



Modeling trophic positions of the alpine meadow ecosystem combining stable carbon and nitrogen isotope ratios

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Abstract

Stable carbon and nitrogen isotope ratios of single tissues or whole bodies were analyzed to establish trophic positions of main consumers living at the alpine meadow ecosystem in the Tibetan Plateau. The results demonstrated that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of vertebrates showed great variations and ranged from -26.83 to -22.51% and from 2.33 to 8.44% , respectively. Plateau pika, root vole, plateau hare, infants of rodents and hatchlings of passerine bird species had the lowest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of omnivorous and insectivorous birds and amphibians showed intermediate. Carnivorous species, steppe polecat and Upland buzzard, and omnivorous Robin accentor and White wagtail possessed extremely higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Omnivorous birds captured in earlier year had significantly less negative $\delta^{13}\text{C}$ and greater $\delta^{15}\text{N}$ values than those captured later. Based on steady angular enrichment between trophic levels, an “alpha and vector model” combining $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values was introduced to reveal trophic positions, the results indicated that Tibetan sheep, Tibetan yak, plateau pika, root vole, plateau hare, infants of small rodents showed the lowest trophic positions (TP 1.81–2.38). While omnivorous and insectivorous birds, their hatchlings and amphibians showed intermediate trophic positions (TP 2.06–2.89), carnivorous species steppe polecat and Upland buzzard, migrant birds possessed extremely higher trophic positions (TP 2.89–3.05). The isotopic investigation of organisms and the introduced “alpha and vector model” successfully demonstrated the same trophic positions and diet prediction of consumers as nitrogen enrichment model at the alpine meadow ecosystem. Besides of this information, the “alpha and vector model” can also be incorporated into multiple isotope signatures to infer trophic relationships. This angular enrichment model has the potential to address basic ecological questions, such as trophic structure, trophic dynamics, and energy flow in other terrestrial ecosystems if properly handled.

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Keywords: Stable carbon and nitrogen isotopes; Alpha and vector model; Trophic relationships; Alpine meadow ecosystem; Tibetan Plateau

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1. Introduction

The stable isotope approach is based on the fact that naturally occurring stable isotope ratios in consumer tissues can be related to those in consumer's diets (DeNiro and Epstein, 1978, 1981). Changes in, or fractionation of stable isotope ratios ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$) occur with trophic level and are of the order of 0–2 and 2–5‰, respectively (Hobson and Clark, 1992; Hilderbrand et al., 1996). Thus, isotope measurement of consumers' tissue can reveal information about their assimilated foods and about trophic positions in systems that are relatively simple and do not involve multiple isotopic inputs. Elevations in an animal's $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ relative to those of the community food base have thus been used to infer a consumer's trophic distance from that food base (Schoeninger and DeNiro, 1984). Wada et al. (1987) have used this approach studying lower animal trophic levels in the Antarctic Ross Sea. On average, the $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios of predators' muscles are increased by 0–2 and 2–5‰ compared with their prey (Hobson and Clark, 1992; Szepanski et al., 1999). In top predators, therefore, the concentration of ^{13}C and ^{15}N are at a maximum. Due to the stepwise ^{13}C and ^{15}N enrichment with increasing trophic level, the ^{13}C and ^{15}N content of predator can be used as time-integrated indicator of their trophic position and relationships. A trophic position-based approach to representing trophic structure incorporates omnivore and weights feeding links according to their relative energetic importance, thereby representing realized trophic structure (Kling et al., 1992). Use of trophic position is likely to improve our ability to model and understand ecosystem process and food web dynamics.

However, lots of information addressing $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and their trophic relationships are constrained to marine ecosystems (Rau et al., 1983; Wada et al., 1987; Hobson and Welch, 1992) and little touches on terrestrial ecosystems (Hilderbrand et al., 1996). Alpine meadow ecosystem, prevailing over Qinghai-Tibetan Plateau, "the third pole of the world", made itself the ideal place of the research of structure, function of alpine meadow ecosystem. However, there is no report on trophic relationships among the ecosystem with the application of ^{13}C and ^{15}N values measurement at the Haibei Alpine Meadow Ecosystem Research Station (HAMERS) of the Chinese Academy

of Sciences (CAS). Our objective was to analyze a single tissue or whole body (i.e., hatchlings and amphibians) to better establish the relationship among animals using the combination of stable carbon and nitrogen isotopes as one indicator, i.e., "alpha and vector model".

