

## Is the maximum carbon number of long-chain *n*-alkanes an indicator of grassland or forest? Evidence from surface soils and modern plants

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The molecular distribution of long-chain *n*-alkanes in 62 soil samples collected from diverse locations across eastern China was analyzed. The long-chain *n*-alkanes were mostly dominated by *n*-C<sub>29</sub> or *n*-C<sub>31</sub>, regardless of the overlying vegetation type at each site. The results were compared with those summarized from the literature, covering more than 100 soil samples within China and more than 300 genera of modern plants distributed worldwide. There were similar *n*-alkane distribution patterns for most genera, with no clear differences among grasses, shrubs, and trees. The evidence from analyses of surface soils and modern plants indicates that the relationship between the molecular distribution of long-chain *n*-alkanes of surface soils and source vegetation is highly complex, and is influenced by many factors. Further, it is suggested that source vegetation types should not be simply inferred from distribution patterns of long-chain *n*-alkanes in sediments.

**surface soils, modern plants, long-chain *n*-alkanes, molecular distribution, vegetation type**

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The evolution of terrestrial landscapes over geological time and modern history, including alternations between grassland and forest, has been driven by changes in the global climate. Efforts to reconstruct the history of terrestrial vegetation have mostly relied on the use of pollen [1,2] and phytolith [3] assemblages. Recently, the characteristic profiles of *n*-alkanes extracted from loess [4–6] and red clay [7,8] sediments have been used to reconstruct changes in vegetation over geological timescales. In some studies, the maximum carbon number (MCN) of long-chain *n*-alkanes, i.e., the carbon number of the most abundant homolog, has been used to indicate vegetation type. More specifically, it was suggested that long-chain *n*-alkanes dominated by *n*-C<sub>27</sub> or *n*-C<sub>29</sub> indicate forest, while those dominated by *n*-C<sub>31</sub>

indicate grassland. Therefore, the ratio of *n*-C<sub>27</sub> to *n*-C<sub>31</sub> (or  $n\text{-C}_{27}+n\text{-C}_{29}/n\text{-C}_{31}+n\text{-C}_{33}$ ) has been used to represent the input of woody versus herbaceous plants.

Leaf waxes of higher terrestrial plants contain abundant long-chain *n*-alkanes with significant odd-to-even carbon number preference [9,10]. There are demonstrable links between the molecular distribution of long-chain *n*-alkanes of modern plants and different seasons, study sites, plant age, and plant organs [9]. Thus, there should be a complex relationship between the molecular distribution of sedimentary long-chain *n*-alkanes from multiple source plants and the source vegetation.

Even all the long-chain *n*-alkanes produced by woody plants are dominated by *n*-C<sub>27</sub> or *n*-C<sub>29</sub>, while those of herbaceous plants are dominated by *n*-C<sub>31</sub>, it is unclear whether the MCN of material from multiple plant sources can be

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used to reconstruct the source vegetation type. It is possible that long-chain *n*-alkanes mixed from sources dominated by *n*-C<sub>27</sub> (woody plants) and *n*-C<sub>31</sub> (herbaceous plants) could be dominated by the *n*-C<sub>29</sub>, because it occurs at relatively high concentrations in both of the source plant types. For example, if a woody plant with a ratio of 10:7:3 for *n*-C<sub>27</sub>, *n*-C<sub>29</sub>, and *n*-C<sub>31</sub> and a herbaceous plant with a ratio of 1:7:10 for *n*-C<sub>27</sub>, *n*-C<sub>29</sub>, and *n*-C<sub>31</sub> contributed equally to a certain sediment, the *n*-alkanes extracted from that sediment would show a ratio of 11:14:13 for *n*-C<sub>27</sub>, *n*-C<sub>29</sub>, and *n*-C<sub>31</sub>. This would erroneously indicate that *n*-C<sub>29</sub> was the dominant homolog in the source vegetation. Therefore, the MCN may not be a robust indicator of vegetation type, and the relative abundance of *n*-C<sub>27</sub> to *n*-C<sub>31</sub> does not indicate equal inputs from woody and herbaceous vegetation. Moreover, the production of long-chain *n*-alkanes may differ between different plants, which further complicate reconstructions of past vegetation.

