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NOTE

Variation in nitrogen isotopic composition in the Selenga river watershed, Mongolia

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Abstract The stable nitrogen (N) isotope ratio (δ^{15}N) has been used to examine the anthropogenic N input (i.e., septic water, wastewater, and manure) to aquatic ecosystems, because anthropogenic N generally has a δ^{15}N signature distinct from that found in nature. Aquatic organisms and the derived organic matter such as sediments are reported to become increasingly enriched in ^{15}N as the human population density increases in watersheds. However, little is known about the relationship in steppe ecosystems, where the livestock population is greater than that of humans. Here, we conducted a preliminary study in the Selenga river mainstream watershed in Mongolia, which covers an area of approximately 300,000 km^2. A multiple regression analysis revealed that the δ^{15}N of the riverine sediment was significantly affected by the human population density and more significantly by livestock population density. The population density, including both humans and livestock, significantly influenced δ^{15}N of the macrophytic *Potamogeton* spp. The results showed that δ^{15}N of riverine organic matter can be an indicator of the human and livestock population density, which is likely associated with the status of N cycles in livestock-dominated watersheds.

Keywords Stable N isotope ratio · Livestock · Population · Watershed · Mongolia

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Introduction

The stable nitrogen isotope ratio (δ^{15}N) has been widely used as a means for understanding the biogeochemistry of nitrogen (N) in aquatic ecosystems because it provides information on the source of N and the processes that occur during N cycles (Heaton 1986; Kendall 1998; Wada and Hattori 1991). It is widely known that the δ^{15}N in N components tends to increase as they undergo kinetic isotope fractionations such as enzymatic and equilibrium reactions. Therefore, anthropogenic N, such as wastewater-derived N, has higher δ^{15}N values because considerable isotope discrimination against ^{15}N occurs during the processes of ammonia volatilization and the denitrification of excreted N during transportation (Heaton 1986; Kendall

1998). For example, the $\delta^{15}N$ of nitrate from wet atmospheric deposition ranges from −11 to +4‰. Meanwhile, the $\delta^{15}N$ of nitrate from septic water, wastewater, and manure varies from +8 to +22‰ (Heaton 1986).

Because of the different N-isotope signatures of nitrogen of anthropogenic and natural origin, a correlation between the $\delta^{15}N$ of lake organisms and the human (and livestock) population density in watersheds has often been observed (Anderson and Cabana 2006; Cabana and Rasmussen 1996). This correlation has also been found in paleolimnological studies. Earlier studies showed that sediments became enriched in ^{15}N when the population (human and livestock) density in the watershed began to increase (Elliott and Brush 2006; Hodell and Schelske 1998; Hyodo et al. 2008; Ogawa et al. 2001). Furthermore, the enrichment of plants and sediments in ^{15}N due to increased anthropogenic N input was found in studies of riverine (Benson et al. 2008; Kohzu et al. 2008, 2009) and estuarine ecosystems (McClelland et al. 1997; Umezawa et al. 2002). These studies suggest that $\delta^{15}N$ of aquatic organisms and organic matter can be a good indicator of anthropogenic impacts on N cycles in watersheds.

Previous studies have examined the anthropogenic N input into aquatic ecosystems in which humans mostly dominate. However, some ecosystems are dominated not by humans but by livestock, such as those in Mongolia. Most of Mongolia consists of steppe grassland where livestock are much more abundant than humans; the human population is 2.75 million and the livestock population is more than 20 million (Boone et al. 2007; Mearns 2004). In Mongolia, the transition from a planned economy to a market economy occurred in the early 1990s. Since then, the rapid increase in the livestock population has been an issue in terms of the sustainable management of grasslands (Saizen et al. 2010). Indeed, it is known that livestock have an impact on N cycles and on N isotopic signatures in some watersheds through the excretion of N (Anderson and Cabana 2006). However, little is known about the applicability of the $\delta^{15}N$ of riverine organic matter as an indicator of the population density (including livestock) in steppe ecosystems.

In this study, we conducted a preliminary study to examine the relationship between the $\delta^{15}N$ of riverine organic matter and population densities of human and livestock in the Selenga River watershed, which covers an area of approximately 300,000 km^2. We measured the N isotopic compositions of riverine organic matter such as river sediments and primary producers (algae and macrophytes) collected from areas of the watershed with different human and livestock population densities. We expected that the correlation between the $\delta^{15}N$ of riverine organic matter and population density would also hold for this watershed.

