TECHNISCHE UNIVERSITÄT MÜNCHEN

Lehrstuhl für Grünlandlehre

Carbon and nitrogen isotope composition of Inner Mongolia grassland: spatio-temporal variations at multiple scales

Maximilian H.O.M. Wittmer

Vollständiger Abdruck der von der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München zur Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften

genehmigten Dissertation.

Vorsitzender: Univ.-Prof. Dr. I. Kögel-Knabner

Prüfer der Dissertation: 1.   apl. Prof. Dr. K. F. Auerswald

                          2.   Univ.-Prof. Dr. J. Schnyder

                          3.   Univ.-Prof. Dr. N. Buchmann,
                               Eidgenössische Technische Hochschule Zürich / Schweiz
                               (nur schriftliche Beurteilung)

Contents

Abstract

Aims. The subject of the present study was the variation of the carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotope composition in the Mongolian-Manchurian grassland. The first aim was to assess the relationship between the carbon isotope discrimination ($^{13}\Delta$) of C3 vegetation and precipitation gradients intended to predict the C3 end-member in isotope mixing models of C3/C4 bulk material. Secondly, the influence of grazing on the fractionation between δ^{13}C and δ^{15}N of vegetation and grazers' tissues were evaluated intended to obtain information about the isotopic composition of bulk vegetation on a larger temporal and spatial scale than by vegetation sampling from the analysis of grazer tissue. Lastly, a spatial pattern of the C4 abundance was established from δ^{13}C of woollen samples and of soil organic carbon (SOC), and was related to environmental and socio-economic gradients intended to reveal the influence of changes in the grazing regime, in atmospheric CO_2 concentration and in climate on the competition between C3 and C4 plants in vast Mongolian-Manchurian grassland.

Material & Methods. Sheep's wool and faeces, top soil and vegetation were sampled in the Mongolian-Manchurian grassland (Inner Mongolia and Republic of Mongolia) covering an area of approximately 700000 km² and at a five-year grazing experiment with variable stocking rates. The samples were analyzed for their δ^{13}C and δ^{15}N and the C4 abundance (P_{C4}) was calculated from δ^{13}C. Maps of P_{C4} and of environmental parameters were geostatistically interpolated.

Results & Discussion. The $^{13}\Delta$ of C3 vegetation exhibited a highly significantly curvilinear increase towards growing period precipitation of the respective sampling year. This relationship was tighter and more consistent than that towards long-term precipitation. A similar response could be found for published values of $^{13}\Delta$ of C3 vegetation in a wide regional range.

The fractionation of δ^{13}C and δ^{15}N between grazed vegetation and grazers' tissues (wool and faeces) was not influenced by stocking rate, nor was any selection towards C3 or C4 vegetation or towards old or young material apparent. Further, there was no effect of differential digestibility of C3 and C4 grassland species on the fractionation. Thus a constant fractionation could be assumed for the reconstruction of δ^{13}C or δ^{15}N of grassland vegetation from grazers' tissue.

From woollen samples originating between 1998 and 2007, a mean P_{C4} of 19% in Inner Mongolian grassland could be calculated. The spatial pattern closely followed isotherms of the respective period. The same was true for P_{C4} calculated from SOC, but the spatial pattern

differed, and mean P_{C4} was 10% lower. The difference between the patterns indicated that P_{C4} has increased, and spread northwards and towards higher altitudes. A comparison of P_{C4} from recent woollen samples from Inner Mongolia and Republic of Mongolia exhibited no difference, despite a distinct difference in the pastoral system (sedentary grazing *versus* transhumance) and stocking densities. Though, the overall spatial pattern of P_{C4} of the Mongolian-Manchurian grassland closely followed recent isotherms and the difference to P_{C4} calculated from SOC could be attributed to the pronounced and regionally varied warming in recent decades.

Conclusions. The $\delta^{13}C$ and $\delta^{15}N$ of grazers' tissues can be used to obtain the respective isotopic composition of the vegetation. This allows for retrospective views, which exhibited an increase in P_{C4} in the Mongolian-Manchurian grassland. The spatial pattern of P_{C4} closely followed that of temperature. This suggests that temperature is the dominant control of C4 abundance on the regional scale.