2. Methods

2.1. Study area

The study was carried out at the Haibei Alpine Meadow Ecosystem Research Station (HAMERS) of the Chinese Academy of Sciences (CAS). It was established in 1976 in order to understand the structure and function of alpine meadow ecosystem, form and development of biodiversity, the adaptive and evolutionary strategies of species, and the impact of global changes on grassland ecosystem. The HAMERS is located in the region of the Tibetan Plateau, in a large valley oriented NW-SE surrounded on all sides by the Qilian Mountains with N latitude $37^{\circ}29'$ – $37^{\circ}45'$ and E longitude $101^{\circ}12'$ – $101^{\circ}33'$. The average altitude of mountainous area is 4000 m above sea level and 3200 m for the valley area. The climate of the HAMERS is dominated by the Southeast monsoon and the higher-pressure system of Siberia. It has a continental monsoon type climate, with severe and long winters and short cool summers. The average air temperature is -1.7°C with extreme maximum of 27.6°C and minimum -37.1°C . During winter months, the average temperature drops to -15 to -20°C in highland area; during summer, the temperature in the warmest month (July) averages 14 – 22°C in the valleys and 4 – 10°C in the mountains. Average annual precipitation ranges from 426 to 860 mm, 80% of which falls in the short summer growing season from May to September. The annual average sunlight is 2462.7 h with 60.1% of total available sunshine. Vegetation was characterized by alpine shrub, alpine meadow, and swamp meadow. The research site was roughly confined in alpine meadow, with *Kobresia humilis* as dominant species and *Polygonum viviparum*, *Carex atro-fusca*, *Saussurea superba*, *Elymus nutans* and *Gentiana straminea* as sub-dominant species. The HAMERS has been expanding as a field open station of CAS in 1988, one of the key stations of the Chinese Ecosystem Research Network (CERN) in 1992, the

unique research stations of International Tundra Experiment (ITEX) in 1998, a member of International Center for Integrated Mountain Development (ICIMOD), and Northern Sciences Network (NSN) in 1999.

2.2. Analysis of stable carbon and nitrogen isotopes

Samples were collected from individuals of vertebrate species for isotope analysis within the station. All samples (the appropriate institutional approval number is no more than 5) were collected during April to August 2002. Muscle samples were air dried indoors to constant weight in an oven at 70 °C for 48 h, ground finely, and dispatch to isotope ratio spectrometer under EAMS (element-analysis meter and spectrometer) condition. Interface between element-analysis meter and spectrometer is ConFlow III. Operation condition: oxidizing furnace temperature is 900 °C, reducing furnace is 680 °C, pillar temperature is 40 °C. The resulting CO₂ and N₂ were purified in a vacuum line and injected in a Finnegan MAT DELTA^{PLUS}XL spectrometer (Finnegan Mat, Bremen, Germany) fitted with double inlet and collector systems. The results are expressed in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ relative to the standards in the conventional δ per mil notation as follows:

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

$$\delta^{15}\text{N} = [(^{15}\text{N}/^{14}\text{N})_{\text{sample}} / (^{15}\text{N}/^{14}\text{N})_{\text{standard}} - 1] \times 1000$$

where $^{15}\text{N}/^{14}\text{N}$ are the isotopic ratios of sample and standard (atmospheric nitrogen); $^{13}\text{C}/^{12}\text{C}$ 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 $\pm 0.2\text{‰}$.

2.3. Model description for estimation of trophic positions

It is well known that the concentration of stable carbon and nitrogen isotopes increase with trophic levels. In this study, two isotope signatures, i.e., $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were measured. In bi-dimensional plane one species is represented by a point for the dual isotope signatures and form a vector from original point. The angle between the vector and the $\delta^{13}\text{C}$ axis is called α and