Currently, there is no evidence that long-chain *n*-alkanes from woody plants are dominated by *n*-C<sub>27</sub> and *n*-C<sub>29</sub>, or that those of herbaceous plants are dominated by *n*-C<sub>31</sub>. Similarly, there is little work showing how the MCN and molecular distribution of sedimentary long-chain *n*-alkanes from higher plants can be used to reconstruct paleovegetation. In this paper, we explore the relationship between the distribution of long chain *n*-alkanes and their respective vegetation sources based on the results from 100 surface soil samples collected from diverse areas across China and more than 300 globally distributed genera of modern plants.

## 1 Material and methods

For this study, a total of 62 surface soil samples were collected from diverse areas across China, extending from Hainan Province in southern China to Heilongjiang Province in northeastern China, spanning latitudes of 18°N to 50°N (Figure 1; Table S1). Vegetation across the study area ranges from tropical rainforest in southern China to temperate coniferous-deciduous broad-leaved mixed forest in northeastern China. The prevailing climate is monsoonal with concurrent rainy and hot seasons, with mean annual precipitation ranging from 500–2500 mm and a mean annual temperature of 0–26°C. The monsoonal climate trends from south to north with a strong dependence on latitude.

Surface soil samples were collected from the top 2–4 cm of weathering crust of basalt or other visible bedrocks in the field, thus avoiding contamination from underground water and allochthonous organic matter. The vegetation at all sampling sites was natural, with little human disturbance. Two to three surface samples were collected from different physiographic locations of each sampling site. A small grassland located in Damaping (114.5°E, 40.9°N, Figure 1), was sampled more intensively (12 samples). All samples

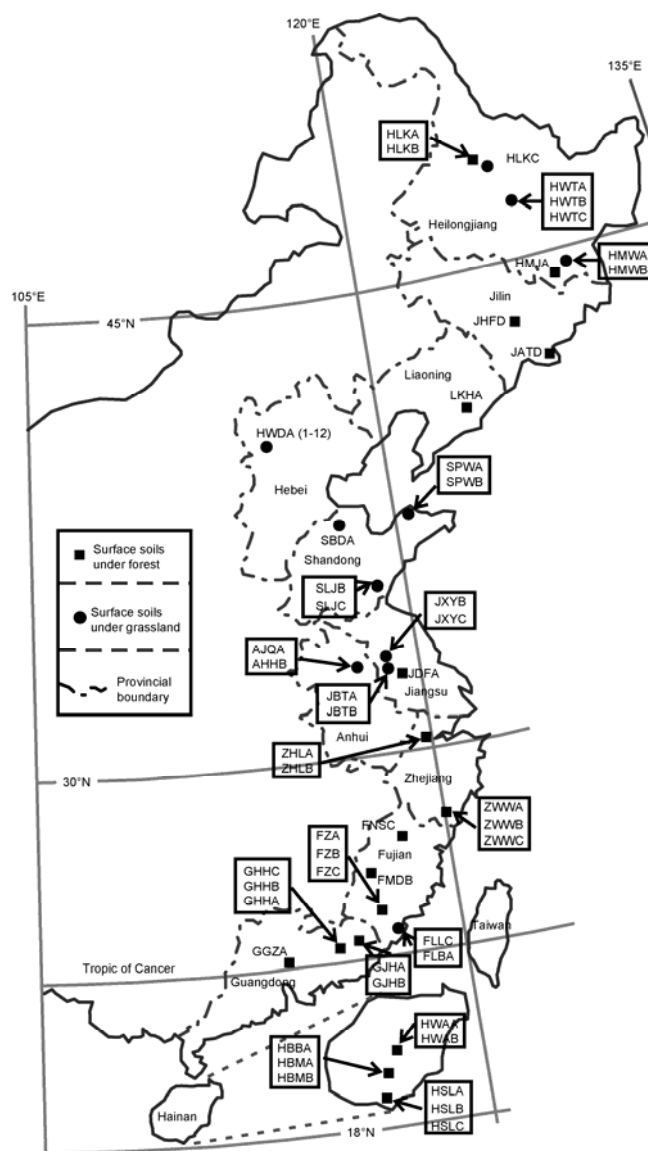


Figure 1 Map of study region and location of sampling sites.

were collected between September and November in 2005.