Zusammenfassung

Zielsetzung. Die vorliegende Arbeit befasst sich mit der Variation der isotopischen Zusammensetzung von Kohlenstoff (δ^{13}C) und Stickstoff (δ^{15}N) im Mongolisch-Mandschurischen Grasland. Zunächst wurde die Veränderung der ^{13}C Diskriminierung ($^{13}\Delta$) von C3 Vegetation entlang eines Niederschlagsgradienten untersucht um das C3 Endglied für den Einsatz in isotopischen Mischmodellen aus gemischtem C3/C4 Material zu schätzen. Ein weiteres Ziel bestand in der Erfassung von Beweidungseinflüssen auf die Fraktionierung zwischen δ^{13}C und δ^{15}N von Vegetation und tierischem Gewebe. Ziel war es, Informationen über die isotopische Zusammensetzung gemischter C3/C4 Vegetation auf einer größeren zeitlichen und räumlichen Skala zu erhalten, als es durch Vegetationsbeprobungen möglich wäre. Zuletzt wurde der C4 Biomasseanteil (P_{C4}) aus dem δ^{13}C von Wollproben und organischem Bodenkohlenstoff (SOC) ermittelt, räumlich interpoliert und mit Umweltgradienten und sozioökonomischen Begebenheiten verglichen. Zielsetzung hierbei war es, die Einflüsse von Veränderungen im Beweidungsregime, in der atmosphärischen CO_2 Konzentration und im Klima auf die Konkurrenz zwischen C3 und C4 Arten im weitläufigen Mongolisch-Mandschurischen Grasland zu untersuchen.

Material & Methoden. Wolle und Kot von Schafen, Oberboden und Vegetation wurden im Mongolisch-Mandschurischen Grasland (Innere Mongolei und Republik Mongolei) verteilt über eine Fläche von ungefähr 700000 km² und von einem Beweidungsexperiment mit variablen Besatzdichten beprobt, und δ^{13}C und δ^{15}N der Proben bestimmt. P_{C4} wurde dann aus δ^{13}C berechnet. Die P_{C4} Karten und Umweltparameterkarten wurden geostatistisch interpoliert.

Ergebnisse & Diskussion. Die ^{13}C Diskriminierung von C3 Vegetation nahm höchstsignifikant gekrümmt mit steigendem Wachstumsperiodenniederschlag des entsprechenden Beprobungsjahres zu. Diese Beziehung war enger und stimmiger als mit dem langfristigen Niederschlag und wurde auch für bereits veröffentlichte Daten von $^{13}\Delta$ auf einer großen räumlichen Skala nachgewiesen.

Die δ^{13}C und δ^{15}N Fraktionierung zwischen beweideter Vegetation und tierischem Gewebe (Wolle und Kot) war nicht von der Besatzdichte abhängig. Es konnte auch keine Selektion auf C3 oder C4 Pflanzen oder altes oder neues Pflanzenmaterial nachgewiesen werden. Verdaulichkeitsunterschiede zwischen C3 und C4 Pflanzen hatten ebenfalls keinen Einfluss auf die Fraktionierung, weshalb diese für Rekonstruktionen von Grasland-δ^{13}C bzw. -δ^{15}N als konstant angenommen werden kann.

Von Wollproben aus den Jahren 1998-2007 wurde ein mittleres P_{C4} von 19% für die Innere Mongolei ermittelt, und das räumliche Muster hat sich mit den entsprechenden Isothermen gedeckt. Eine ähnliche Beziehung zu den entsprechenden Isothermen konnte auch für P_{C4} aus SOC entwickelt werden, jedoch war das mittlere P_{C4} um 10% geringer. Der Unterschied in der räumlichen Struktur lässt eine Ausdehnung von C4 nach Norden und zu höheren Lagen vermuten. Ein Vergleich von rezenten Wolldaten aus der Inneren Mongolei und der Republik Mongolei konnte keinen Unterschied erkennbar machen, obwohl die Art der Beweidung (Sesshaftigkeit gegenüber Transhumanz) und die Besatzdichten sich unterschieden. Jedoch folgte das räumliche Muster von P_{C4} des Mongolisch-Mandschurischen Graslands wiederum eng dem Muster der Isothermen. Die Differenz zu dem aus SOC berechneten P_{C4} konnte auf die ausgeprägte und räumlich variierende Erwärmung innerhalb der letzten Dekaden zurückgeführt werden.

