^{15}N fractionation between vegetation, soil, faeces and wool is not influenced by stocking rate

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Abstract Understanding stable isotope fractionation in trophic networks is important for the interpretation of stable isotope composition of ecosystem components. This work explores the influence of grazing pressure on the nitrogen isotope composition (δ^{15}N) of vegetation (standing biomass), soil, and sheep's faeces and wool in a three-years (2005–2007) experiment with different stocking rates (0.375–2.25 sheep ha^{-1} year^{-1}) in semi-arid Inner Mongolia grassland. The ^{15}N of wool (from a yearly shearing) reflects vegetation at the whole-year grazing grounds-scale while faeces reflect that of the area grazed within a few days. Stocking rate had no effect on δ^{15}N of vegetation and soil, and sheep's faeces and wool, although nitrogen content of bulk vegetation increased with stocking rate. Furthermore, δ^{15}N of vegetation and diet did not differ between stocking rates. Hence, ^{15}N fractionations between vegetation and faeces ($\varepsilon_{\text{veg-faeces}}$), vegetation and wool ($\varepsilon_{\text{veg-wool}}$), faeces and soil ($\varepsilon_{\text{faeces-soil}}$) and soil and vegetation ($\varepsilon_{\text{soil-veg}}$) were constants, with $\varepsilon_{\text{veg-faeces}}=3.0‰$ ($\pm0.1‰$, 95% confidence interval), $\varepsilon_{\text{veg-wool}}=5.3‰$ ($\pm0.1‰$), $\varepsilon_{\text{faeces-soil}}=1.1‰$ ($\pm0.4‰$) and $\varepsilon_{\text{soil-veg}}=-4.1‰$ ($\pm0.3‰$). This finding is useful as it means that δ^{15}N of wool or faeces can be used to estimate the ^{15}N of grazed vegetation, even if grazing pressure is unknown.

Keywords 15-N · Enrichment · Trophic shift · Diet · C/N ratio · Plant-animal-soil system · Cycling

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Introduction

The nitrogen isotope composition (δ^{15}N) of vegetation varies strongly along gradients of climate (Amundson et al. 2003; Craine et al. 2009a), nitrogen loads and cycling (Frank et al. 2004; Schwertl et al. 2005; Watzka et al. 2006) and disturbance (Evans and Ehleringer 1993; Craine et al. 2009b; Handley et al. 1994). Variation in vegetation δ^{15}N reflects the net effect of different processes in the local nitrogen cycle, including the rate and δ^{15}N of nitrogen inputs/sources and losses, and associated fractionation effects inside the system (Högberg 1997). Such effect may result from N

fixation (Ledgard and Steele 1992), ammonia vola-tilization or assimilation (Frank and Evans 1997; Kerley and Jarvis 1996), nitrification/denitrification (Mariotti et al. 1981; Stevens and Laughlin 1998), or the plants' nitrate and ammonia uptake (Kahmen et al. 2008) among others. Thus, knowledge of the $\delta^{15}N$ of grassland can reveal important information on biogeochemical cycling of nitrogen in an ecosystem (Robinson 2001).

Many aspects of nitrogen cycling (and associated isotopic signals) are subject to strong spatio-temporal variation. In grassland, such variation concerns small-scale heterogenity caused by excrement patches (Auerswald et al. 2010; Augustine and Frank 2001), large-scale redistribution of excrements often associated with topography (Burke 1999; Schnyder et al. 2010), or spatio-temporal variation in soil water status (Handley et al. 1994). Augustine and Frank (2001) noted that large herbivores altered the distribution of soil N at every spatial scale from that of single plants to that of the landscape. Variation at low spatial scales can challenge the collection of information at higher spatio-temporal scales if the sampling is done by vegetation collection. In these cases, more integrated system-scale measures of vegetation ^{15}N composition could be useful. One way to collect system-scale information in grasslands is by making use of grazers. Grazers integrate isotopic signals by grazing extensive areas (Auerswald et al. 2009; De Niro and Epstein 1981; Hobson 1999), thus levelling out small-scale variation. Notably, different grazer tissues can provide different scales of temporal and spatial integration: for example, faeces reflect the area grazed in a few days (Weston 1988), while wool obtained from a yearly shearing gives the isotopic signal from the whole grazing ground. Keratinous tissues, such as horn, hair or hooves, are particularly useful, since they grow continuously and become stable after formation. Thus, they incorporate and record temporal changes in diet isotopic signals, which can be resolved by segmental analysis and interpreted retrospectively regarding temporal changes of dietary isotopic signals (Cerling et al. 2009; Schwertl et al. 2005).