Rootlets and gravel were removed from the samples, and then they were ground and sieved through an 80- $\mu$ m mesh sieve. After immersion in dichloromethane for ~2 h, approximately 10 g powdered soil was ultrasonically extracted for 10 min. This process was repeated three times and the combined solvents were then concentrated by rotary evaporation. The total lipid extract was separated using silica gel flash-column chromatography. The sample was eluted with hexane to obtain the saturated hydrocarbon fraction containing long-chain *n*-alkanes.

The long-chain *n*-alkanes were analyzed using an HP 6890 gas chromatograph equipped with an HP-5 MS silica capillary column (30 m  $\times$  0.32 mm  $\times$  0.25  $\mu$ m). The oven temperature program was as follows: 80°C for 3 min, increasing to 200°C at a rate of 10°C/min, then increasing at

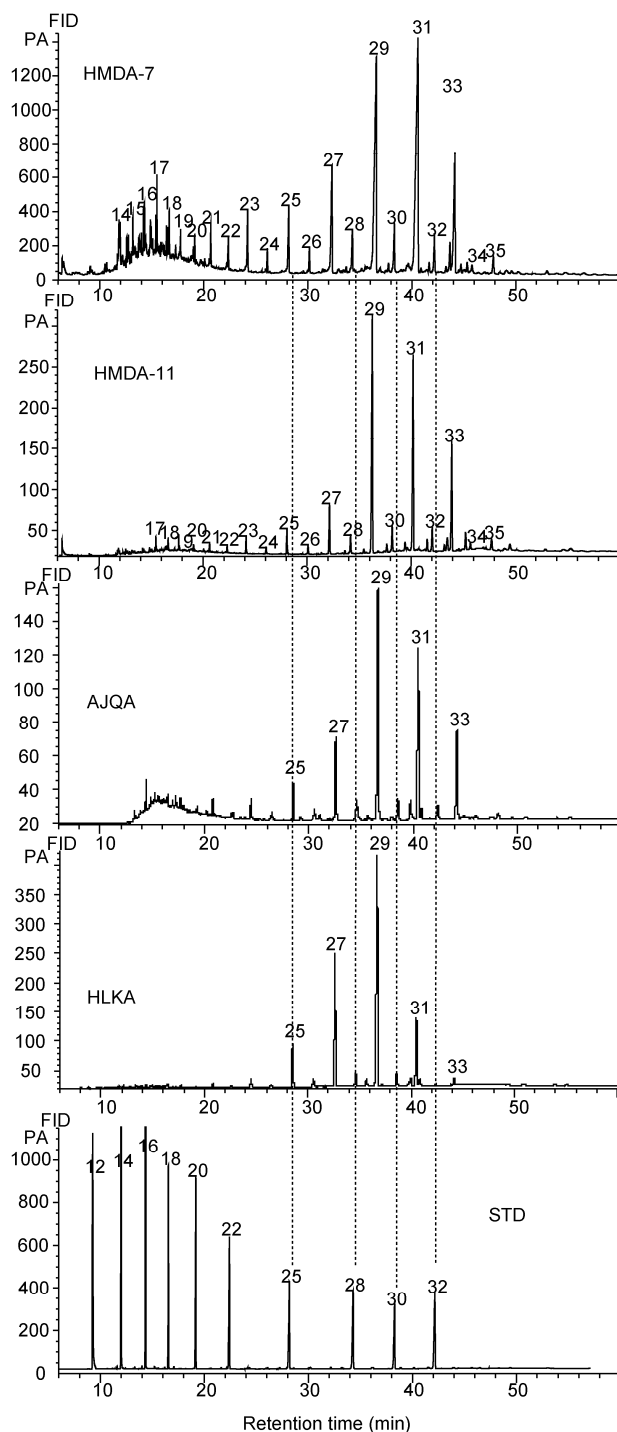
3°C/min to 290°C, and hold for 30 min. A reference material-Indiana STD (a set of *n*-alkanes consisting of 10 homologs; *n*-C<sub>12</sub>, *n*-C<sub>14</sub>, *n*-C<sub>16</sub>, *n*-C<sub>18</sub>, *n*-C<sub>20</sub>, *n*-C<sub>22</sub>, *n*-C<sub>25</sub>, *n*-C<sub>28</sub>, *n*-C<sub>30</sub>, and *n*-C<sub>32</sub>; Figure 2) was analyzed under exactly the same conditions. The carbon numbers of *n*-alkanes extracted from surface soil samples were determined by comparing their retention times with those of known alkanes in the reference material (Figure 2). The areas underneath *n*-alkane peaks were used to determine relative abundance.

