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海北高寒草甸土壤有机碳同位素组成及 C_3/C_4 碳源的变化*

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摘　要:通过对高寒嵩草草甸土壤剖面不同深度(0～5 cm,5～15 cm,15～25 cm,25～35 cm,35～50 cm,50～65 cm)有机碳稳定性碳同位素的测定发现,土壤有机碳稳定性同位素($\delta^{13}C$)随土壤深度的增加而变大. 表层土壤(0～5 cm,定义为现代土壤)的 $\delta^{13}C$ 值最小,基本上接近现代植被的碳同位素特征. 在土层 5～10 cm 深度以下(粗略地定为古土壤),土壤有机碳稳定性同位素骤然上升,与表层土壤的同位素特征明显不同. 考虑到影响土壤碳同位素的诸多因素,通过稳定性碳同位素的质量平衡模型计算,得出初步结果:来自 C_4(或CAM)植物的碳源随土壤深度的增加而增大. 进一步推测,该地区植被可能经历由 C_4 植物占优势的群落向 C_3 植物占优势的群落演化的过程. 在这个过程中,大气碳同位素的变化和土壤有机质的形成过程(有机质淋溶过程)等也会引起土壤碳同位素的升高,因此质量平衡模型可能会过多地估算 C_4 组分,而低估 C_3 组分.

关键词:$\delta^{13}C$;C_3/C_4 碳源;高寒草甸
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Stable Carbon Isotopic Composition in Soil Organic Carbon and C_3/C_4 Source Variations at the Haibei Alpine Meadow

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Abstract: Stable carbon isotopes of organic matter originated from different soil layers (0～5 cm, 5～15 cm, 15～25 cm, 25～35 cm, 35～50 cm, 50～65 cm) were investigated in the Haibei Alpine Meadow Ecosystem Research Station of the Chinese Academy of Sciences. The preliminary results indicated that $\delta^{13}C$ values of soil organic matter increased with increased soil depth. $\delta^{13}C$ of soil organic carbon in 0～5 cm layer showed the lowest value, -25.09‰; while 50～65 cm soil layer possessed the lower $\delta^{13}C$ value, -13.87‰. Based on mass balance model of stable isotopes, it was proposed that the percentage of C_4 carbon source tend to increase with increased soil depth. The preliminary study indicated that alpine meadow might have undergone a successive process from C_4-dominated community to C_3-dominated one. However, changing $\delta^{13}C$ values in atmospheric CO_2 overtime and different processes of soil organic carbon formation (or eluviation) might somewhat contribute to increasing $\delta^{13}C$ values. In this case, mass balance model would underestimate C_3 community and overestimate C_4 community.

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Key words: $\delta^{13}C$; C_3/C_4 source; alpine meadow

Agren *et al.*[1] recently have pointed out that the isotopic composition of soil organic carbon (SOC) is a powerful characteristic of the development of soil organic matter. It is well established that the natural carbon isotope composition of soil organic matter varies with soil depth[2,3]. $\delta^{13}C$ values of SOC have been previously used to document shifts in community composition and distribution[4]. Stable carbon isotopes of soil organic matter was considered directly related to isotopic signatures of vegetations on it[5~10]. Different vegetations will generate different stable carbon isotopic signatures of soil organic carbon[11]. It is well known that C_3 and C_4 plants possess significantly different stable carbon isotopic signatures, and stable carbon isotopic ratios of C_3 plants range from - 17‰ to - 11‰ and C_4 plants from - 34‰ to - 25‰[12,13]. Because the $\delta^{13}C$ value of soil organic matter is approximately equal to that of the plant material from which it originates[14], the isotopic composition of soil organic carbon (SOC) can be used to indicate where vegetation has shifted from dominance by one photosynthetic pathway type to the other[2]. This difference in stable carbon isotopic ratios between photosynthetic types can be used to quantify the proportion of C_3 and C_4 species contributing to a mixture of plant material[15~17]. The $\delta^{13}C$ value of soil organic matter should also reflect the relative importance of C_3 and C_4 plants in the community. Theoretically, the $\delta^{13}C$ value of the soil organic matter should be identical to the existing vegetation at a site if (1) the existing vegetation has remained unchanged for a period equal to that of the oldest carbon in the soil profile; (2) the $\delta^{13}C$ value of atmospheric CO_2 has remained constant with time; (3) no fractionation of carbon isotopes has taken place in the soil as a result of either decomposition processes or differential preservation of plant biochemicals. If assumptions 2 and 3 hold true and there is a difference in the $\delta^{13}C$ value between the soil and the vegetation, then in all likelihood there has been some degree of compositional change in the C_3/C_4 biomass of the community[18]. Based on this acknowledgement, we can re-evaluate relative abundances of C_3 and C_4 plants in soil organic carbon even vegetation variation history using stable carbon isotopic patterns of soil derived from different soil depth.

