干旱、半干旱区土壤蚯蚓稳定性碳同位素组成与轮作模式的关系

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摘要:对 10 个不同耕地生境中蚯蚓(*Lumbricus terrestris*)的稳定性碳同位素进行了分析,研究了豫西洛阳干旱、半干旱地区土地轮作模式和耕作历史。结果表明,耕地表层土壤(0 ~30cm)中蚯蚓的 $\delta^{13}C$ 介于 -18.3‰和 -25.6‰之间,变化幅度较大。经稳定性同位素质量平衡模式计算,10 个生境中蚯蚓取食 C_3 作物的比例在 40.1% 和 99.4% 之间波动,蚯蚓的取食生态受到土地上 C_3/C_4 作物轮作模式的影响。C_4 作物轮作频率与蚯蚓稳定性碳同位素比值之间呈正相关。土壤动物的稳定性碳同位素比值可较客观地反映出耕作制度和轮作模式。

关键词:稳定性碳同位素;蚯蚓;轮作模式;C_3 植物;C_4 植物

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Stable carbon isotopes of earthworms to reveal dominant C_3 and C_4 crop sources and different crop rotation systems in arid and semi-arid areas

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Abstract: Stable carbon isotopes of earthworms in ten different habitats were analyzed to reveal crop rotation history in arid areas, Luoyang, Henan Province, China. The results of the present study indicated that $\delta^{13}C$ values of earthworms ranged from -18.3‰ to -25.6‰ and show great variations. Based on the mass balance theory of stable isotopes, the distribution percentages of C_3 crops incorporated into earthworms' diets are ranged from 40.%1 to 99.4%, respectively in the ten different habitats. The above data suggest that digested diets of earthworms are dependent on different C_3/C_4 crop rotation model. We found a close correlation between C_4 crop percentage revealed from $\delta^{13}C$ values of earthworms and rotation frequencies by C_4 corns based on the oral interview with farmers. $\delta^{13}C$ values of earthworms basically reflected the carbon isotopes of winter wheat in habitats never undergoing rotation by corn in recent 10 years. However, $\delta^{13}C$ values of earthworms become significantly less negative in habitats where alternative rotation by corn in recent 10 years. It seemed that $\delta^{13}C$ of earthworms actually reflected cropping system in the research habitats.

Key Words: stable carbon isotope; earthworm; rotation system; C_3 crop; C_4 crop

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Soil fauna play an important role in nutrient dynamics and earthworms are considered to be important ecosystem engineers in terrestrial ecosystems [1]. Earthworms in particular have a strong effect on both soil properties and biogeochemical cycles through their interactions with microbial populations. As the earthworm passes through the soil it eats, decomposes, and deposits the castings. Their burrowing activity improves soil structure, fertility, soil aeration, porosity, and permeability while creating holes for root growth and allowing air, water and fertilizer to enter the soil. Tests have shown that crops grown in earthworm-inhabited soil increased yields from 25% to over 300% than in earthworm-free soil [2]. Knowledge of the feeding ecology of earthworms is essential for understanding many of the ecosystem functions performed by these important soil invertebrates, in particular their role in biogeochemical cycling [3]. Earthworm feeding ecology has attracted much scientific inquiry during the last decade [4,5].

Isotopic techniques have proved to be a powerful tool in soil ecology studies by proving valuable information about nutrition flux and ecosystem levels. The stable isotope approach is based on the fact that naturally occurring stable isotope ratios in consumer tissues can be related to those in consumers′ diets [6,7]. Thus, isotope measurement of consumers′ tissue can reveal information about their ingested foods and vegetation characteristics and history in ecosystems that are relatively simple. Stable isotope techniques at natural abundance levels measure net assimilated diets rather than just ingested diets; they are relatively sensitive and accurate, analytically automated and have proven particularly to be useful for the analysis of complex detrital food webs and animals which are hard to observe [8]. However, several researchers used the stable isotopes of organic C to identify types of plants or their resulting soil organic matter fractions as sources of assimilated C in earthworms [9–12].