Materials and methods

Study site

The Selenga River, which is the largest river flowing into Lake Baikal, with a discharge of 32 km^3/year (∼50% of the total influx into the lake), drains an area that includes the northern part of Mongolia and southern Siberia (Kozhov 1963). The watershed covers about 500,000 km^2, most of which is occupied by grasslands and coniferous forests. Annual precipitation in this area is about 300 mm (Ma et al. 2003). The Selenga River system consists of one mainstream and two tributaries, the Tuul and Orhon.

Sample collection and isotope analyses

We collected sediments, filamentous green algae, and macrophytes (*Potamogeton pectinatus*, *P. rostratus*, *P. compressus*, *Polygonum amphibia*, *Myriophyllum verticillatum*, *Ranunculus* sp., and *Sparganium* sp.) from six sites in the upper reaches of the Tuul tributary through the junction with the Orhon tributary to the junction with the Selenga mainstream in July 2000 (from St.B to St.F in Fig. 1) and in July 2003 (St.A). We also conducted sampling at 10 sites in the Selenga mainstream in July 2002 (from St. 1 to 10). Algae were collected from 1–3 sites on the riverbed at each study site. 1–4 individuals of one species of macrophyte were collected near the riverbank at each site.

Sediments collected from the riverbed were passed through a 500 µm sieve, retained on a 25-µm mesh net, and dried at 60°C (Kohzu et al. 2009). These sediments were ground, decarbonated with 0.5 N HCl, and rinsed with distilled water (Ogawa et al. 2001). Filamentous green algae and whole macrophytes were rinsed with distilled water, dried at 60°C, and pulverized.

To analyze stable N isotopes, the samples were folded into tin capsules. The stable N isotope ratio was measured using a mass spectrometer (Finnigan MAT Delta S or Delta^{plus} XP, Germany) coupled to an elemental analyzer. Natural abundances of ^{15}N were expressed as a per mil (‰) deviation from the international standard; $\delta^{15}N = (R_{sample}/R_{standard} - 1) \times 1000$, where R is $^{15}N/^{14}N$. Atmospheric nitrogen was used as the standard. The precision of the online procedure was ±0.2‰ better.

Estimation of population density and statistical analyses

The total area of subwatersheds that drained into each sampling site was determined manually based on a topographic map from the National Atlas of the Mongolian People's Republic (Anonymous 1990). The human population density and livestock (camel, horse, cattle, yak,