Alpine meadow ecosystem, prevailing over Qinghai-Tibet Plateau, "the third pole of the world", made itself the ideal place for the research of structure, function of alpine meadow ecosystem. Qinghai-Tibet Plateau has undergone a long-term uplift and succession. To reveal vegetation properties is difficult via traditional measures. Preliminary study[19] revealed that no C_4 plants found at the alpine meadow. Stable carbon isotopes of paleosols would indicate the abundance of C_4 plants in vegetation history of alpine meadow. However stable carbon isotope has been used to investigate vegetation variations, there are still more problems to handle, such as relationship between carbon isotope of modern soil and vegetation ecology and carbon isotope fractionation during succession from paleosols to modern soils. In this study, $\delta^{13}C$ values of soil organic carbon were investigated to reveal vegetation variation history at the alpine meadow compared with $\delta^{13}C$ values of modern plants.

1 Materials and methods

1.1 Study location

The study was conducted in the Haibei Alpine Meadow Ecosystem Research Station (HAMERS) of Chinese Academy of Sciences. It was established in 1976 in order to understand the structure and function of alpine meadow ecosystem, form and development of bio-diversity, the adaptive and evolutionary strategies of species, and the impact of global changes on grassland ecosystem. The HAMERS has been expanding as a open field station of CAS in 1988, one of the key stations of the Chinese Ecosystem Research Network (CERN) in 1992, the unique research station of International Tundra Experiment (ITEX) in 1998, a member of

International Center for Integrated Mountain Development (ICMOD), and Northern Sciences Network (NSN) in 1999.

The HAMERS is situated in the northeast of Qinghai-Tibet Plateau at an altitude of 3 200 m, 37°29′～37°45′N, 101°12′～101°33′E, with an average annual temperature -1.7℃. There is no apparent four seasons with only warm season from May to October and cold season from November to April. The annual average air temperature is -1.7℃ with extremes of maximum at 27.6℃ and minimum at -37.1℃. During winter months, the average temperature can drop to -15℃ to -20℃ in highland area. In summer, the average temperature in the warmest month (July) is 14℃ to 22℃ in the valleys and 4℃ to 10℃ in the mountains. Average annual precipitation ranges from 426～860 mm, 80% of which falls in the short summer growing season from May to September. The annual average sunlight is 2 462.7 hours with 60.1% of total available sunshine.

The soil types of the HAMERS are dominated by mollic-gryic cambisols and matcryo-sod soil in three rich elements as nitrogen, phosphorus and potassium. They are characterized by higher organic matter content, a thinner soil layer, and lower mineralization rate.