As the carbon isotopic composition of animals is correlated with that of plants, their muscles have the potential to provide an average isotopic signal for particular environments [3–15] due to its fast turnover rate. Terrestrial plants perform three distinct photosynthetic pathways (C_3, C_4 and CAM) that produce characteristic $\delta^{13}C$ tissue values [6]. Plants using the C_4 (Hatch-Slack) pathway have markedly higher $\delta^{13}C$ values (−17‰—−11‰) than plants employing the C_3 pathway (−34‰—−25‰) [7–19]. Mean $\delta^{13}C$ values of −13.1‰ ± 1.2‰ for C_4 plants and −27.1‰ ± 2.0‰ for C_3 plants are reported for a large number of species grown under a variety of conditions [6]. These distinct isotopic signatures in C_3 and C_4 plants are passed on to the tissues of consumers with a small change or fractionation related to biochemical processes within the animals, which provide a concrete basis for identify C_3 and C_4 plant contributions to given ecosystems [20].

Human activities are expected to alter the agricultural biogeochemical cycle and land use. These changes in land use can have a substantial influence upon the soil fauna and carbon flow in soil ecosystem. The objectives of this work were to establish stable carbon isotope patterns of earthworms and C_3 and C_4 crop sources assimilated by earthworms in ten different arid habitats, and tentatively to evaluate tillage effect on soil fauna and to reveal different historical tillage (rotation) systems based on isotopic analyses.

1　Materials and methods

1.1　Sample preparation

There are two basic types of worms, those that feed on the surface and those that feed in the subsurface. The surface feeders were collected because they mainly eat plant residue. Earthworms were collected in 0—30cm soil deeps in ten different arid habitats in Luoyang, Henan province, Chian. The uniform species (*Lumbricus terrestris*) were selected for isotopic analyses. Captured earthworms were starved indoors for 24—48 hours to ensure that they completely egest the residue in their stomach and gut [20]. The whole body of earthworms was diluted with 5% HCl to get rid of the interference of inorganic matters [20].

In Luoyang areas, peasants have formed a long-term tillage system in order to use their land effectively. C_3

(wheat) and C_4 (mainly corn and seldom millet) crops were extensively rotated in some areas, i. e., from October to June for winter wheat or other C_3 crops and from July to September for corn or millet. Ten croplands were selected for earthworm collection and for subsequent isotopic analysis. The croplands were sorted into three types based on interviewing with local peasants: (1) never rotation by corn in recent 10 years; (2) occasional rotation by corn (2 −4 times) in recent 10 years and (3) alternative rotation by corn (5 −10 times) in recent 10 years, and they were symbolized as 1, 2 and 3, respectively, as indicated in Table 1.

1.2 Measurement of stable carbon isotopes

Muscle samples of 3 −5 earthworms captured in each habitat were mixed and air dried indoors to constant mass in an oven at 70℃ for 48 h, ground finely, and dispatch to isotope ratio spectrometer under EAMS (element-analysis meter and spectrometer) condition [10]. Interface between element-analysis meter and spectrometer is ConFlow III. Operation condition for the oxidizing furnace was a temperature of 900℃, reducing furnace at 680℃, pillar temperature at 40℃. The resulting CO_2, were purified in a vacuum line and injected in a Finnigan MAT DELTA^{PLUS} XL spectrometer (Finnigan Mat, Bremen, Germany) fitted with double inlet and collector systems. The results are expressed in $\delta^{13}C$ relative to the standards in the conventional δ per mil notation as follows [10]:

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

where $^{13}C : {}^{12}C$ are the isotopic ratios of sample and PDB (Peedee Belemnite formation from South Carolina, USA) standard. The overall (sample preparation plus analysis) analytical precision is ±0.2‰.

1.3 C_3 and C_4 crop sources distribution

C_3 and C_4 crop sources distribution (CSD) can be retrieved from stable carbon isotopic values of earthworms as shown in the following equation [11]:

$$CSD_{C4}(\%) = (\delta^{13}C_{earthworm} - E - \delta^{13}C_{C3}) / (\delta^{13}C_{C4} - \delta^{13}C_{C3}) \times 100$$

where CSD_{C4} indicates relative percentage of C_4 plants; $\delta^{13}C_{earthworm}$ refers to average stable isotopic value of earthworm muscles; $\delta^{13}C_{C3}$ and $\delta^{13}C_{C4}$ represent average values of C_3 winter wheat (−26.74‰) and C_4 corn (−14.36‰) respectively (measured in this study); E indicates the fractionation factor between diets and muscles, where 1.05 is used to offset isotopic enrichment during carbon assimilation [12].