calculated from arctangent $|\delta^{15}\text{N}/\delta^{13}\text{C}|$ (we called it as “alpha and vector method”). In tridimensional or multiple dimensional plane if multiple isotopes are introduced, the α could also be figured out. Consequently, primary producers and primary consumers will form an angle called $\alpha_{\text{primary producer}}$ and $\alpha_{\text{primary consumer}}$, respectively. Consequently, isotope signature of one given consumer can also form an angle called α_{consumer} . Fractionation of stable isotope ratios ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$) occurs with trophic level and is of some order in marine ecosystem (Hobson and Clark, 1992; Hilderbrand et al., 1996). Despite of few studies on fractionation of stable isotopes in terrestrial ecosystems, we found isotopic enrichments of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ along trophic levels based on stomach content analyses in this study, which are about $1.15 \pm 0.50\text{‰}$ (calculated through three rodent species and one insect species living at the HAMERS and their known isotopic diets or stomach contents) and $5.27 \pm 0.50\text{‰}$ (calculated through three rodent species and two ruminant species and their known isotopic diets or stomach contents) (Table 1). The average $\delta^{13}\text{C}$ of primary producers is about $-26.51 \pm 0.72\text{‰}$ and calculated through 102 plant species grown at the HAMERS (Table 2). The average of $\delta^{15}\text{N}$ is $-1.388 \pm 0.90\text{‰}$ and calculated through 11 N₂-fixing and non-N₂-fixing plant species (Table 3). Because of great variations in stable carbon and nitrogen isotope signatures of primary producers and differences between stomach contents and average vegetation, primary consumers (some herbivorous small mammals) are treated as the baseline for trophic position estimation. Based on the stable isotopic patterns of primary consumers ($\delta^{13}\text{C} = -25.31 \pm 0.49\text{‰}$, $\delta^{15}\text{N} = 3.34 \pm 0.57\text{‰}$ for small mammals) and enrichment of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ along trophic levels ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of primary producers were increased by 1.15 and 5.27‰, respectively), we obtained an angular enrichment with trophic levels against isotope signatures of primary consumers, $\Delta\alpha$, i.e., $19.62 - 7.52 = 12.10^\circ$, which stands for an angular enrichment along trophic levels (Fig. 1). Trophic position of one given animal is calculated according to the following equation:

$$\text{TP} = 2 + (\alpha_{\text{consumer}} - \alpha_{\text{primary consumer}}) / \Delta\alpha$$

where TP represents trophic position of a given species; $\alpha_{\text{primary consumer}}$ is equal to 7.52° .

Table 1
Stable carbon and nitrogen isotope ratios of some animals and their stomach contents

Common names	$\delta^{13}\text{C}$ (‰) (average)	S.D.	$\delta^{15}\text{N}$ (‰) (average)	S.D.	Sample sizes
Root vole	-25.48 (-26.45) ^a	0.06	2.33 (-1.92)	0.06	4
Plateau hare	-24.67 (-26.16)	0.02	2.51 (-1.87)	0.02	2
Plateau pika	-24.77 (-26.77)	0.02	3.08 (-1.81)	0.02	3
Tibetan yak	-25.50 (-)	0.03	4.30 (-1.55)	0.03	2
Tibetan sheep	-24.92 (-)	0.10	5.34 (-1.66)	0.04	2
Meadow worm	-25.23 (-25.82)	0.05	2.71 (-)	0.24	4

^a Numbers in parentheses are stable carbon and nitrogen isotope ratios of stomach contents.

Table 2
 $\delta^{13}\text{C}$ values of modern plants grown at the alpine meadow

Family	Ranges of $\delta^{13}\text{C}$ value (‰)	Family	Ranges of $\delta^{13}\text{C}$ value (‰)
Boraginaceae (2) ^a	-27.44 to -26.13	Primulaceae (2)	-27.12 to -26.41
Chenopodiaceae (1)	-26.17	Ranunculaceae (8)	-27.09 to -25.32
Compositae (13)	-28.24 to -25.12	Rosaceae (6)	-26.51 to -25.84
Cruciferae (3)	-27.52 to -26.19	Rubiaceae (2)	-25.93 to -25.74
Cyperaceae (10)	-27.63 to -26.13	Salicaceae (1)	-25.82
Dipsacaceae (1)	-25.25	Scrophulariaceae (6)	-28.20 to -24.87
Equisetaceae (1)	-25.32	Thymelaceae (1)	-24.97
Gentianaceae (12)	-28.11 to -26.03	Umbelliferae (2)	-27.62 to -25.36
Gramineae (11)	-27.45 to -4.87	Violaceae (1)	-25.45
Iridaceae (1)	-26.47	Liliaceae (1)	-27.86
Labiatae (3)	-26.92 to -25.79	Papaveraceae (3)	-28.22 to -27.16
Leguminosae (6)	-27.75 to -24.84	Plantaginaceae (1)	-26.13
Polygonaceae (4)	-27.74 to -26.06	Average	-26.51

^a Data in parentheses indicate numbers of plant species measured.