1.2 δ^{13}C measurements of soil and plant organic carbon

Soil samples were collected from *Kobresia humilis* meadow using a push probe at 5～10 cm increments to a depth of 65 cm. Six sets of samples were collected randomly during the exuberant season (July to August) in 2002. Soil samples were separated from live roots and air-dried indoors to constant mass in an oven at 70℃, and ground to pass a 2 mm sieve. Inorganic carbon-free samples (samples for SOC δ^{13}C analysis were treated with 1 mol·L^{-1} HCl at 25℃ for three days to remove carbonate C) and finely grounded plant samples were dispatched to Finnigan MAT (Bermen, Germany) DELTA^{PLUS}XL isotopic ratio spectrometer under EAMS (Element analyzer and mass spectrometer) conditions. Interface between element-analysis meter and spectrometer is Conflow III (continuous flow III). Operation condition: oxidizing furnace temperature is 900℃, reducing furnace is 680℃, pillar temperature is 40℃. Standards consist of the Peedee Belemnite (PDB) formation from South Carolina, USA. The results are expressed in δ^{13}C relative to the PDB standard in the conventional δ per mil notation as follows:

$$\delta^{13}C = [(^{13}C/^{12}C)_s/(^{13}C/^{12}C)_{sta} - 1] \times 1000,$$

where $^{13}C/^{12}C$ are the isotopic ratios of sample (s) and PDB standard (sta). The overall (sample preparation plus analysis) analytical precision is ±0.2‰.

2 Results

2.1 δ^{13}C values of modern plants

One hundred and two plant species, representing 70 genus and 25 families have been examined. The results showed that the δ^{13}C values of all plant species measured had a narrow range from -28.24‰ to -24.84‰ and averaged at -26.51‰ (Table 1), which indicated no C_4 (or CAM) plants found at the alpine meadow, all plant species perform C_3 photosynthetic pathway.

Table 1 δ^{13}C values of modern plants grown at the alpine meadow

Family	Ranges of δ^{13}C value (‰)	Family	Ranges of δ^{13}C value (‰)
Boraginaceae (2)*	-27.44～-26.13	Primulaceae (2)	-27.12～-26.41
Chenopodiaceae (1)	-26.17	Ranunculaceae (8)	-27.09～-25.32
Compositae (13)	-28.24～-25.12	Rosaceae (6)	-26.51～-25.84
Cruciferae (3)	-27.52～-26.19	Rubiaceae (2)	-25.93～-25.74
Cyperaceae (10)	-27.63～-26.13	Salicaceae (1)	-25.82
Dipsacaceae (1)	-25.25	Scrophulariaceae (6)	-28.20～-24.87
Equisetaceae (1)	-25.32	Thymelaceae (1)	-24.97
Gentianaceae (12)	-28.11～-26.03	Umbelliferae (2)	-27.62～-25.36
Gramineae (11)	-27.45～-24.87	Violaceae (1)	-25.45
Iridaceae (1)	-26.47	Liliaceae (1)	-27.86
Labiatae (3)	-26.92～-25.79	Papaveraceae (3)	-28.22～-27.16
Leguminosae (6)	-27.75～-24.84	Plantaginaceae (1)	-26.13
Polygonaceae (4)	-27.74～-26.06	Average	-26.51

Note: * Data in parentheses indicate numbers of plant species measured.

2.2 $\delta^{13}C$ values of different soil layers at the alpine meadow

Table 2 showed that the 0-5 cm SOC was enriched with the heavier isotope ($\delta^{13}C$ = - 25.09‰) relative to the plant samples by approximately 1.4‰ (102 plants investigated showed C_3 photosynthetic pathway and possessed $\delta^{13}C$ values ranged from - 28.24‰ to - 24.84‰, and averaged at - 26.51‰). However, $\delta^{13}C$ values of paleosol (5～65 cm) organic carbon ranged from - 21.73‰ to - 13.87‰, which were significantly higher than the average $\delta^{13}C$ value of modern vegetation and modern soil (One-Sample Test, t = 4.914, df = 4, P = 0.008, and t= 3.941, df = 4, P = 0.017 respectively).