2 Results and analysis

The results of this study show that $\delta^{13}C$ values of earthworms ranged from −18.3‰ to −25.6‰ and show great variations (Table 1). Based on the mass balance theory of stable isotopes, the distribution percentages of C_3 crops to earthworms' diets are ranged from 40.1 and 99.4%, respectively in the different habitats. The above data suggest that digested diets of earthworms are dependent on different C_3/C_4 crop rotation model. Based on the oral interview with farmers, we found a close correlation between C_4 crop percentage revealed from $\delta^{13}C$ values of earthworms and rotation frequencies by C_4 corns ($r^2 = 0.910$, $df = 10$, $P < 0.01$). $\delta^{13}C$ values of earthworms basically reflected the carbon isotopes of winter wheat in habitats never undergoing rotation by corn in recent 10 years (Table 1). However, $\delta^{13}C$ values of earthworms become significantly less negative in habitats where alternative rotation by corn in recent 10 years.

3 Discussion

Patterns of muscle $\delta^{13}C$ values can be attributed to inclusion of varying amounts of C_3 and C_4 carbon sources in animal diets. The variability in muscle $\delta^{13}C$ values observed among the earthworms suggests that stable carbon isotope analyses can contribute to dietary information and crop distribution and rotation system in the different habitats. The more negative muscle $\delta^{13}C$ values reveal that earthworms rely more on C_3 plants than C_4 types. The results indicate that digested diets of earthworms are dependent on different food supply and C_3/C_4 crop rotation model (Table 1), which firmly reflects our interview with farmers who possess the farmland. The stable isotope approach is based on the fact that naturally occurring stable isotope ratios in consumer tissues can be related to those in consumers' diets[6]. Stable carbon isotopic patterns in muscles can reveal food base information of animals in given ecosystems. It seemed that earthworms actually reflected cropping system in the research habitats. The results also indicated that farmland seldom undergone high frequency rotation by corn in arid areas where irrigation is not available, because farmers will usually take the risk of few precipitation in summer or/and autumn and encounter lower yields of autumn crops and even severe reduction of winter wheat output in the next year. Lower level of rotation by corn proved to be a trade-off of farmers in arid areas.

Table 1　The calculated percentages of C_3 and C_4 crops as revealed by $\delta^{13}C$ values of earthworms in different habitats

Habitats	$\delta^{13}C$ (‰)	SD (‰)	C_3 crops (%)	C_4 crops (%)	Rotation frequency
Ⅰ	-22.48 (8)	0.06	74.07	25.93	2
Ⅱ	-18.27 (8)	0.11	40.06	59.94	3
Ⅲ	-25.62 (8)	0.24	99.43	0.565	1
Ⅳ	-23.25 (8)	0.16	80.29	19.71	2
Ⅴ	-20.14 (8)	0.26	55.17	44.83	3
Ⅵ	-24.68 (8)	0.14	91.84	8.158	2
Ⅶ	-25.58 (8)	0.18	99.11	0.889	1
Ⅷ	-19.43 (8)	0.12	49.43	50.57	3
Ⅸ	-24.28 (8)	0.21	88.70	11.30	2
Ⅹ	-25.01 (8)	0.17	94.55	5.45	2

However, there might be an uncertainty in the present study. First, stable carbon isotopic patterns of other C_3 crops, such as soybean and peanut and C_4 crops as millet are not measured in this study, because of their small planting areas. Their isotopic values are theoretically estimated as the average value of winter wheat and corn, respectively. On the other hand, turnover rate of stable carbon isotopes and enrichment factors of earthworms are also not investigated due to condition limitation. But, as mentioned above, mean $\delta^{13}C$ values of $-13.1‰ \pm 1.2‰$ for C_4 plants and $-27.1‰ \pm 2.0‰$ for C_3 plants are reported for a large number of species grown under a variety of conditions[6], and great variations among C_3 or C_4 photosynthetic groups are seldom detected in the same growing conditions. The steady enrichment of 1.05‰ was used in this study to offset the trophic level enrichment, because the changes in, or fractionation of, stable carbon isotope ratios occur with trophic level and are often of the order of 1‰—2‰[23-26]. The whole body stable carbon isotopic data of earthworms suggest that it is reasonable to use earthworms to reveal the abundance and distribution of different photosynthetic pathway crops. Consequently, stable carbon isotopic data of soil fauna (especially for earthworms) could be utilized to illustrate energy and nutrition flow within the agricultural ecosystems.

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