Schlussfolgerungen. δ^{13}C und δ^{15}N von tierischem Gewebe lassen Rückschlüsse auf die entsprechende Signatur des Graslands zu. Dadurch war es möglich, einen Anstieg von P_{C4} im Mongolisch-Mandschurischen Grasland nachzuweisen. Da das räumliche Muster von P_{C4} weitgehend dem räumlichen Muster der Temperatur entsprach, ist es wahrscheinlich, dass die Temperatur der dominante Kontrollfaktor für das regionale Vorkommen von C4 Graslandarten ist.

List of Figures

List of Tables

CHAPTER I – General introduction

The present work is part of the second phase of the DFG founded project *Forschergruppe 536 MAGIM* (**Ma**tter fluxes in **g**rasslands of **I**nner **M**ongolia as influenced by stocking rate, www.magim.net). The project was intended to understand how extensive grazing regimes and environmental changes – as prevailing in Inner Mongolian grassland – provoke the degradation of the grassland and promote the associated alterations in e.g. soil erosion and desertification rate as well as in vegetation community structure. Therefore eleven subprojects were established to shed light how different effects of grazing regimes and environmental changes affect the Inner Mongolian steppe. The goal was to offer a possible implementation of a sustainable grazing system to prevent the grasslands of further degradation, just as to allow for recovery of the grasslands. This work extends the focus of the MAGIM group to the Mongolia-Manchurian grassland to cover a wide range of environmental and socio-economic gradients which cannot be analysed on the local scale. This also may allow predicting the boundary conditions under which (some) results obtained on the local scale are still valid. This work further extends the focus of the MAGIM group temporarily and allows for retrospective views, which should help to identify changes in grassland composition due to the pronounced changes in grazing system and intensity, in climate and in CO_2 concentration during the last century.

Grasslands, the carbon and nitrogen balance and the human impact

With its 50 million km² (about 40% of land surface), the global extent of grassland embraces more than that of forests, which cover an area of approximately 40 million km² (about 32% of land surface) (Loveland *et al.*, 2000; FAO, 2005). Altogether, the global grasslands store 57.5 gigatonnes (Gt) of carbon (C) in living vegetation and another 613 Gt C in the soil beneath it (calculated after Houghton, 1999). The yearly amount of C cycled within the grasslands is tremendous, with an estimated unidirectional net C flux from the atmosphere of about 0.5 Gt yr^{-1} (Schimel, 1995; Scurlock & Hall, 1998). For nitrogen (N), only tentative estimates of the global storage exist, as for instance the amount of gaseous losses and fixation by legume species are hard to capture as well as the magnitude of other potential nitrogen sources and sinks (Galloway, 1998). A simplified appraisal can be done by assuming a C:N ratio of 20 for

vegetation and 10 for soil. Hence one yields about 2.9 Gt N in stored in living vegetation and about 30.6 Gt N stored in the soil.

However, none of these pools and fluxes is globally constant, but they are subject to distinct variations on the temporal and on the spatial scale. Precisely, human activity is distinctly altering the global balance and budget. Concerning carbon, this happens globally due to fossil fuel burning and the associated rise in the atmospheric CO_2 concentration and thus global warming. On the regional scale carbon cycle is affected by land-use changes like deforestation or intensification, and subsequent processes like erosion or desertification.

Changes in the N cycle induced by the effects of fossil fuel burning on the C cycle act on the global scale while production and application of nitrogen fertilizers and cultivation of nitrogen-fixing legumes act more regionally. Especially N addition to grasslands can cause a feed-back, as it has been observed that this will lead to species-poor communities with a dominance of nitrogen-demanding species (Vittousek *et al.*, 1997).

The effect of human induced changes is especially crucial to quantify, as it can vary strongly between different regions but also between individual users, whose impact is manifold and difficult to capture. Despite of the individual human activity being difficult to appraise, various studies report a gross C flux to the atmosphere of about $6.5 - 9.2$ Gt year^{-1} (Gifford, 1994; Schimel, 1995; Schimel *et al.*, 2001) due to human induced changes, while energy production, fertilizer production and cultivation of legume crops lead to a additional nitrogen fixation of about 0.2 Gt year^{-1} (Galloway, 1998).