## 2 Results and discussion

### 2.1 Long-chain *n*-alkanes extracted from surface soils under different vegetation types

The carbon numbers of *n*-alkanes extracted from most surface soil samples ranged from 14 to 35 with significant bimodal distribution (Figure 2). The short chain components without strong odd-to-even carbon number preference were dominated by either *n*-C<sub>17</sub> or *n*-C<sub>19</sub>. All samples showed relatively high concentrations of *n*-C<sub>23</sub> to *n*-C<sub>34</sub> alkanes with strong odd-to-even preference, with *n*-C<sub>27</sub>, *n*-C<sub>29</sub>, and *n*-C<sub>31</sub> being the most dominant. The CPI (carbon preference index) of the long-chain *n*-alkanes extracted from all 62 surface soil samples ranged from 3 to 12.8 with an average value of 6.3. As reported in previous studies, *n*-C<sub>15</sub> to *n*-C<sub>20</sub> alkanes without significant odd-to-even preference and dominated by *n*-C<sub>17</sub> or *n*-C<sub>19</sub> alkanes are mainly derived from lower organisms [11], while terrestrial higher plants produce abundant long-chain *n*-alkanes with significant odd-to-even preference (such as *n*-C<sub>27</sub>, *n*-C<sub>29</sub>, and *n*-C<sub>31</sub>) and with a CPI value exceeding 5 [9,12]. According to these classifications, the molecular distributions of *n*-alkanes extracted from our surface soil samples are characteristic of both higher plants and lower organisms, while the long-chain *n*-alkane components are derived from terrestrial higher plants.

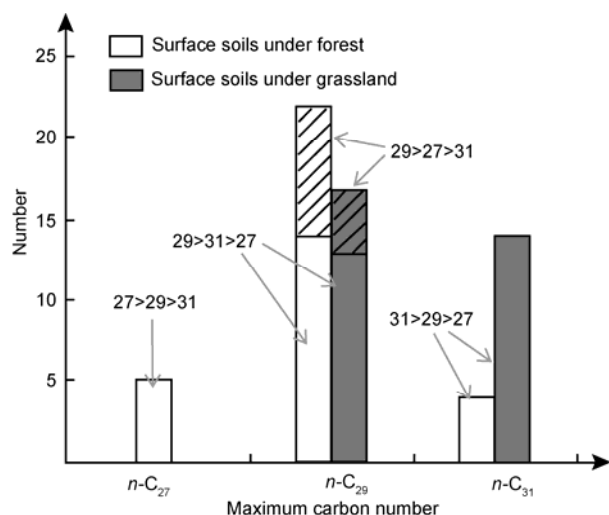
We analyzed the MCN and relative abundance of the three main homologs (*n*-C<sub>27</sub>, *n*-C<sub>29</sub>, and *n*-C<sub>31</sub>) of long-chain *n*-alkanes in each sample. Half of the samples (31) were collected from forested locations. The MCNs of *n*-alkanes in these samples were 27, 29, or 31, reflecting dominance of *n*-C<sub>27</sub>, *n*-C<sub>29</sub>, or *n*-C<sub>31</sub> alkanes, respectively (Table S1; Figure 3). Of these 31 samples, 22 were dominated by *n*-C<sub>29</sub>, 14 of which showed a relative abundance of *n*-C<sub>29</sub>>*n*-C<sub>31</sub>>*n*-C<sub>27</sub>, and 8 of which showed a relative abundance of *n*-C<sub>29</sub>>*n*-C<sub>27</sub>>*n*-C<sub>31</sub>. Of the remaining 9 samples, 5 were dominated by *n*-C<sub>27</sub> with a relative abundance of *n*-C<sub>27</sub>>*n*-C<sub>29</sub>>*n*-C<sub>31</sub>, and 4 were dominated by *n*-C<sub>31</sub> with a relative abundance of *n*-C<sub>31</sub>>*n*-C<sub>29</sub>>*n*-C<sub>27</sub>. Half of the samples (31) were collected from grassland. These had an MCN of 29 or 31, reflecting dominance of *n*-C<sub>29</sub> or *n*-C<sub>31</sub> alkanes, respectively. Of these 31 samples, 14 were dominated by *n*-C<sub>31</sub>