One difficulty in the assessment of vegetation $\delta^{15}N$ from herbivore tissues is uncertainty on the exact value of isotopic enrichment between grassland vegetation and grazer tissue (Männel et al. 2007; Sponheimer et al. 2003a; b). The accuracy of $\delta^{15}N$ estimation of vegetation from animal tissues depends on the variability of the enrichment between vegetation and tissues and the accuracy with which this enrichment is known. Several factors affect this relation.

In particular, an isotopic offset between diet and standing vegetation can result from selective behaviour of the grazer. Grazers tend to select particularly palatable species, such as legumes, and plant parts, such as young nitrogen-rich leaves (Grant et al. 1985; Lu 1988). Especially legumes will differ in $\delta^{15}N$ from bulk vegetation (Ledgard and Steele 1992). The grazer tissues would then reflect the diet but not the vegetation. Notably, opportunities for selection may be variable, changing with grazing pressure. At high grazing pressure opportunities for selective grazing are minimal. Conversely, where grazing pressure is small, there are ample opportunities for selection of the most palatable feed (Lu 1988). As grazing pressure varies strongly in the grassland biome (Augustine et al. 2003; Fernandez-Gimenez and Allen-Diaz 2001; Kawamura et al. 2005), it could generate variable selection-related isotopic enrichments. Ignorance of such putative effects could cause errors in the estimation of grassland ^{15}N composition, particularly in retrospective studies where grazing pressure is unknown.

Another factor is the variation in ^{15}N enrichment during metabolism of the ingested feed. The enrichment between diet and tissue decreases with increasing nitrogen content N content (or increasing dietary value or decreasing C:N ratio) of the diet (Adams and Sterner 2000; Pearson et al. 2003; Robbins et al. 2005; Vanderklift and Ponsard 2003). This is presumably caused by the fact that the relation between faecal and urinal nitrogen excretion changes with nitrogen content of the feed (Del Rio and Wolf 2005). Robbins et al. (2005) report a change of 0.1% if the N content in the feed changes by 1‰. This relation was established for a N content in feed ranging from 2.5 to 12%. Whether this change in enrichment is relevant depends on the magnitude of variation in N content of the feed. It may be influenced by selection but it may also be influenced by a feed-back mechanism. Removal of leaves initiates regrowth and reduces leaf age. Young leaves have a higher N content than old leaves (Mattson 1980) and thus the range in N content also depends on grazing intensity. However, both influences, selection and regrowth, act in different

directions and potentially cancel out: A low grazing intensity potentially allows for the selection of N rich plant parts while in heavily grazed areas such selection becomes unimportant but the N content of the bulk vegetation increases due to the stimulation of regrowth.

The magnitude of the individual processes and their overall effect are hence difficult to estimate and thus are a source of uncertainty in the interpretation of the δ^{15}N of grazer tissues. Thus, we test the following hypotheses:

1. Differences in nitrogen content and in δ^{15}N of the vegetation exist within grazed plots, which can cause selection and affect apparent enrichments between vegetation and grazer tissues.
2. Nitrogen content of vegetation increases and diet-tissue enrichment decreases with grazing pressure.

We examined these hypotheses using an experiment in which grazing pressure was varied from very low (0.375 sheep ha^{-1} year^{-1}), providing ample opportunity for selection, to very high (2.25 sheep ha^{-1} year^{-1}), where practically all aboveground biomass was consumed with no opportunity for selection by the sheep. This experiment was carried out within the Mongolian-Manchurian grassland where typically grazing intensity ranges from almost ungrazed areas where drinking water is not available to heavily grazed areas around watering places and near settlements (Fernandez-Gimenez and Allen-Diaz 2001). Opportunities for selection arise especially due to the coexistence of C3 and C4 photosynthetic types. C4 plants start growth later at higher temperature than C3 plants (Ehleringer et al. 1997) and thus are in a younger stage of development than the co-occurring C3 plants. The delay in development may be longer than one month (Bai et al. 2004; Liang et al. 2002), causing young, palatable, and highly digestible C4 plants to co-exist near less digestible C3 plants as indicated e.g. by leaf nitrogen content (Liu 1993).