Table 3
 $\delta^{15}\text{N}$ values of some dominant grasses grown at the alpine meadow ecosystem

Species	$\delta^{15}\text{N}$ (‰)	Species	$\delta^{15}\text{N}$ (‰)
<i>Kobresia humilis</i>	-2.88	<i>Oxytropis ochrocephala</i>	-1.84
<i>Carex atrofusca</i>	-0.75	<i>Oxytropis kansuensis</i>	-1.67
<i>Gentiana straminea</i>	0.54	<i>Gueldensaedtis diversifolia</i>	-1.98
<i>Saussurea superba</i>	-0.79	<i>Astragalus adsurgens</i>	-1.80
<i>Elymus nutans</i>	0.40	<i>Thermopsis lenceolata</i>	-2.20
<i>Polygonum viviparum</i>	-2.30		

3. Results

3.1. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the species living at the HAMERS

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of 42 vertebrate samples were measured and shown in Table 4. Their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values showed great variations and ranged from -26.83‰ of hatchlings of Black redstart to -22.51‰ of White wagtail and from 2.33‰ of root vole captured in May 2002 to 8.44‰ of Robin accentor, respectively. Plateau pika, root vole, plateau rabbit, infants of rodents

and hatchlings of bird species had the lowest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, i.e., from -26.83 to -25.04‰ and 2.33 to 5.27‰, respectively (Table 4). While $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of omnivorous and insectivorous birds, Chinese forest frog, and Chinese big toad showed intermediate. Carnivorous species Steppe polecat and Upland buzzard, and omnivorous Robin accentor and White wagtail possessed extremely higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (from -23.09 to -22.51‰ and 8.09 to 8.44‰, respectively) (Table 1). Omnivorous birds captured in earlier the year had significantly less negative $\delta^{13}\text{C}$ and greater $\delta^{15}\text{N}$ values than those captured later respec-

Table 4
Table 1 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of species living at the HAMERS