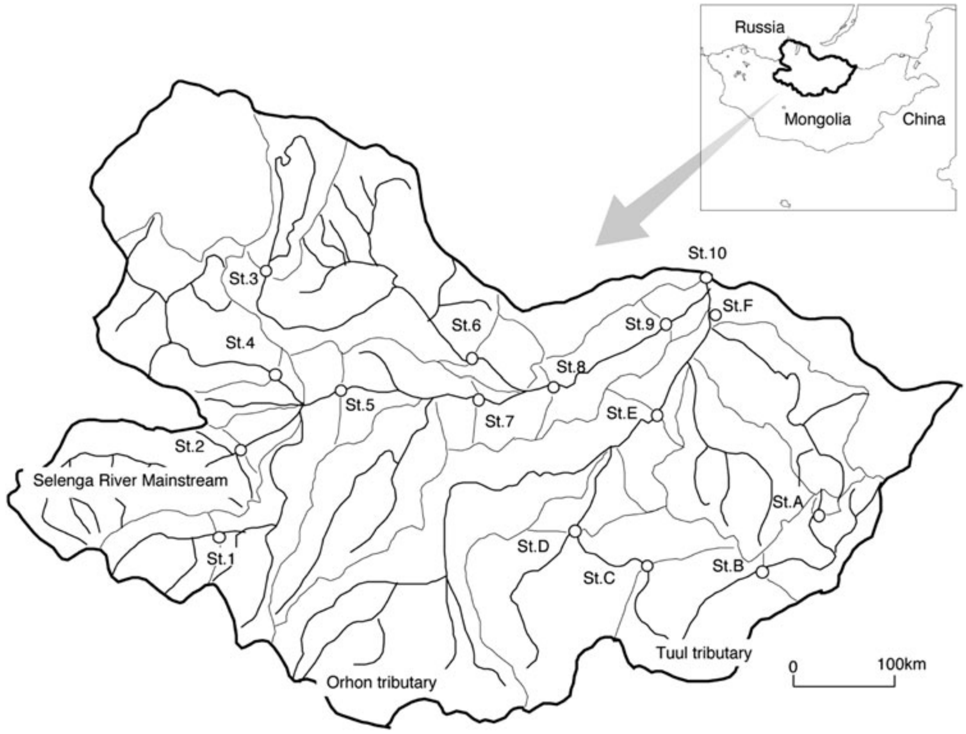

Fig. 1 Sampling sites in the Selenga River mainstream watershed

sheep, and goat) population density were estimated for each subwatershed using ArcGIS 9.3 with the statistical dataset for the year 2001, provided by the National Statistical Office of Mongolia (Saizen et al. 2010; Tachiiri et al. 2008). For this estimation, we converted the numbers of five kinds of livestock (sheep, goat, cattle, horse, and camel) to equivalent numbers of humans as follows. First, we used the standard stocking unit (Mongolian sheep unit or sheep forage unit), which has been traditionally used in Mongolia to manage livestock numbers, and it allows for an approximate comparison of the forage requirements of five kinds of livestock (Humphrey and Sneath 1999; Neupert 1999; Retzer et al. 2006; Retzer and Reudenbach 2005). The unit denotes the number of livestock, based on sheep, using the following proportions: sheep = 1, goat = 0.9, cattle = 5, horse = 6, camel = 7 (Humphrey and Sneath 1999). Subsequently, in order to estimate population including both human and livestock (thereafter called human + livestock population), we converted the population of sheep to that of humans by multiplying by a factor of 0.83, assuming that the average weight of a sheep is 50 kg and that of a human is 60 kg (Schmidt-Nielsen 1984).

We performed simple linear regression analysis to examine the relationships of the δ^{15}N values of sediments and primary producers (algae and macrophytes). In this analysis, we evaluated the δ^{15}N of macrophytes as the average value of the species of macrophyte collected at the sampling site. We also conducted statistical analysis on two genera of macrophytes, *Potamogeton* spp. and *Sparganium* sp., both of which were collected from more than five sites. For *Potamogeton* spp., we pooled the three species of the genus and used the average δ^{15}N value at each site. In addition, we used multiple regression analysis to examine the effects of human population density and livestock population density on the δ^{15}N values of sediments, algae, macrophytes, *Potamogeton* spp. and *Sparganium* sp. We also conducted simple linear regression analysis to assess the relationship between the population densities of humans, livestock or humans + livestock and the δ^{15}N of riverine organic matter. Statistical analyses were conducted using the statistical software JMP (version 8.0.2 for Macintosh; SAS Institute, Cary, NC, USA).

Results and discussion

The δ^{15}N values of riverine sediments, filamentous green algae, and macrophytes ranged from 4.1 to 8.1, −1.5 to 10.0, and 5.1 to 10.4‰, respectively (Table 1 and the "Appendix"). The δ^{15}N value of sediments had a significant positive correlation with the values for the two primary producers ($r^2 = 0.435$, $P = 0.0272$, $n = 11$ for algae and $r^2 = 0.552$, $P = 0.0088$, $n = 11$ for macrophytes). When the macrophytes were classified into genera, the δ^{15}N of the *Potamogeton* spp. had a significant relationship with that of the sediment ($r^2 = 0.564$, $P = 0.0122$, $n = 10$). This pattern was not found in *Sparganium* sp. ($r^2 = 0.145$, $P = 0.456$, $n = 5$).

Table 1 The population density and the $\delta^{15}N$ values of sediments and primary producers at each site

Site	Watershed area ($\times$100 km^2)	Human population density (person/km^2)	Livestock population density (individual/km^2)	Human + livestock population density (person equivalents/km^2)	$\delta^{15}N$ (‰)		
					Sediment	Algae (mean ± SD)	Macrophytes (mean ± SD)
StA	4	0.41	13.96	14.37	4.1	nd	nd
StB	81	80.96	51.61	132.58	5.4	4.4 ± 1.7 (3)	nd
StC	210	28.89	46.53	75.42	7.0	7.0 ± 1.9 (3)	nd
StD	453	15.18	57.09	72.27	8.1	10.0 ± 0.2 (4)	10.4 ± 0.2 (2)
StE	974	8.89	61.58	70.47	7.5	9.3 ± 2.1 (3)	7.4
StF	1312	8.05	51.77	59.82	6.6	nd	5.8
St1	71	1.03	25.53	26.56	5.6	nd	5.1 ± 1.1 (4)
St2	189	1.37	26.46	27.83	6.8	8.8 ± 0.4 (2)	9.0 ± 0.1 (2)
St3	60	0.42	11.82	12.24	6.0	2.5	5.1 ± 1.4 (4)
St4	188	1.05	27.18	28.22	6.0	nd	nd
St5	622	1.39	33.75	35.14	5.9	−1.5	7.8 ± 0.5 (2)
St6	368	0.96	17.97	18.92	4.6	nd	5.6 ± 0.9 (2)
St7	925	1.40	40.97	42.38	6.8	nd	7.8 ± 1.0 (2)
St8	1374	1.26	34.00	35.25	6.6	7.0	8.2 ± 1.9 (3)
St9	1455	1.26	33.43	34.69	5.8	5.2 ± 0.5 (2)	6.3 ± 2.0 (2)
St10	2917	4.53	41.80	46.33	6.2	8.8	nd

nd no data. The numbers in the parentheses indicate the number of samples analyzed for the isotopic compositions