Material and methods

Grazing experiment and sampling procedure

The grazing experiment was situated in a typical semi-arid steppe at 116°40'E and 43°33'N in the Xilin River Basin, Inner Mongolia Autonomous Region,

People's Republic of China. It was operated by the Inner Mongolian Grassland Ecosystem Research Station (IMGERS), the Institute of Botany, Chinese Academy of Sciences, and the Deutsche Forschungs-gemeinschaft research group 536 MAGIM (www. magim.net). The soils were classified as Calcic Chernozems. The vegetation was dominated by perennial C3 grasses (*Stipa grandis*, *Leymus chinensis*, *Agropyron cristatum*, *Carex korshinskyi* and *Achnatherum sibiricum*), but perennial C4 grasses (mainly *Cleistogenes squarrosa* and *Festuca dahurica*) and annual C4 herbs (mainly *Salsola collina*) were also present. Legumes (*Oxytropis myriophylla*, *Melilotoides ruthenica*, *Astragalus galactites*, *Lespedeza davurica*), although present, accounted for less than 1% of total biomass. The growing period in Inner Mongolia lasts from April/May to September/October. During this period, the mean nitrogen content of aboveground dry matter biomass decreases from about 2.1% to 0.7% (Liu 1993). Growth of C4 plants starts about 30 to 50 days later compared to C3 plants (Bai et al. 2004; Liang et al. 2002).

The grazing experiment included six different stocking rates (fixed stocking rates of 0.375 to 2.25 sheep ha^{-1} year^{-1}, in steps of 0.375 sheep ha^{-1} year^{-1}) with four replicates. This yielded a total of 24 plots, each of about 2 ha in size to allow a minimum of three sheep per plot at the lowest stocking rate (Fig. 1). The experiment covered a total area of approximately 4 km². The sheep (*Ovis aries*) were about 2 years old, non-pregnant, and non-lactating ewes from the local Mongolian fat-tail breed. The
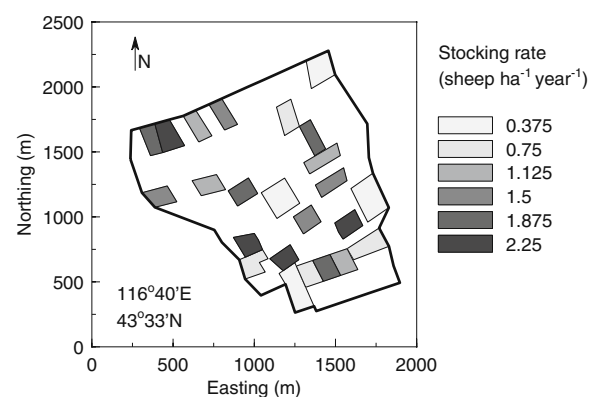

Fig. 1 Layout of the grazing experiment in Xilingol League in the Autonomus Region of Inner Mongolia, China. Six stocking rates were replicated four times

sheep grazed the pasture from beginning of June until September. Until 2003 the whole area was moderately grazed by local semi-nomadic flocks of sheep and goats. Thereafter the grass sward was left to recover for 2 years before the beginning of the experiment in 2005.

Vegetation samples of standing biomass from each plot were taken at around the 1st to the 16th of July, August, or September of 2005, 2006, and 2007. Bulk vegetation samples were obtained from inside three 0.5-m² pasture cages (hereafter termed 'cage') and from the grazed pastures ('pasture'). In both cases, vegetation was clipped at 1 cm stubble height. After clipping, the cages were moved to a previously grazed site within each plot to determine subsequent growth. In total, 217 vegetation samples from cages and 212 vegetation samples from the continuously grazed pastures were analyzed. Additionally, in 2007 we sampled leaves of *S. grandis* ($n=72$), *L. chinensis* ($n=48$) and *C. squarrosa* ($n=72$), which combined accounted for about 75% of total standing biomass. Other species accounted for less than 10% of biomass and were not collected.