Table 2 $\delta^{13}C$ values of organic carbon in different soil layers at the alpine meadow

Soil layers (cm)	$\delta^{13}C$ values (‰)	SD
0～5	- 25.09 (6) *	0.04
5～15	- 21.73 (6)	0.25
15～25	- 21.65 (6)	0.07
25～35	- 20.53 (6)	0.17
35～50	- 18.93 (6)	0.11
50～65	- 13.87 (6)	0.10

Note :* Sample size

3 Discussion

3.1 Definition of modern soils and paleosols

We tentatively defined 0～5 cm layer as modern soil layer because their $\delta^{13}C$ values did not show significant difference between those of modern plants (One-Sample Test, t = - 1.419, df = 101, P = 0.159). The other 5～65 cm soil were defined as paleosol due to their significant higher $\delta^{13}C$ values compared with modern plant $\delta^{13}C$ values (One-Sample Test, t = - 40.233, df = 101, P = 0.000 ; One-Sample Test, t = - 41.147, df = 101, P = 0.159 ; One-Sample Test, t = - 54.082, df = 101, P = 0.159 ; One-Sample Test, t = - 72.560, df = 101, P = 0.159 ; One-Sample Test, t = - 130.997, df = 101, P = 0.159, respectively).

3.2 Basis of $\delta^{13}C$ values of soil to reveal vegetation variation history

Stable carbon isotopes of soil organic matter was directly related to isotopic composition of vegetations grown on it[7,8,10]. Different vegetations will result in different stable carbon isotopic signatures of soil organic matters[11]. $\delta^{13}C$ values of modern soil organic carbon and modern vegetation are similar even identical due to that modern soil organic carbon mainly derived from modern vegetation. The difference between them is related to microorganism respiration and mineralization after death of plants. Stable carbon isotopic fractionation during transformation of plant litter to soil organic matters should be concerned. It was reported that carbon fractionation caused by soil and microorganism respiration is very small[20], i.e., about 1‰～2‰, which would result in elevation of $\delta^{13}C$ values of modern soil organic carbon[18,20,21]. Recent study[22] demonstrated that stable carbon isotopic fractionation mainly occurred at the beginning of decomposition of plant litter and would remain stable after decomposition. Soil organic matters are composed of carbon derived from plant, bacteria and water organisms. Some studies[23,24] have showed that bacteria and water organisms contributed little carbon to soil organic matters and could be neglected. Based on these investigations, it was reasonable to propose that it was effective and objective to utilize carbon isotope of paleosols to recovery vegetation history.

3.3 Use of $\delta^{13}C$ values to reveal C_3/C_4 sources of soil organic carbon

Because soil organic carbon is originated from vegetation on it, the photosynthesis pathways of plants, air pressure, $\delta^{13}C$ values of atmosphere, temperature, solar radiation, and soil moisture will influence $\delta^{13}C$ values of soil organic carbon[25～28]. This provides us a basis to infer vegetation succession at the alpine meadow. After 5～15 cm soil layer, $\delta^{13}C$ values of soil organic carbon (paleosol layers) maintained steady and ranged from - 21.73‰ to - 13.87‰. The abrupt elevation of $\delta^{13}C$ values of soil organic carbon indicated that there must be some carbon source with higher $\delta^{13}C$ values adding to the deeper layers, i.e., C_4 or CAM sources. Based on mass balance model of stable carbon isotope, 2.78%, 25.03%, 25.56%, 32.98%, 43.58%, and 77.09% of carbon from C_4 or CAM sources would be incorporated into modern and paleosol organic carbon pool, respectively (Table 3). Modern soil layer (0～5 cm) at alpine meadow only contained 2.78% organic carbon from C_4 source, which was accordant with our previous work[19] and the results of Wang and Yang[29]. These data firmly indicated that modern soil organic carbon was mainly originated from C_3 plants, rather than C_4 plants as revealed by $\delta^{13}C$ values of soil organic carbon and modern vegetation. On the contrary, $\delta^{13}C$ values of soil organic carbon (beneath 5～15 cm layer) tended to increase with soil depths, proportion of C_4 contribution increased with depth reaching approximately 77.09 % at the 50～65 cm depth, which informed us that there might have been a dominant duration for C_4 plants and C_4 plants might have undergone a long time to decrease.