**Figure 2** GC chromatogram of reference material and four typical surface soil samples (refer to Figure 1 and Table S1 for detailed information on soil samples).

with a relative abundance of *n*-C<sub>31</sub>>*n*-C<sub>29</sub>>*n*-C<sub>27</sub>. The other 17 samples were dominated by *n*-C<sub>29</sub>; 13 samples showed a relative abundance of *n*-C<sub>29</sub>>*n*-C<sub>31</sub>>*n*-C<sub>27</sub> and 4 samples showed a relative abundance of *n*-C<sub>29</sub>>*n*-C<sub>27</sub>>*n*-C<sub>31</sub>.

The MCN and relative abundance of the three main homologs of long-chain *n*-alkanes varied among surface soil



**Figure 3** Maximum carbon number and relative abundance of three main homologs ( $n-C_{27}$ ,  $n-C_{29}$ , and  $n-C_{31}$ ) of long-chain  $n$ -alkanes extracted from 62 surface soil samples collected from across eastern China.

samples from the same location under the same vegetation. At the Damaping site (Figure 1), the bedrock is basalt and the top soil layer is too thin to support the growth of woody plants. Indeed, no woody plants were observed in the study area. Therefore, this location may have been influenced by grassland for a relatively long time. Of the 12 samples collected from this sampling site, 10 were dominated by  $n-C_{31}$  with a relative abundance of  $n-C_{31} > n-C_{29} > n-C_{27}$  (HMDA-7 in Figure 2). The remaining two samples were dominated by  $n-C_{29}$  with a relative abundance of  $n-C_{29} > n-C_{31} > n-C_{27}$  (HMDA-11 in Figure 2).

Most of the soil samples collected from across eastern China were dominated by  $n-C_{29}$  and  $n-C_{31}$  (only 5 out of 62 samples were dominated by  $n-C_{27}$ ). This trend has also been reported in other studies. In a similar smaller study of 26 surface soil samples from China and Mongolia, most samples were dominated by  $n-C_{29}$  and  $n-C_{31}$  with  $n-C_{31}$  as the most frequent MCN, and only few samples were dominated by  $n-C_{27}$  [13]. In another study,  $n$ -alkanes of 10 surface soil samples from different climatic and vegetation zones were analyzed. Four from forested locations (three of which were plantation forests) were dominated by  $n-C_{29}$ , which were suggested to reflect a mixture of woody and herbaceous source plants, and 6 samples from grassy locations were dominated by  $n-C_{31}$  [14]. Long-chain  $n$ -alkanes extracted from surface soil in the Heshang Cave, Qingjiang, Hebei Province, were dominated by  $n-C_{31}$  [15]. Long-chain  $n$ -alkanes extracted from 9 surface soil samples collected from across the Loess Plateau were mainly dominated by  $n-C_{29}$  and  $n-C_{31}$ , while only few samples were dominated by  $n-C_{27}$  [16]. All of these surface soil samples were obtained from diverse locations with various overlying vegetation types and plant assemblages across a huge geographical region; however, the long-chain  $n$ -alkanes extracted from these samples were mainly and consistently dominated by

$n-C_{29}$  and  $n-C_{31}$ .

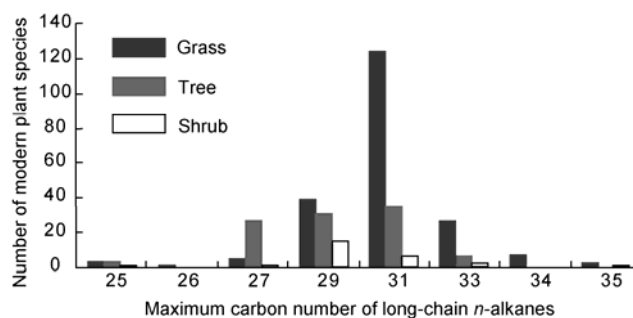
## 2.2 Long-chain $n$ -alkanes extracted from different modern plants