Fresh faeces were sampled at the time of vegetation sampling in 2005 and in 2006. In 2007, faeces sampling was performed between 20th and 30th of each month in June, July, and August. In total, we analyzed 152 faeces samples. Sheep were shorn just before the start of the grazing period (June) and again at the end of the grazing period (late September) of 2005, 2006, and 2007. Hair samples were taken from shearings in September ($n=40$). Top soil samples (0–10 cm) were taken in September 2007 ($n=24$).

Nitrogen isotope and elemental composition

Vegetation, faeces, and soil samples were dried prior to milling: vegetation and soil samples for 48 h at 60°C and faeces for 48 h at 40°C. Vegetation and faeces samples were ground to homogeneity with a ball mill and 0.7–0.8 mg of vegetation or faeces material per tin cup were analyzed. The distal first centimetre of the wool samples was discarded to avoid artefacts from the hair stubble, identified by dye which was placed on back of the animal just after the June shearing, and hair located in the hair channel at the June shearing and hair synthesized from body pools immediately after shearing. The remaining 4 to 6 cm-long staple of wool was cleaned as per the procedure of Schwertl et al. (2003),

ground to homogeneity with a ball mill, and 0.2–0.4 mg woollen material was packed into tin cups for analysis. Soil samples were sieved prior to analysis (2 mm), ball milled, and 7–15 mg of dry soil was packed into tin cups for analysis.

The nitrogen isotope and elemental compositions were determined with an elemental analyzer (NA 1110; Carlo Erba, Milan) interfaced (ConFlo III; Finnigan MAT, Bremen) to an isotope ratio mass spectrometer (Delta Plus; Finnigan MAT). Nitrogen content in vegetation is given as mass ratio in dry matter (%N, 100 g g^{-1} or %). Nitrogen isotopic data are presented as δ^{15}N, with δ^{15}N$=(R_{sample}/R_{standard})-1$, where R is the ^{15}N/^{14}N ratio and standard refers to nitrogen in air. Each sample was measured against a laboratory working standard N$_2$ gas, which was previously calibrated against an IAEA secondary standard (IAEA-NO3, accuracy of calibration 0.19‰ standard deviation SD). After every tenth sample, a solid internal laboratory standard (SILS) with similar C/N ratio as the sample material (fine-ground wheat flour for vegetation, soil, and faeces samples; protein powder for wool samples) was run as a blind control. The SILS were previously calibrated against an international standard (IAEA-NO3). The precision for sample repeats was better than 0.2‰ (SD).

Nitrogen isotope enrichments between a certain source (indexed S) and a certain product (indexed P) were calculated:

$$\varepsilon = \frac{\delta_P - \delta_S}{1 + \delta s} \tag{1}$$

The δ^{15}N of the diet (indexed D) was calculated from isotopic mass balance of standing vegetation in the cage (representing feed on offer; indexed C) and on the pasture (residual feed; indexed R).

$$\delta_D = \frac{m_C \delta_C - m_R \delta_R}{m_C - m_R} \tag{2}$$

However, a close approximation of the vegetation-diet enrichment is given by the difference of the δ^{15}N of vegetation in the cage and on the pasture ($\varepsilon' = \delta_C - \delta_R$).

Statistical methods

The datasets were evaluated by ordinary least squares linear regression. The coefficients of determination

were tested with a two-sided test for the significance of the regressions. Significance thresholds of $P \geq 0.05$ (n.s.), $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***) were used. The location and spread are reported as arithmetic mean with its 95% confidence interval prefixed by a plus-minus sign. Hypothesis testing on equal means of groups used Student's t-test. All statistical analyses were performed with GNU R 2.9.0 (R Development Core Team 2009) and followed standard protocols (Sachs and Hedderich 2006). We excluded one outlier (vegetation sample from pasture) from a total of 837 samples.