Even though it is essential to quantify the extent of human induced alteration of the carbon and nitrogen cycle, the primary changes especially of the additional carbon are already obvious: an increase in atmospheric CO_2 will foster climatic change, and thus leads to an increase in temperature and a spatial shift in temperature gradients. Thinking of mixed grasslands with variable proportions of C3 and C4 photosynthetic types that changes would result in variation in the C3/C4 ratio as an increase in CO_2 concentration favours C3 plants, while increasing temperature favours C4 plants (Ehleringer & Bjorkmann, 1977; Ehleringer *et al.*, 1997; Sage & Kubien 2003). The C3/C4 ratio is further closely coupled to the nitrogen cycle, as C4 grasses tend to dominate where nitrogen is scarce, owed to a higher nitrogen-use efficiency (Yuan *et al.*, 2007). Although the overall influence of the converse effects is hard to appraise, a variation in the C3/C4 ratio entails changes in the seasonal distribution and magnitude of biomass production, soil carbon storage, water use and nutrient cycling (Bird & Pousai, 1997; Tieszen *et al.*, 1997; Epstein *et al.*, 1998; Sage & Kubien, 2003; Semmartin *et al.*, 2004). The largest changes should occur in grasslands, where both photosynthetic types

coexist and allow identifying the drivers and mechanisms behind these changes. Such mixed C3/C4 grasslands occupy about 15% of the grassland area (Parton *et al.*, 1995). Small changes in the drivers may have pronounced effects on the C3/C4 competition and thus affect the sustainability of land-use regionally and even lead to globally relevant feedback mechanisms.

The Inner Mongolia grassland

The semi-arid grassland of Inner Mongolia belongs to Mongolian-Manchurian grassland, a part of the Eurasian steppe, one of the most contiguous biomes of the world. Of the total land area of 1.2 million km² in Inner Mongolia, 0.8 million km² are covered with grassland, including meadow steppe, typical steppe and desert steppe (Xiao *et al.*, 1995a). This grassland is dominated by perennial C3 grasses (e.g. *Stipa grandis, S. baicalensis* or *Leymus chinensis*), but other perennial C3 vegetation (e.g. shrubby legumes like *Caragana microphylla* and xerophytic shrubs like *Reaumuria soongorica*), perennial C4 grasses (e.g *Cleistogenes squarrosa*), annual C4 grasses (e.g. *Chloris virgata*) and annual C4 dicotyledons (e.g. *Salsola collina*) are also present and are often co-dominant members of the grassland community (Bai *et al.*, 2002).

For centuries, the Inner Mongolia grassland was used for traditional livestock keeping with nomadic lifestyle. The herds consisted of mainly sheep and goats for the production of wool and meat, horses and camels for riding and transport and a few cows. However, in the late 1950s, following the politically entailed collectivization of the livestock, the herders were forced to change their lifestyle and become sedentary. In the early 1980s, the collectivization was annihilated again and the pastoralists gained ownership of their livestock but remained sedentary (Humphrey & Sneath, 1995; Sneath, 1998). This also induced changes in the composition and size of the herds. Especially livestock numbers increased in an attempt to keep pace with the increasing non-rural incomes. For instance, the number of sheep increased by about 1250% from 4.6 million heads in 1950 to 57.2 million heads in 2007 (Inner Mongolia Statistical Yearbook, 2008). Thus, Inner Mongolia faced the increase in atmospheric CO_2 concentration, the rising temperatures, and increasing grazing pressure. Hence one might expect a pronounced impact on the C3/C4 ratio of Inner Mongolia grassland on the temporal scale, but also on the spatial scale.