Common names	Scientific names	Sample size	$\delta^{13}\text{C}$ (‰)	S.D.	$\delta^{15}\text{N}$ (‰)	S.D.	Trophic position ^a	Trophic position ^b
Root vole (02.05) ^c	<i>Microtus oeconomus</i>	4	-25.48	0.01	2.33	0.06	1.81	1.81
Plateau hare (02.08)	<i>Lepus oiostolus</i>	2	-24.67	0.11	2.51	0.02	1.86	1.84
Plateau pika (02.07)	<i>Ochotona curoniae</i>	3	-24.77	0.04	3.08	0.02	1.96	1.95
Root vole (02.07)	<i>Microtus oeconomus</i>	3	-25.92	0.44	3.25	0.03	1.97	1.98
Plateau pika (02.05)	<i>Ochotona curoniae</i>	4	-25.07	0.55	3.24	0.04	1.99	1.98
Twite (hatchling) (02.07)	<i>Acanthis flavirostris</i>	2	-26.69	0.10	3.53	0.28	2.00	2.04
Horned lark (hatchling) (02.07)	<i>Eremophila alpestris</i>	2	-26.08	0.32	3.78	0.11	2.06	2.08
Plateau pika (infant) (02.07)	<i>Ochotona curoniae</i>	3	-24.76	0.28	3.41	0.25	2.03	2.01
Root vole (infant) (02.07)	<i>Microtus oeconomus</i>	3	-25.99	0.14	3.64	0.18	2.04	2.06
Tibetan yak (02.06)	<i>Bos grunniens</i>	2	-25.50	0.03	4.30	0.03	2.17	2.18
Horned lark (02.07)	<i>Eremophila alpestris</i>	3	-24.48	0.08	4.90	0.15	2.31	2.30
Hodgson's Pipit (hatchling) (02.08)	<i>Anthus roseatus</i>	1	-25.78	-	4.95	-	2.28	2.31
Small Skylark (hatchling) (02.07)	<i>Alauda gulgula</i>	2	-26.52	0.07	4.98	0.02	2.26	2.31
Plateau zokor (02.04)	<i>Myospalax fontanierii</i>	4	-25.78	0.36	5.27	0.24	2.33	2.37
Twite (02.07)	<i>Acanthis flavirostris</i>	2	-26.25	0.13	5.32	0.12	2.33	2.38
Tibetan sheep (02.06)	<i>Ovis ammon hodgsoni</i>	2	-24.92	0.10	5.34	0.04	2.38	2.38
Yellow-headed Wagtail (hatchling) (02.07)	<i>Motacilla citreola</i>	2	-26.62	0.21	5.50	0.16	2.34	2.41
Long-billed Calandra lark (hatchling) (02.07)	<i>Melanocorypha</i>	3	-24.26	0.12	5.78	0.07	2.49	2.46
Long-billed calandra lark (02.07)	<i>Melanocorypha</i>	4	-24.54	0.32	6.01	0.02	2.52	2.51
Small Skylark (02.05)	<i>Alauda gulgula</i>	2	-23.76	0.32	6.00	0.11	2.55	2.50
Przevalski's Rosefinch (02.07)	<i>Urocynchramus</i>	1	-24.35	-	6.19	-	2.56	2.54
Chinese Forest Frog (02.07)	<i>Rana temporaria</i>	4	-24.11	0.02	6.18	0.04	2.57	2.54
Przevalski's Rosefinch (hatchling) (02.07)	<i>Urocynchramus</i>	2	-25.04	0.15	6.43	0.03	2.57	2.59
Black Redstart (02.07)	<i>Phoenicurus ochruros</i>	2	-23.83	0.04	6.67	0.00	2.67	2.63
Tree Sparrow (02.07)	<i>Passer montanus</i>	3	-24.27	0.15	7.00	0.18	2.71	2.69
Hodgson's pipit (02.08)	<i>Anthus roseatus</i>	1	-24.16	-	7.05	-	2.72	2.70
Red-billed Chough (hatchling) (02.06)	<i>Pyrrhocorax</i>	2	-25.39	0.10	7.10	0.05	2.67	2.71
Robin Accentor (hatchling) (02.07)	<i>Prunellidae</i>	2	-25.47	0.13	7.23	0.09	2.69	2.74
Horned Lark (02.04)	<i>Eremophila alpestris</i>	3	-24.18	0.28	7.35	0.07	2.78	2.76
Hume's Ground Jay (02.07)	<i>Pseudopodoces humilis</i>	5	-24.25	0.28	7.37	0.22	2.78	2.76
Yellow-headed Wagtail (02.07)	<i>Motacilla citreola</i>	3	-23.46	0.08	7.56	0.16	2.86	2.80
Upland Buzzard (02.04)	<i>Buteo hemilasius</i>	2	-22.80	0.69	7.53	0.08	2.89	2.80
Hume's Ground Jay (hatchling) (02.06)	<i>Pseudopodoces</i>	2	-24.60	0.21	7.59	0.04	2.80	2.81
Brandt's Rosy Finch (02.04)	<i>Leucosticte brandti</i>	3	-24.08	0.30	7.77	0.21	2.86	2.84
Black Redstart (hatchling) (02.07)	<i>Phoenicurus ochruros</i>	3	-26.83	0.14	7.82	0.11	2.72	2.85
Twite (02.05)	<i>Acanthis flavirostris</i>	5	-24.28	0.20	7.90	0.16	2.87	2.87
White Wagtail (02.04)	<i>Motacilla citreola</i>	3	-22.51	0.11	7.98	0.08	2.99	2.88
Tree Sparrow (02.04)	<i>Passer montanus</i>	3	-24.16	0.07	7.97	0.09	2.89	2.88
Chinese big toad (02.07)	<i>Bufo bufo</i>	1	-24.22	-	8.01	-	2.89	2.89
Steppe Polecat (02.07)	<i>Mustela eversmanni</i>	2	-23.90	0.18	8.09	0.11	2.92	2.90
Robin Accentor	<i>Prunellidae</i>	2	-24.90	0.07	8.44	0.15	2.93	2.97
Yellow-browed Warbler (02.05)	<i>Phylloscopus inornatus</i>	1	-23.91	-	8.82	-	3.05	3.04

^a Calculated from "alpha and vector model".