Results

N content and isotopic composition of standing biomass

Effects of species and leaf-age

The nitrogen content (%N in dry biomass) within a grazed plot varied by about a factor of three and the largest contrast occurred between different cohorts of leaves of a species. For instance leaves of *C. squarrosa* of different age sampled at the same time differed between 1.4% and 3.9%, with higher %N in younger leaves ($r^2 = 0.66$, $n = 35$). Also, species differed in %N: *C. squarrosa* had consistently higher %N than *S. grandis*, but similar %N as *L. chinensis*. The difference between *S. grandis* and the other species was especially large in July when they differed in %N by 0.6%.

Moreover there were large differences in $\delta^{15}N$ between species but also between leaf-age classes of one species. *C. squarrosa* had a consistently lower $\delta^{15}N$, than *S. grandis* and *L. chinensis* (Table 1). The contrast between leaves of different ages were even larger. For instance, leaves of *C. squarrosa* of

different age deviated by up to 8.0‰. However, these deviations between cohorts of leaves were undirected (e.g. correlation with leaf nodal positions yielded $r^2 = 0.009$), perhaps due to temporal variation in the $\delta^{15}N$ of the nitrogen source.

Effects of stocking rate on nitrogen content

Significant differences also occurred between plots. The %N in bulk vegetation ranged from 1.3 to 2.4%. In general %N was highest in July ($1.74 \pm 0.09\%$) and lowest in September ($1.52 \pm 0.07\%$). The largest influence however was caused by stocking rate. The %N in bulk vegetation of cage samples increased from about 1.4% to 1.9% with increasing stocking rate (Fig. 2 top). In the same way, the %N of bulk vegetation increased with stocking rate (Fig. 2 bottom). A pair-wise t-test per stocking rate showed that vegetation %N in the cage was not significantly different from that in the pasture (all $P > 0.05$). Also the regression coefficients were not significantly different.

^{15}N relationships between animal tissues, vegetation, diet and soil

There were no significant correlations between stocking rate and $\delta^{15}N$ from the cages, pasture, soil, faeces or wool ($\delta^{15}N_{cage}$, $\delta^{15}N_{pasture}$, $\delta^{15}N_{soil}$, $\delta^{15}N_{faeces}$ or $\delta^{15}N_{wool}$, respectively) (all $P > 0.05$, Fig. 3). The means averaged over all stocking rates were $0.56 \pm 0.09‰$ for $\delta^{15}N_{cage}$, $0.64 \pm 0.09‰$ for $\delta^{15}N_{pasture}$, $4.61 \pm 0.32‰$ for $\delta^{15}N_{soil}$, $3.61 \pm 0.12‰$ for $\delta^{15}N_{faeces}$, and $5.94 \pm 0.22‰$ for $\delta^{15}N_{wool}$ (Fig. 3). The means of the species were $1.33 \pm 0.16‰$ for *S. grandis*, $1.19 \pm 0.20‰$ for *L. chinensis* and $-0.29 \pm 0.13‰$ for *C. squarrosa*. Further, a pair-wise t-test per stocking rate showed that $\delta^{15}N_{cage}$ was not significantly different from $\delta^{15}N_{pasture}$ (all $P > 0.05$). Thus, no net isotopic effects (i.e. enrichment or depletion) were evident that

Table 1 Mean nitrogen content (%N) and mean $\delta^{15}N$ in the dry matter of leaves for the main species ($n = 24$ per month; ± denotes the 95% confidence interval of the mean)

Parameter	Species	June	July	August	Mean
%N (%)	*C. squarrosa*	2.40±0.07	2.97±0.11	2.47±0.09	2.61±0.08
	L. chinensis		2.81±0.13	2.62±0.15	2.71±0.01
	S. grandis	2.20±0.08	2.37±0.10	2.23±0.12	2.26±0.06
$\delta^{15}N$ (‰)	*C. squarrosa*	-0.45±0.20	-0.23±0.27	-0.20±0.21	-0.29±0.13
	L. chinensis		1.13±0.25	1.26±0.33	1.19±0.21
	S. grandis	1.35±0.29	1.40±0.26	1.25±0.29	1.33±0.16