Methodological aspects concerning the reconstruction of the C3/C4 ratio from bulk carbon isotope signatures of samples

The C3 and C4 vegetation patterns can be inferred from the carbon isotope composition ($\delta^{13}C$) of a suitable sample, due to the pronounced difference in ^{13}C discrimination ($^{13}\Delta$) of C3 and C4 plants during photosynthesis (O`Leary, 1981; Farquhar *et al.*, 1989). Even though a variety of expedient samples exist (e.g. bulk vegetation, soil carbon or animal tissues), the general approach is the same: from the signature $\delta^{13}C_b$ of a bulk sample (i.e. the signature of the sample) and know C3 ($\delta^{13}C_3$) and C4 ($\delta^{13}C_4$) end-members, the proportion of C3 (P_{C3}) and C4 ($P_{C4} = 1 - P_{C3}$) derived carbon to the bulk signature can be calculated from a rearrangement of the mass-balance equation

$$\delta^{13}C_b = P_{C3} \times \delta^{13}C_3 + P_{C4} \times \delta^{13}C_4 \tag{I.1}$$

which thus delivers

$$P_{C4} = \frac{\delta^{13}C_b - \delta^{13}C_3}{\delta^{13}C_4 - \delta^{13}C_3} = 1 - P_{C3} \quad . \tag{I.2}$$

However, several points need to be addressed prior to calculation of P_{C4} which relate to environmental impacts on the non-constant end-members that can cause some uncertainty in the mass balance calculation despite the wide spacing of the end-members. Firstly, a change in atmospheric $\delta^{13}C$ ($\delta^{13}C_a$) can influence the $\delta^{13}C$ of a plant ($\delta^{13}C_p$) (Farquhar *et al.*, 1989). This is vitally important on the temporal scale of decades as $\delta^{13}C_a$ constantly decreases since the beginning of the record, especially strong since the 1960s (Geist *et al.*, 2005; Barbosa *et al.*, 2009a) but it is also important on the seasonal scale due to the fluctuations caused by seasonally changing photosynthesis and respiration. This problem can be overcome by calculating $^{13}\Delta$, defined as

$$^{13}\Delta = \frac{\delta^{13}C_a - \delta^{13}C_s}{1 + \delta^{13}C_s} \tag{I.3}$$

which 'normalises' the respective $\delta^{13}C$ of the sample ($\delta^{13}C_s$) (Farquhar *et al.*, 1989). Secondly, $\delta^{13}C_3$ and $\delta^{13}C_4$ are unknown. The plain use of a single mean value for each on a broad spatial scale (e.g. as reported by Witt *et al.*, 1998) is a oversimplification, as it is known

that $\delta^{13}C$ of C3 species (and therefore $\delta^{13}C_3$) follows altitudinal gradients (e.g., Körner *et al.,* 1988; 1991; Männel *et al.*, 2007) as well as precipitation gradients (e.g., Schulze *et al.*, 1998; Swap *et al.*, 2004; Liu *et al.*, 2005; Zheng & Shangguan, 2006; Murphy & Bowman, 2009). To some degree, the same applies to $\delta^{13}C$ of C4 species (and therefore $\delta^{13}C_4$), as again it appears that there is a relationship to precipitation gradients (e.g. Buchmann *et* al., 1996; Schulze *et al.*, 1996; Murphy & Bowman, 2009), although the absence of any relationship to precipitation gradients has also been reported (e.g. Swap *et al.*, 2004). In any case, influences of altitude and of aridity on the end-members need to be quantified to get a resilient estimate of P_{C4} or P_{C3} on the spatial scale.

Isotopic composition of animals tissue and soil carbon and nitrogen as proxies for isotopic composition of vegetation

Since "you are what you eat, isotopically" (De Niro & Epstein, 1978; 1981) animals tissue have gained attention for studying the isotopic dietary history of an animal. However, in doing so one must consider a tissue-specific isotopic fractionation between the diet and the tissue (e.g. Cerling & Harris, 1999; Männel *et al*., 2007), which can be referred to as discrimination in case of carbon (Auerswald *et al*., 2009a) or enrichment in the case of nitrogen (Robinson, 2001). The situation however is more complicated. For nitrogen there is evidence for a change in enrichment with a change in dietary nitrogen content (Adams & Sterner, 2000; Pearson *et al*., 2003; Vanderklift & Ponsard, 2003; Robbins *et al*., 2005) which needs to be considered. Furthermore, selective grazing and differential digestibility, e.g. between C3 and C4 species, can lead to biases between the isotopic composition of aboveground biomass (i.e. the vegetation which reflects the diet on offer) and the ingested diet. Further, depending on the tissue regarded, the spatial and temporal integration can vary considerably. For instance, a sheep grazing the semi-arid grassland of Inner Mongolia integrates the whole grazing area and the whole grazing time in its hair between two shearings. On the other hand, the passage rate for faeces is less than one week (Raymond, 1948), hence the integrated grazing area and grazing time is considerably lower for faeces, compared to that of hair.