Table 3 Percentage of soil organic carbon derived from C_4 plants *

Soil layers(cm)	$\delta^{13}C$ values (‰)	C_3 plants (%)	C_4 plants (%)
0～5	-25.09	97.22	2.78
5～15	-21.73	74.97	25.03
15～25	-21.65	74.44	25.56
25～35	-20.53	67.02	32.98
35～50	-18.93	56.42	43.58
50～65	-13.87	22.91	77.09

Notes: * Calculation was based on the following equation (mass balance model): $\delta^{13}C_{soil} = \delta^{13}C_{C_4 plant} \times P + \delta^{13}C_{C_3 plant}((1-P)$, where P refers to percentage of C_4 plants as carbon source to soil organic carbon. Average $\delta^{13}C$ values of C_4 and C_3 plants were defined as -12.5‰ [11～13,30] and -26.51‰, respectively; while $\delta^{13}C$ value of soil organic carbon ($\delta^{13}C_{soil}$) were decreased by 1‰ due to fractionation during soil respiration, microorganism respiration, and mineralization[20].

3.4 Other factors influencing $\delta^{13}C$ values of soil organic carbon

As summarized in the introduction, several hypotheses have been proposed to explain changing $\delta^{13}C$ values with soil depth, including changing $\delta^{13}C$ values in atmospheric CO_2 over time and different processes of SOC formation. On this point, eluviation of soil organic carbon would, in certain degree, contribute to the elevation of stable carbon isotopic ratios of soil in deeper layers. Some researches[31] supports a C-mixing hypothesis (i.e., that $\delta^{13}C$ value in SOC are influenced by the mixing of new C inputs with existing and older SOC pools). Surface litter inputs (which have lower $\delta^{13}C$ values relative to SOC), in combination with subsurface C inputs from microbial decomposers may contribute to a shift in $\delta^{13}C$ values with soil depth. According to the fractionation hypothesis, SOC is enriched in $\delta^{13}C$ values as a result of discrimination against ^{13}C and preferential utilization of ^{12}C compounds by soil microorganisms during decomposition[2]. This discrimination most likely occurs during enzymatic reactions associated with metabolism by soil microorganisms. Assuming that kinetic isotope effects prevail at the presence of large available C pools, then compounds containing ^{12}C are more readily metabolized than those containing ^{13}C[32]. As a result, residual SOC becomes more enriched in ^{13}C[2]. Furthermore, the "Suess effect"[33], which is essentially a dilution of atmospheric $^{13}CO_2$ by $^{12}CO_2$ from fossil fuel combustion and biomass burning, could have contributed to lower $\delta^{13}C$ values in surface soil by lowering $\delta^{13}C$ values in plant, litter inputs[34]. The recent addition of CO_2 depleted in ^{13}C, from biomass burning and fossil fuel combustion, has changed the annual average $\delta^{13}C$ value of atmospheric CO_2 from -6.5‰ to about -7.8‰. The "Suess effect" could be partly responsible for changing $\delta^{13}C$ values with soil depth[3]. In this case, the mass balance model will underestimate C_3 community and overestimate C_4 community. There is still room remained for evaluating plant community variation at the alpine meadow.

In summary, analysis on stable carbon isotope values of soil organic carbon will provide direct chemical evidence, which will allow acceptance or rejection of the hypothesis that C_3 plants might have replaced C_4 plants at the Haibei Alpine Meadow Ecosystem. In this study, $\delta^{13}C$ values of SOC increased with depth in a predictable pattern that serves as a surrogate for different patterns of C_3/C_4

source into soil organic matter. The data show that SOC $\delta^{13}C$ is a time integrated signal and provides evidence on the temporal C_3 and C_4 community dynamics where these plant communities co-exist.

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