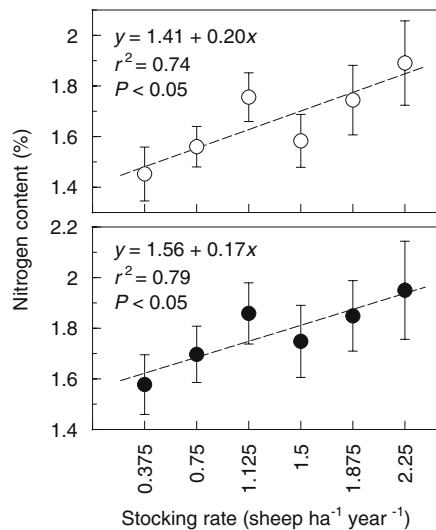**Fig. 2** Nitrogen content in bulk vegetation from the cages (○, *top*) and from the pasture (●, *bottom*) as related to stocking rate. Error bars denote the 95% confidence interval of the mean. Lines denote linear regressions

could be related to selection and stocking rate. This was true despite a difference of about 1.5‰ between the dominant species, which together accounted for more than 75% of aboveground biomass. Consequently, the $\delta^{15}N$ of the diet was similar to the $\delta^{15}N$ of the vegetation and there was no evidence of any ^{15}N enrichment between vegetation and diet. Accordingly, diet ^{15}N composition could be equated with that of vegetation on pasture and in the cage, and the $\delta^{15}N$ of vegetation ($\delta^{15}N_{veg}$) was calculated as the mean of $\delta^{15}N_{cage}$ and $\delta^{15}N_{pasture}$. The overall mean $\delta^{15}N_{veg}$ was 0.60±0.06‰.

The ^{15}N enrichment between individual components of the plant-animal-soil N cycle and in wool were then calculated (Fig. 4). The largest enrichments occurred between vegetation and soil ($\varepsilon_{veg-soil}$=4.1‰), and vegetation and wool ($\varepsilon_{veg-wool}$=5.3‰). The enrichment between vegetation and faeces was also substantial ($\varepsilon_{veg-faeces}$=3.0‰), but that between faeces and soil was small ($\varepsilon_{faeces-soil}$=1.1‰).

Discussion

No selective grazing

The vegetation demonstrated systematic variation of %N within (leaf-age) and between species offering an

opportunity for selecting a N-rich diet in agreement with our first hypothesis. Young leaves had higher %N than old leaves, consistent with many studies (e.g. Mattson 1980 and citations therein). Also, *C. squarrosa*, which started to grow later than the C3 species, had higher %N particularly in young leaves. Hence a potential benefit would result from selection of the more nutritious (N-rich) vegetation components and according to Robbins et al. (2005) this would
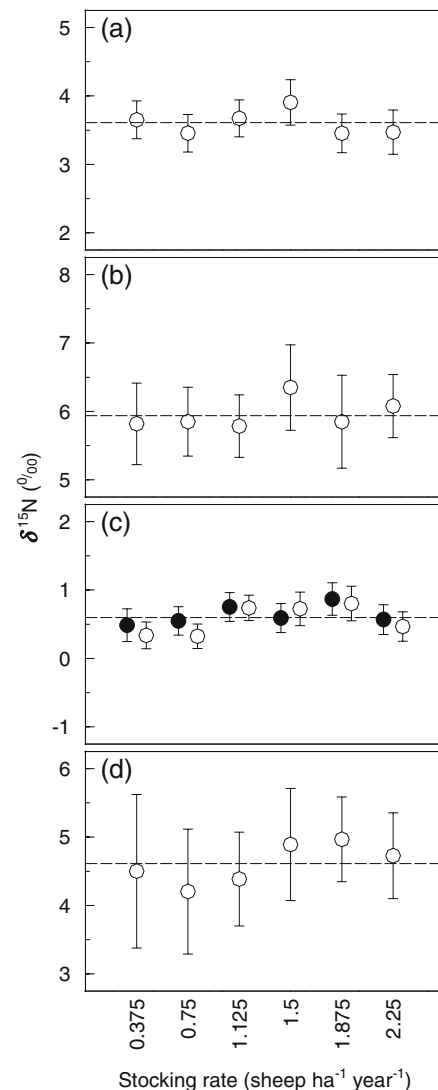