Soil carbon – organic carbon or from carbonates – can also be used for retrospective views on the C3/C4 ratio of grasslands (Cerling *et al*., 1989; Monger et al., 2009). Depending on whether soil organic carbon or soil carbonates are regarded, the other fraction needs to be removed prior to analysis (e.g. Harris *et al.* 2001; Kennedy *et al.*, 2005) given that organic

and carbonate carbon differ isotopically even if derived from the same initial carbon source. On the other hand, soil organic carbon is turned over within decades (Jenkinson *et al.*, 1990), hence a definite temporal allocation is not possible, and thus no time course can be detracted. For instance, top-soil organic carbon delivers a $\delta^{13}C$ signal integrating that of $\delta^{13}C$ of aboveground biomass over several years to decades or even centuries (Wynn & Bird, 2007). The nitrogen isotopic composition ($\delta^{15}N$) of the soil tends to reflect the $\delta^{15}N$ of the vegetation (Cheng *et al.*, 2009), but this relationship changes with changing climatic conditions (Amundson *et al.*, 2003). The latter mainly acts on the global scale, as regional variation in temperature and precipitation is mostly too small. Regional variation is thus mainly associated with landuse variations, e.g. in stocking rate or human induced disturbance.

Aims

The subject of the present study was the variation of $\delta^{13}C$ and $\delta^{15}N$ in the vast Mongolian-Manchurian grassland. The first aim was to establish a relationship between $^{13}\Delta$ of C3 vegetation and precipitation gradients intended to predict the C3 end-member in isotope mixing models of C3/C4 bulk material (cf. eqn. I.2). Secondly, the influence of grazing on the fractionation between $\delta^{13}C$ and $\delta^{15}N$ of vegetation and grazers' tissues were evaluated intended to obtain information about the isotopic composition of bulk vegetation on a larger temporal and spatial scale than by vegetation sampling from the analysis of grazer tissue. Lastly, a spatial pattern of the C4 abundance was established from $\delta^{13}C$ of woollen samples and of SOC, and was related to environmental and socio-economic gradients intended to reveal the influence of changes in the grazing regime, changes in atmospheric CO_2 concentration and changes in climate on the competition between C3 and C4 plants in vast Mongolian-Manchurian grassland.

The first part of this thesis evaluates how the $\delta^{13}C$ of the C3 end-member relates to precipitation gradients. Precisely, a geostatistical method to derive site-specific and year-specific growing period precipitation in a thin meteorological network was established and demonstrated that this can be used to derive annually and spatially resolved values of $^{13}\Delta$ of the C3 end-member of equation I.2 despite the large regional and inter-annual variation in growing-season precipitation in semi-arid areas like Inner Mongolia (Chapter II; hereafter referenced as Wittmer *et al.*, 2008).

The second part evaluates the influence of selective grazing and differential digestibility of C3 and C4 species on the ^{13}C discrimination between vegetation and sheep wool and faeces

(Chapter III; hereafter referenced as Wittmer *et al.*, 2010a). This part was carried out within a long-term grazing experiment with the MAGIM framework (for further information see Schönbach *et al.*, 2009).

The third part evaluates the influence of grazing pressure and the resultant change in nitrogen content of the vegetation on selectivity of grazing and on the ^{15}N enrichment between vegetation, soil and sheep wool and faeces was studied. This part was – in analogy to carbon – carried within the long-term grazing experiment (Chapter IV).

The last and largest part then retrives recent C3/C4 vegetation patterns in Inner Mongolia from carbon isotope analysis of wool originating from the years 1998 to 2007 and how this relates to temperature and other climate gradients (Chapter V, hereafter referenced as Auerswald *et al.*, 2009). This pattern was then compared to a 'former-times' pattern derived from carbon isotope analysis of soil organic carbon (Chapter VI, hereafter referenced as Wittmer *et al.*, 2010b) and related to long-term gradients of temperature and atmospheric CO_2 concentration. This part is completed by a regional-scale quantification of the influences of grazing pressure and transhumant *versus* sedentary grazing on the C3/C4 ratio of vegetation by comparison of the contrasting but neighbouring countries, the Autonomous Region of Inner Mongolia and the Republic of Mongolia (Chapter VII) and by a local scale quantification of the influences of grazing pressure on the C3/C4 ratio from the grazing experiment (Chapter VIII).