We conducted a systematic evaluation of long-chain  $n$ -alkane distribution from 334 genera of modern plants reported in published studies [2,5,17–41] and unpublished data (Rao et al. personal unpublished data; Table S2). Most plants are dominated by  $n-C_{29}$  and  $n-C_{31}$ , even when they are divided into different groups such as trees, shrubs, and grasses (Table S2). Of the 207 modern grasses, 124 were dominated by  $n-C_{31}$  and 39 by  $n-C_{29}$ . Of the 101 modern trees, 35 genera showed an MCN reflecting  $n-C_{31}$  and 31 showed an MCN reflecting  $n-C_{29}$ . Of the 26 modern shrubs, 6 showed an MCN reflecting  $n-C_{31}$  and 15 showed an MCN reflecting  $n-C_{29}$  (Figure 4). Overall, 80% of grasses, 65% of trees, and 80% of shrubs were dominated by either  $n-C_{31}$  or  $n-C_{29}$ . Although this is a small sample size compared with the number of plant species worldwide, the data highlights that plants show common characteristics in the molecular distribution of long-chain  $n$ -alkanes.

## 2.3 Discussion

The relationship between the composition of long-chain  $n$ -alkanes in surface soils and the corresponding vegetation is complex, and there are many sources of uncertainty. It is possible that soils located under grasslands are affected by surrounding forests, and/or by deposition of atmospheric aerosols that act as a source of long-chain  $n$ -alkanes [42,43]. Considering the consistent odd-to-even preference and molecular distribution of long-chain  $n$ -alkanes from surface soils, it is likely that their variability results from overlying vegetation. For example, the  $n$ -alkane distribution in soils mainly dominated by  $n-C_{29}$  and  $n-C_{31}$  is similar to that identified in modern plants, regardless of the type of vegetation and plant.

Several studies on  $n$ -alkanes in soils have been carried out on red earth in southern China, including 12 Pleistocene samples from Changxing, Zhejiang Province [8], 52 Pleistocene samples from Xuancheng, Anhui Province [7] and



**Figure 4** Maximum carbon number of long-chain  $n$ -alkanes extracted from modern plants, summarized from data in Table S2.

some Pleistocene samples from Xiushui, Jiangxi Province [44]. All of those samples were dominated by  $n\text{-C}_{29}$  or  $n\text{-C}_{31}$ . Analyses of samples from the Chinese Loess Plateau in northern China, including the Yuanbao profile in Linxia [45], the Dadiwan profile in Qin'an [5], the Caoxian profile [46], the Xifeng profile [47], and the Luochuan profile [4,6], showed a predominance of  $n\text{-C}_{31}$ , with only few samples dominated by  $n\text{-C}_{29}$ . Thus, in the red earth areas in southern China and loess areas in northern China, almost all samples were dominated by  $n\text{-C}_{29}$  and  $n\text{-C}_{31}$ , although it is unclear whether red earth or loess directly affects the  $n$ -alkane distribution in surface soils and modern plants in those areas. Together, these data indicate that there is a complex relationship between the molecular distribution of long-chain  $n$ -alkanes in surface soil and sediment derived from multiple plant sources and the type of source vegetation. There are several factors that control the molecular distribution of sedimentary  $n$ -alkanes, and therefore, affect their relationship with modern vegetation. These factors can be summarized as follows:

(1) Production of  $n$ -alkanes in plants: This can strongly influence the  $n$ -alkane profile, because a plant species that produces greater amounts of  $n$ -alkanes will bias the distribution of  $n$ -alkanes in sediments. Thus, the  $n$ -alkane profile of the sediment will not reflect the dominant vegetation. Differences in  $n$ -alkane production among plants in China have been reported [20]. In one study, the concentration of total  $n$ -alkanes from 93 gramineous plants ranged from 6.9 to 1860  $\mu\text{g/g}$  with an average value of 164.1  $\mu\text{g/g}$  [18]. Therefore, it is possible that the distribution of  $n$ -alkanes in sedimentary sequences reflects the plant source(s) with greatest  $n$ -alkane production rates, rather than those contributing the most biomass. Indeed, there is no evidence to suggest that the dominant plant shows the highest rate of  $n$ -alkanes production. On the contrary, there were no detectable  $n$ -alkanes in fresh pine needles of Lodgepole Pine (*Pinus contorta*), the dominant plant in a Canadian coniferous forest [19]. Likewise, the concentrations of  $n$ -alkanes extracted from some coniferous needles from Scandinavian regions were too low to permit isotopic measurement [17].