^b Calculated from nitrogen enrichment model.

^c Numbers in parentheses are sampling dates (year and month).

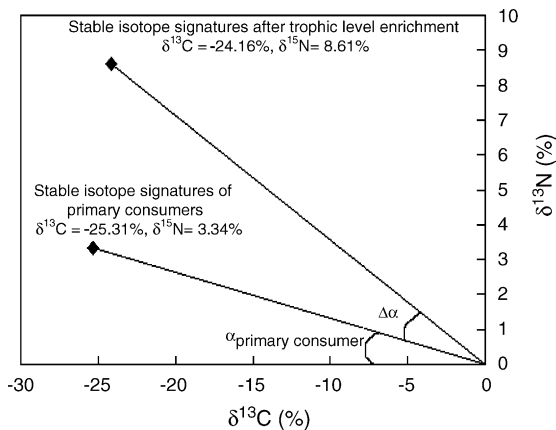


Fig. 1. A graphic illustration for modeling trophic positions with combination of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

tively. While herbivorous mammals Tibetan sheep and Tibetan yak possessed extremely higher $\delta^{13}\text{C}$ values (from -23.47 to -22.51‰) (Table 4).

3.2. Trophic position of vertebrates living at the HAMERS

Based on the simple model incorporating diet-tissue stable carbon and nitrogen isotope fractionations established through several animals and known isotopic diets, the results indicated that Tibetan sheep, Tibetan yak, plateau pika root vole, plateau rabbit infants of rodents showed the lowest trophic positions (TP 1.81–2.38) for muscles. While omnivorous and insectivorous birds and their hatchlings, Chinese forest frog, and Chinese big toad showed intermediate trophic positions (TP 2.06–2.89), carnivorous species steppe polecat and Upland buzzard, omnivorous Robin accentor, Yellow-browed warbler and White wagtail possessed extremely higher trophic positions (TP 2.89–3.05).

4. Discussion

4.1. Pattern of stable isotope of vertebrate species living at the HAMERS

^{13}C and ^{15}N trophic enrichment of about 0–2 and 2–5‰ had been reported for marine systems (DeNiro and Epstein, 1978, 1981; Rau et al., 1983; Dickson, 1986). However, there are few reports on ^{13}C and ^{15}N

trophic enrichment for terrestrial systems (Harding, 2001). Our study showed discernible enrichment after the level of plants. Tibetan sheep and yak, plateau pika, root vole, plateau zokor, and plateau hare had the lowest $\delta^{15}\text{N}$ values for muscles (Table 4). Nitrogen isotope fractionations between plateau pika *Ochotona curzoniae*, root vole *Microtus oeconomus*, plateau zokor *Myospalax fontanierii* and their diets were more than the results of DeNiro and Epstein (1978, 1981). Stable carbon and nitrogen signatures of small mammals (plateau pikas, plateau zokors and root voles) showed similar patterns to other small mammals (such as Keen's deer mice, *Peromyscus keeni* and long-tailed vole, *Microtus longicaudas*) captured from other places (Ben-David et al., 1997a,b). This suggested that all these small mammals were mainly dependent on C_3 food chains (Yi et al., 2003a). After the level of rodents, omnivorous birds, insectivorous birds (e.g., *Pseudopodoces humilis*), Chinese forest frog *Rana temporaria*, and Chinese big toad showed intermediate $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Table 4). At the top predators, ^{13}C and ^{15}N concentrations reached maximum due to stable carbon and nitrogen isotope enrichments. Table 4 showed carnivorous species *Mustela eversmanni* and *Buteo hemilasius* stand near the highest level of ^{13}C and ^{15}N concentrations with exceptions of Robin Accentor and White Wagtail because they are rodent-eaters. Greater fractionations of nitrogen isotope (more than 5‰) between plateau pika *Ochotona churzoniae*, root vole *Microtus longicaudas*, plateau zokor *Myospalax fontanierii* and their diets may be due to different nutrient pathways involving proteins (Ambrose and Norr, 1993). Table 4 also revealed great difference of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between organisms captured in early and late the year. This maybe is related to different food qualities and food availabilities in winter. The stable carbon isotope enrichments between Tibetan sheep and Tibetan yak and their diets were much more than the reported data of 1–2‰, which may be due to different nutrient pathways involving carbohydrates (Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Hobson and Stirling, 1997).