Several reports have shown that as the population density in a watershed increases, riverine and lacustrine organic matter progressively become enriched in ^{15}N (Cabana and Rasmussen 1996; Kohzu et al. 2008, 2009). These studies were mostly conducted in watersheds with human population densities of >500 people/km^2. In the Selenga River watershed, the human population density is much lower than those investigated in the previous studies, and ranges from 0.41 to 80.96 people/km^2. On the other hand, the livestock population ranges from 13.96 to 61.58 individuals/km^2, and the human + livestock population density varies from 12.24 to 132.58 person equivalents/km^2.

Multiple regression analysis showed that the human and livestock population densities explain the wide variation that occurs in the sediment $\delta^{15}N$ ($r^2 = 0.632$, $F = 11.19$, $P = 0.0015$, $n = 16$; Fig. 2). The $\delta^{15}N$ was significantly affected by the human population ($F = 4.94$, $P = 0.044$) and even more significantly affected by the livestock population ($F = 22.39$, $P = 0.0004$), as indicated by the high F value. Simple regression analysis showed that the relationships between the $\delta^{15}N$ value of the sediments and the population densities of humans and of humans + livestock were not significant. However, livestock population density was significantly related to the sediment $\delta^{15}N$.

The multiple regression analysis found that the human and livestock population densities had no significant influence on the $\delta^{15}N$ of algae ($r^2 = 0.330$, $F = 1.725$, $P = 0.246$, $n = 10$) or macrophytes ($r^2 = 0.272$, $F = 1.499$, $P = 0.280$, $n = 11$). In addition, neither the human, the livestock, nor the human + livestock population density was significantly related to the $\delta^{15}N$ of algae ($r^2 = 0.0009$, $P = 0.935$, $n = 10$; $r^2 = 0.246$, $P = 0.145$, $n = 10$; and $r^2 = 0.0374$, $P = 0.592$, $n = 10$, respectively) or macrophytes ($r^2 = 0.246$, $P = 0.120$, $n = 11$; $r^2 = 0.252$, $P = 0.116$, $n = 11$; and $r^2 = 0.267$ $P = 0.104$, $n = 11$, respectively).

When the macrophytes were classified to genus level, multiple regression analysis also showed that neither the human nor the livestock population significantly explained the variation in $\delta^{15}N$ of *Potamogeton* spp. ($r^2 = 0.413$, $F = 2.460$, $P = 0.155$, $n = 10$) or that of *Sparganium* sp. ($r^2 = 0.391$, $F = 0.965$, $P = 0.475$, $n = 6$). However, the human + livestock population density had significant relationships with that of the $\delta^{15}N$ of *Potamogeton* spp. (Fig. 3). This relationship was not observed in *Sparganium* sp., probably due to the small number of sampling sites ($r^2 = 0.392$, $P = 0.184$, $n = 6$; $r^2 = 0.329$, $P = 0.233$, $n = 6$; and $r^2 = 0.330$, $P = 0.230$, $n = 6$, for the human population, the livestock population and the human + livestock population densities, respectively). These results may also be related to differences in life form and N source between the two genera; i.e., *Potamogeton* spp. are classified as emergent plants and *Sparganium* sp. is classified as a submerged plant (Grubov 2001).

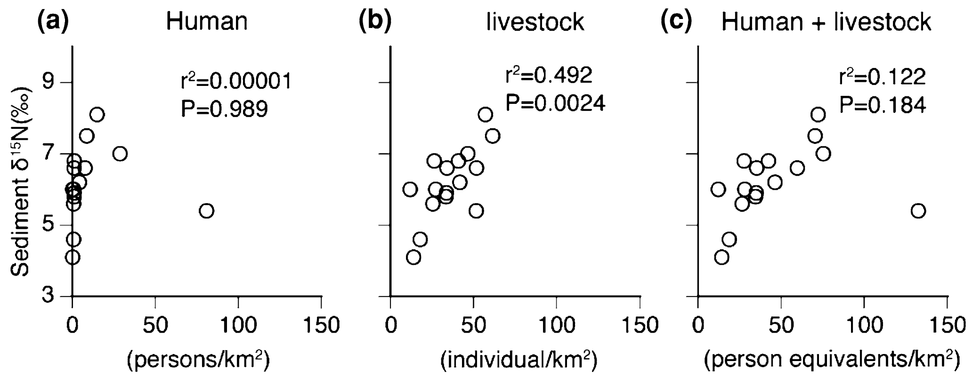

Fig. 2a–c Relationships of human (**a**), livestock (**b**) and human + livestock (**c**) population densities to δ^{15}N of sediments. See the main text for the conversion of the livestock population to a human population