(2) Mixing effect of different sources: as mentioned above, a mixture of long-chain  $n$ -alkanes dominated by  $n\text{-C}_{27}$  and  $n\text{-C}_{31}$  may produce a signal indicating dominance of  $n\text{-C}_{29}$ , as all of the vegetation sources may contain relatively high concentrations of  $n\text{-C}_{29}$ . This is one possible reason why  $n\text{-C}_{29}$  is frequently the dominant component in surface soils and sediments. A recent study highlights this, as the molecular distribution of terrestrial  $n$ -alkanes in lake sediments was not characteristic of the dominant vegetation around the lake [17]. In that study, the authors analyzed diverse vegetation types and surface lake sediments collected from a wide geographical range (northern Finland to southern Italy). Their results may reflect, at least partly, the mixing process of  $n$ -alkanes.

(3) Variation of long-chain  $n$ -alkanes from the same

plant under different environmental conditions, and post-depositional alteration: Some studies have argued there is no significant variation in the MCN and the molecular distribution of long-chain  $n$ -alkanes during the decomposition process [16] or in different growth seasons [15]. However, there is a large body of evidence that variation and alterations do occur [17,19,35,38,39]. Results from Europe indicate that the molecular distributions of  $n$ -alkanes within the same species vary among different study sites, and the average chain length (ACL) of  $n$ -alkanes from deciduous tree leaves increases from northern to southern Europe [17]. Some studies have reported varied molecular distribution of  $n$ -alkanes from the same species across different study sites (Table S2). Together, these findings indicate that the molecular distribution of  $n$ -alkanes in modern plants varies significantly depending on the prevailing climatic and environmental conditions.

All of these factors result in a very complex relationship between the molecular distribution of sedimentary long-chain  $n$ -alkanes and their respective source vegetation. At present, it is uncertain whether it is valid to use molecular distributions of long-chain  $n$ -alkanes, and particularly the MCN of long-chain  $n$ -alkanes, as indicators of source vegetation.

### 3 Conclusions

In this study, analyses of long-chain  $n$ -alkanes in 62 surface soil samples highlighted that the molecular distribution of long-chain  $n$ -alkanes of surface soils is highly variable, even among different sites with the same type of vegetation (forest or grassland). However, there were similar molecular distributions of long-chain  $n$ -alkanes in surface soils under different vegetation types (forest and grassland). Of the 62 samples, most (57) were dominated by  $n\text{-C}_{29}$  or  $n\text{-C}_{31}$  (only 5 were dominated by  $n\text{-C}_{27}$ ), regardless of the type of overlying vegetation. This is consistent with the findings of other surface soil studies [13–16]. More importantly, the patterns of  $n$ -alkane distribution in more than 100 surface soils are consistent with those reported for more than 300 modern plant species [2,5,17–41], because most modern plants, whether they are trees, grasses, or shrubs, are also dominated by  $n\text{-C}_{29}$  and  $n\text{-C}_{31}$ . These results confirm that long-chain  $n$ -alkanes extracted from soils or sediments are derived from terrestrial higher plants. However, they cannot accurately predict the source vegetation because of the complex relationship between the molecular distribution of sedimentary long-chain  $n$ -alkanes (including both surface soils and sediments) and the type of source vegetation. That is, there is no simple model of interpretation. Therefore, for a given and specific set of long-chain  $n$ -alkanes that are dominated by  $n\text{-C}_{29}$  or  $n\text{-C}_{31}$ , it is almost impossible to determine whether they are derived from herbaceous or woody plants, or whether they represent forest or grassland vegetation.

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## Supporting Information

**Table S1** Detailed information on 62 surface soil samples collected from across eastern China, and composition of long-chain *n*-alkanes

**Table S2** Maximum carbon number of long-chain *n*-alkanes extracted from modern plants and references

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