Fig. 3 Nitrogen isotope composition ($\delta^{15}N$) of faeces **a** and wool **b** of sheep, vegetation **c** from the pasture (●) and the cages (○) and soil **d** in relation to stocking rate. Error bars denote the 95% confidence interval of the mean. Dashed lines denote the mean over all respective samples. Total *n* is 152, 40, 211, 217 and 24, for faeces, wool, pasture and cage, and soil samples. To allow for comparison, the y-axis spans always 3.0‰

$\varepsilon_{\text{veg-wool}} = 5.3 \pm 0.1‰$

$\delta^{15}N_{\text{wool}}$

$\delta^{15}N_{\text{diet}}$

$\varepsilon_{\text{veg-diet}} = 0‰$

$\varepsilon_{\text{veg-faeces}} = 3.0 \pm 0.1‰$

$\delta^{15}N_{\text{veg}}$

$\delta^{15}N_{\text{faeces}}$

$\varepsilon_{\text{soil-veg}} = -4.1 \pm 0.3‰$

$\varepsilon_{\text{faeces-soil}} = 1.1 \pm 0.4‰$
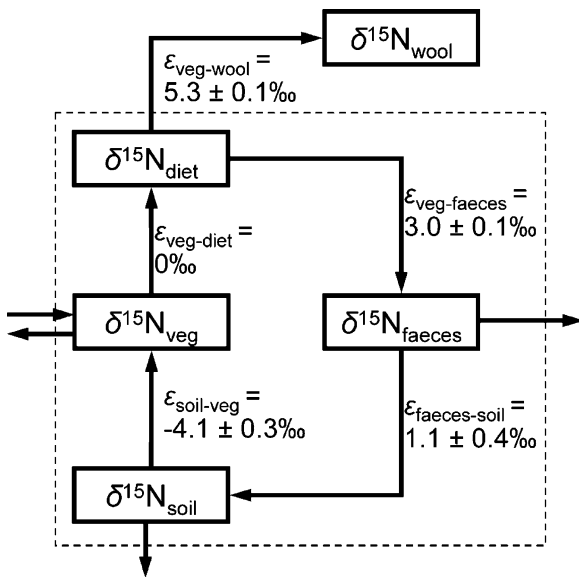
$\delta^{15}N_{\text{soil}}$

Fig. 4 Conceptual model of nitrogen isotope enrichment between vegetation, faeces, soil and wool. The dashed line-box gives the boundary of the plant-animal-soil system. Exports and imports are denoted by arrows out or in of the dashed box. Enrichments are reported together with the 95% confidence interval of the mean

decrease diet-tissue enrichment. Furthermore $\delta^{15}N$ of *C. squarrosa* (C4) was 1.0‰ lower than that of the main C3 species. Selection of the N-rich *C. squarrosa* would thus also result in a lower apparent tissue enrichment compared to bulk vegetation and act in the same direction as the higher %N. Both mechanisms should cause a large effect if selection for *C. squarrosa* happens consistently. However, we found no indication for such a selection. This finding is consistent with data from carbon isotope analysis, which presented no evidence for selective grazing for C3 or C4 vegetation (Wittmer et al. 2010).

Our work presents no evidence for an effect of vegetation/diet N content on $\varepsilon_{\text{veg-fec}}$ or $\varepsilon_{\text{veg-wool}}$ within the range of bulk vegetation in our study (0.9 to 3.7%), which is typical for the semi-arid grassland of Inner Mongolia (Chen et al. 2007; Liu 1993). Following the regression by Robbins et al. (2005), who used a much wider range of diet N contents (2.5 to 12% w/w) to develop their relation, our range in % N_{veg} should only cause a variation in enrichment of 0.3%. For the means of the different stocking rates (Fig. 2), which better reflect the diet of free ranging animals and the attenuating effect of nitrogen turnover times (e.g. more than 8 weeks for new hair growth

reported by Sponheimer et al. 2003b), the expected effect would only be <0.1%, which is too small to be detected. Furthermore, the higher %N on the intensively grazed plots, where selection was less possible, might have a similar effect as the selection of N-rich parts on the plots of low stocking rates, although even there such a selection was not detectable (Fig. 2). Hence, effects of grazing intensity on selection and isotopic enrichment were not apparent and if existing they are possibly counteracting.

