody plants (sig. or p value < 0.05), [a1] is by t test, [a2] is by visual observation.
[b] SCBG = South China Botanical Garden, BGUT = Botanical Gardens of the University of Turin.
[c] Dash (–) indicates it cannot distinguish by visual.
[d] The climate classification refers to Peel et al. (2007) and Kottek et al. (2006).

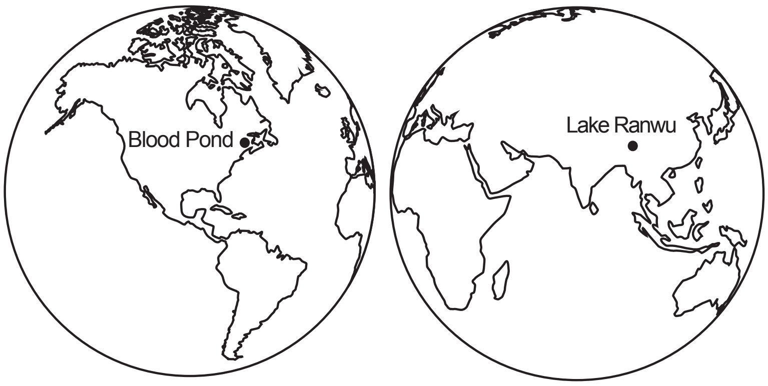


Figure 1. Locations of Blood Pond in the northeastern United States and Lake Ranwu on the southeastern Tibetan Plateau, China.

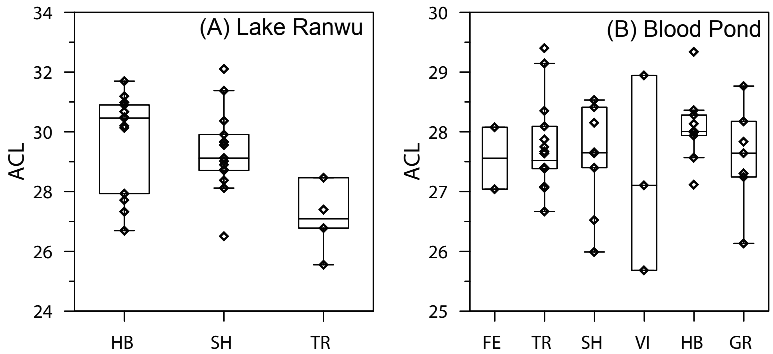


Figure 2. Distribution of ACL values for modern plants at Lake Ranwu **(a)** and Blood Pond **(b)**. HB, herbs; SH, shrubs; TR, trees; FE, ferns; VI, vines; GR, grasses. Box plots represent median (horizontal line), upper and lower quartiles (boxes).

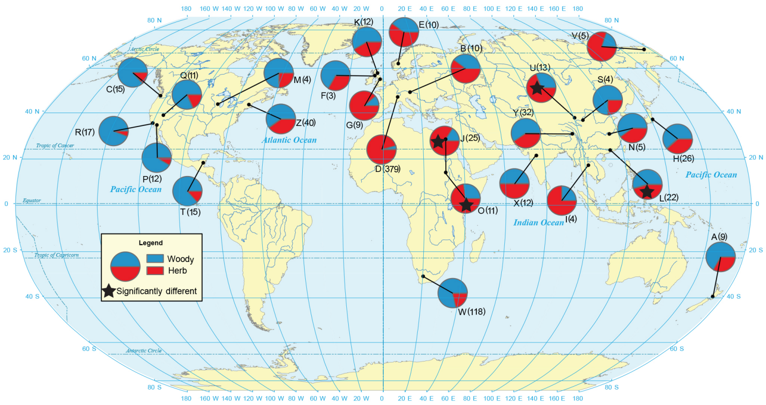
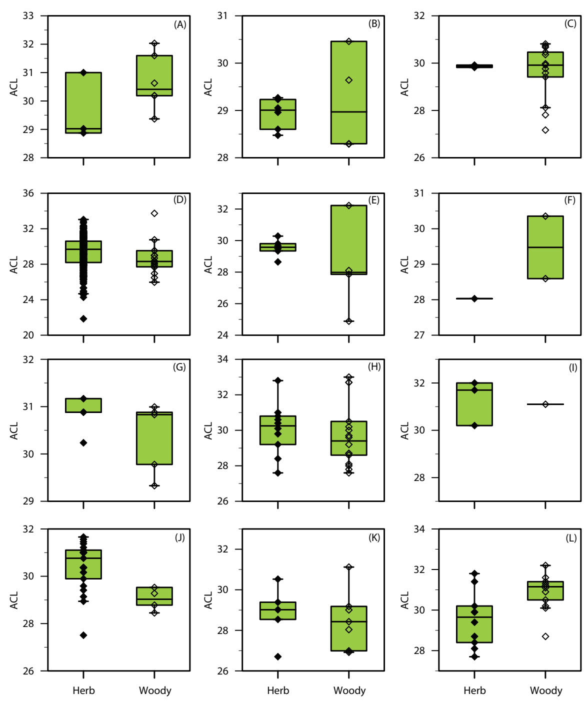


Figure 3. Compiled dataset of modern plants from around the world (including Lake Ranwu and Blood Pond in this study). Letters correspond to the sites in Table 3, and the numbers of samples at each site are listed in parentheses. Pie charts show the percentage of woody and non-woody plants at each site. Stars denote the fact that there is a statistically significant difference in ACL values between the two types of plants.



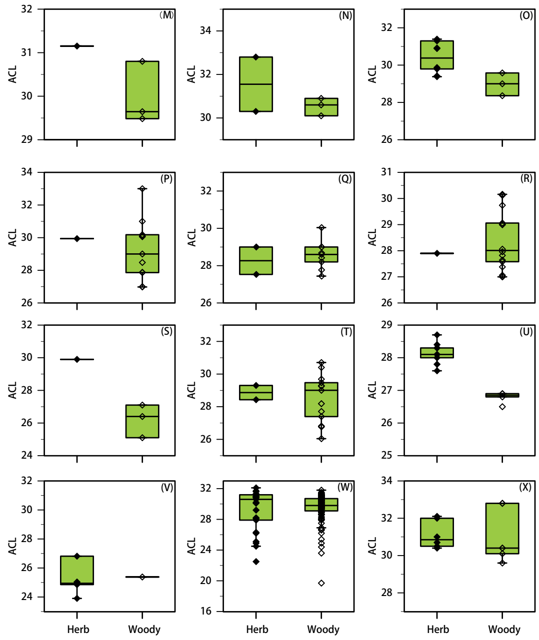


Figure 4. Distribution of ACL values for modern plants at 24 sites around the world. Solid diamonds represent non-woody plants (including graminoids/grasses and forbs), and open diamonds represent woody plants (including shrubs and trees). Letters correspond to the sites in Table 3 and Fig. 3.