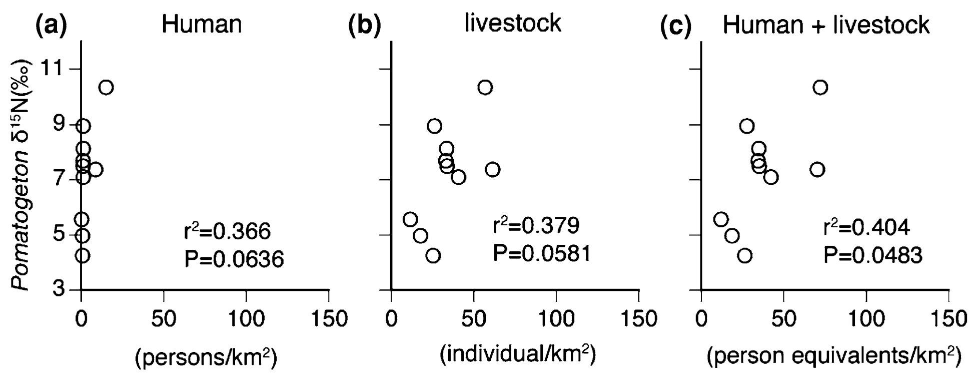

Fig. 3a–c Relationships of human (**a**), livestock (**b**) and human + livestock (**c**) population densities with δ^{15}N of *Potamogeton* spp. See the main text for the conversion of the livestock population to a human population

Overall, we demonstrated that the relationship between the δ^{15}N of aquatic organic matter and population density is also applicable to watersheds where the population of livestock is greater than humans. At this stage, it is difficult to propose precise mechanisms for how such a low human and livestock population density influences the δ^{15}N of riverine organic matter in the watersheds. Meanwhile, the present results have some implications. Like the human population, the livestock population affected the δ^{15}N of aquatic organic matter, despite potential differences between these two populations (e.g., N released from livestock is not subjected to sewage treatment). Changes in the human population most likely accompany the alteration of land use in a watershed, such as agricultural and industrial activities, which could represent additional sources of N loading. The relationship between the δ^{15}N of aquatic organic matter and human and livestock population density suggests that the number of animals could be an important influence on the ^{15}N of riverine organic matter at the watershed level. In addition, in this preliminary study, we focused on riverine organic matter and did not measure the N concentration (e.g., the ammonia and nitrate) of river water. This study showed that investigating the δ^{15}N of riverine organic matter could be the first step in an examination of N cycles at the watershed level. Further studies on δ^{15}N of nitrate and ammonium in river water would provide insight into N sources and transformations, such as denitrification and nitrification processes, in livestock-dominated watersheds (Kendall 1998; Kohzu et al. 2008).

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Appendix

See Table 2.

Table 2 δ^{15}N (‰) of macrophyte species at each sampling site

Site	*Potamogeton* spp. (mean ± SD)	*Sparganium* sp.	*Myriophyllum verticillatum*	*Ranunculus* sp.	*Polygonum amphibia*
StA	nd	nd	nd	nd	nd
StB	nd	nd	nd	nd	nd

Table 2 continued

Site	*Potamogeton* spp. (mean ± SD)	*Sparganium* sp.	*Myriophyllum verticillatum*	*Ranunculus* sp.	*Polygonum amphibia*
StC	nd	nd	nd	nd	nd
StD	10.4 ± 0.2 (2)	nd	nd	nd	nd
StE	7.4	nd	nd	nd	nd
StF	5.8	nd	5.8	nd	nd
St1	4.2	6.4	5.6	nd	4.1
St2	8.9	nd	nd	9.0	nd
St3	5.6 ± 1.2 (3)	nd	3.6	nd	nd
St4	nd	−4.1	nd	nd	nd
St5	8.1	7.5	nd	nd	nd
St6	5.0	nd	nd	6.2	nd
St7	7.1	8.6	nd	nd	nd
St8	7.5 ± 2.2 (2)	9.5	nd	nd	nd
St9	7.7	4.8	nd	nd	nd
St10	nd	nd	nd	nd	nd

nd no data. The numbers in the parentheses indicate the number of samples analyzed to obtain the isotopic compositions

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