4.2. Trophic positions of vertebrate species living at the HAMERS

The inferred trophic relationships of the various organisms sampled, based on the isotope contents of

muscle or other tissues, generally match what we know of these species. That is, these results showed that the four species of rodents are trophically lowest, which are known to be herbivores. From the results of nearly the same $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, we also found that there was a substantial overlap of diets for the rodents. Stable carbon and nitrogen isotopic evidence suggested that omnivorous birds fed at a higher trophic level than the only granivorous birds (*Acanthis flavirostris*). Granivorous birds, twice stands higher trophic positions at the early year because ripe plant seeds had greater $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values than the fresh one (Saurer et al., 1999). Carnivorous species *Buteo hemilasius* and *Mustela eversmanni*, top predators living at the meadow, stand extremely higher trophic positions among carnivores (Table 4). Based on stable carbon isotopic patterns and enrichments, the results indicated that steppe polecat mainly preyed on small mammals as food supply (Yi et al., 2004). Upland buzzards, however, possessed higher $\delta^{13}\text{C}$ values; we proposed that upland buzzards derived their food sources from passerines birds living at the alpine meadow (Yi et al., 2003b). Table 4 demonstrated that migrant omnivorous bird species (i.e., White wagtail, Robin accentor and Yellow-browed warbler captured in April to May, 2002) showed the highest trophic positions among the overall species collected because they were mainly dependent on water insects. Aquatic insects (or organisms) possess extremely higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values than terrestrial organisms (Hobson and Welch, 1992) because their carbon sources mainly originated from carbonates. On the other hand, the newcomers maybe still carrying with the information of stable isotope of previous feeding partially resulted in highest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and trophic positions (Hobson, 1999).

4.3. Comparison with nitrogen enrichment model

The isotope investigation of organisms living at the HAMERS demonstrated that the approximate $\delta^{13}\text{C}$ trophic enrichment of the ecosystem is similar to the estimates of 0–2‰ reported for other systems (DeNiro and Epstein, 1978, 1981), which stood at $1.15 \pm 0.50\text{‰}$. However, $\delta^{15}\text{N}$ trophic enrichment ($5.27 \pm 0.50\text{‰}$) of the ecosystem is slightly higher than the reported values 2–5‰ in marine ecosys-

tems (Hobson and Welch, 1992). We firmly know that the trophic enrichment factor derived from wild data is only as good as our knowledge of the trophic linkage, however, captive studies are not carried out due to limitations. Stable nitrogen enrichment of $5.27 \pm 0.50\text{‰}$ seems to be a little bit higher based only on stomach content analyses, which calls for further captive studies. In general, $\delta^{15}\text{N}$ was used to infer trophic level of organisms rather than $\delta^{13}\text{C}$ (Hobson and Welch, 1992) in marine ecosystems. However, few studies touched on trophic positions in terrestrial ecosystems based on nitrogen enrichment approaching. According to the nitrogen enrichment model (TP = $2 + (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{primary consumer}}) / \text{enrichment factor}$) actively used in marine and aquatic ecosystems, we found that $\delta^{15}\text{N}$ alone generate the same trophic relationship information as the “alpha and vector model” in alpine meadow ecosystem (Table 4), which indicated that the combination of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values as an indicator to reveal trophic position estimation of organisms living at the HAMERS is highly recommended. Compared with the $\delta^{15}\text{N}$ enrichment method used in marine ecosystems, we suggest that the “alpha and vector model” may be more efficient for analyzing trophic relationships in terrestrial ecosystems because of incorporation of another stable isotope, i.e., $\delta^{13}\text{C}$ for trophic flow. In light of this, a stable isotope-based trophic position to represent trophic structure becomes an alternative to connect food webs and food chain-based models, which remain document paradigms in community and ecosystem ecology. We call for further studies on other stable isotopes to make the “alpha and vector model” more perfect. Mathematically, multiple isotopes, such as δD , $\delta^{18}\text{O}$, $\delta^{34}\text{S}$ and $\delta^{87}\text{Sr}$, could be incorporated into our “alpha and vector method” model to reach a more accurate angular enrichment and address basic ecological questions, such as trophic structure and energy flow in other terrestrial ecosystems.

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