Enrichments between N compartments of grazed ecosystems

The ^{15}N enrichment between vegetation and wool ($\varepsilon_{\text{veg-wool}}$) of 5.3‰ was similar to that reported by Sponheimer et al. (2003b) for goats fed on a pure *Medicago* diet (5.0‰) and the 5.2‰ found in several free ranging domestic animals (e.g. sheep, goat, yak) in Mongolia (Kohzu et al. 2009). However, it was higher than the $\varepsilon_{\text{veg-wool}}$ of 3.6‰ for sheep grazing pure C3 vegetation reported by Männel et al. (2007), which was based on a much more restricted data set obtained in a onetime sampling campaign. In our experiment $\varepsilon_{\text{veg-faeces}}$ was 3.0‰, the same as in sheep fed on a pure *Medicago* diet (Sutoh et al. 1993). For goats, Sutoh et al. (1987) reported slightly higher $\varepsilon_{\text{veg-faeces}}$ of about 3.6‰. But again, for llama, fed either a pure *Medicago* or *Cynodon* diet $\varepsilon_{\text{veg-faeces}}$ was 3.0‰ (Sponheimer et al. 2003a). This close similarity of $\varepsilon_{\text{veg-faeces}}$ accross a wide range of environmental and feeding conditions suggests that both factors had little impact on the physiological processes underlying the $\varepsilon_{\text{veg-faeces}}$ phenomenon in ungulate ruminants. Accordingly, modification of faecal $\delta^{15}N$ after deposition, e.g. by gaseous losses like ammonia volatilization (Högberg, 1997), must have been small. Indeed, faeces mainly consist of protein, lignin and other indigestible components (van Soest 1994), which degrade only slowly after deposition and with little isotopic fractionation (Holst et al. 2007; Ma et al. 2007). Rapid drying of faeces in the semiarid environment may have further reduced degradation and associated ^{15}N fractionation. This may also explain the tight 95% confidence interval of only 0.1% (Fig. 3) associated with $\varepsilon_{\text{veg-faeces}}$.

The $\varepsilon_{\text{veg-soil}}$ of 4.1‰ was similar to that reported by Cheng et al. (2009), which reported 4.5‰ for a transect study in Inner Mongolia. The value is also similar to that obtained from the data of Amundson et

al. (2003) for the semi-arid Inner Mongolian grassland. Furthermore, a meta-analysis of a global data set of grasslands, forests and woodlands (supporting material of Amundson et al. 2003) yielded a mean on $\varepsilon_{\text{soil-veg}}$ of -4.8‰, with no influence of mean annual precipitation on $\varepsilon_{\text{soil-veg}}$. Watzka et al. (2006) investigated the relationship between soil and vegetation ^{15}N in grassland subject to a large range of fertilizer regimes and found a constant $\varepsilon_{\text{soil-veg}}$ of about -3.7‰. Thus it appears that $\varepsilon_{\text{veg-soil}}$ is similar in a wide range of environmental conditions and biomes. Remarkably, this also suggests that the pathway of nitrogen cycling in the plant-soil system (e.g. via litter fall or faeces of grazers) has little or no systematic effect on $\varepsilon_{\text{soil-veg}}$. This would also agree with the present finding that $\varepsilon_{\text{veg-soil}}$ was constant (at least in the time-frame of the 3-year study) over a wide range of grazing intensities in which the ratio of faeces production to litter fall varied greatly.

Conclusions

This work found distinct ^{15}N fractionation effects in the soil-plant-animal N cycle of semi-arid grassland in Inner Mongolia. These effects were unrelated to a wide range of stocking rates and associated variation in herbage N contents. The finding means that vegetation ^{15}N of Inner Mongolia grassland can be estimated from knowledge of ^{15}N in grazer tissues (wool and faeces), even if local stocking rates and past changes in stocking rate are unknown. This is particularly helpful, as grazer tissues provide an integrated measure of vegetation ^{15}N, faeces reflecting the area grazed in several days (hectares) and sheep that of the whole grazing season (km[2]).

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