The work is completed in the Annex by a review of statistical problems arising from retrieving the isotopic composition of vegetation from consumers' tissues (Auerswald *et al.*, 2010; Annex).

CHAPTER II – Carbon isotope discrimination of C3 vegetation in Central Asian grassland as related to long term and short term precipitation patterns[1]

Abstract

The relationship between carbon isotope discrimination ($^{13}\Delta$) of C3 vegetation and long-term (30 years) and short-term (growing period) precipitation was investigated. Different species of *Stipa*, a dominant grass genus in the (semi-)arid Asian steppes, and other C3 species were collected along aridity gradients in Inner Mongolia in 2005 (11 sites, 71 samples) and in the Republic of Mongolia in 2006 (40 sites, 45 samples). The data set was expanded with published and unpublished data of *Stipa* and other C3 species (11 studies covering 8 years, including 64 observations of *Stipa*, and 103 observations of other C3 species) and C3 community bulk-samples (11 samples). Weather data were geostatistically interpolated for all sampling sites and years. $^{13}\Delta$ of *Stipa* followed different relationships for the individual years when related to mean annual precipitation due to large anomalies between annual and long-term average precipitation patterns. However, the $^{13}\Delta$ response to rainfall converged when the (long-term) mean annual precipitation was replaced by year-specific mean daily precipitation during the growing period ($\mathbb{P}_G$). Remarkably, the $^{13}\Delta$-response to $\mathbb{P}_G$ for C3 species as a whole (including herbaceous dicots, semi-shrubs and grasses) and also the C3 community-level response were virtually identical to that of *Stipa*. The relation was also valid outside the geographical and climatic range where it was developed, giving proof of its robustness.

Introduction

The Central Asian Grassland is the largest continuous biome of the world and includes the steppes of the Republic of Mongolia (1.3 Mio km² of a total land area of 1.6 Mio km², Kerven *et al.*, 1996) and of the Inner Mongolia autonomous region (0.8 Mio km² of a total land area of 1.2 Mio km², Xiao *et al.*, 1995a and citations therein) of the People's Republic of China. This region has a continental climate with most of the rain falling from June to August. By far, the largest part of this grassland is situated in arid and semiarid zones, and the amount and distribution of rainfall is a major factor controlling vegetation composition, functioning and services, such as the quality and availability of herbage for grazing livestock (Xiao *et al.*, 1995a and citations therein). The Central Asian Grassland flora contains a large number of C4 species (approx. 20% of the flora; mostly dicots; Pyankov *et al.*, 2000), but the vegetation is generally dominated by C3 species, mostly perennial grasses. In particular, species of the genus *Stipa* are a frequent and often dominant component of most grassland types in the region.

The carbon isotope composition (δ^{13}C) of C3 vegetation generally correlates negatively with water availability. This is primarily attributed to the leaf-level response of ^{13}C discrimination ($^{13}\Delta$) to water availability in C3 plants (Farquhar *et al.*, 1989), which translates to community and higher scales (e.g., Kaplan *et al.*, 2002; Randerson *et al.*, 2002; Schnyder *et al.*, 2006). The carbon isotope composition of plants (δ^{13}C$_P$) is related to $^{13}\Delta$ as

$$\delta^{13}C_p = \frac{\delta^{13}C_a - {}^{13}\Delta}{1 + {}^{13}\Delta} \qquad\qquad (II.1)$$

where δ^{13}C$_a$ is the δ^{13}C of atmospheric CO$_2$ (Farquhar *et al.*, 1989). Rearranging Eq. II.1 leads to $^{13}\Delta = (\delta^{13}C_a - \delta^{13}C_p)/(1 + \delta^{13}C_p)$ and shows that $^{13}\Delta$ can be calculated directly from δ^{13}C$_p$ if δ^{13}C$_a$ is also known.

Precipitation is highly variable in time and space in the Mongolian grassland. Mean annual precipitation (MAP) varies from several hundred millimeters per year (mm yr^{-1}; mainly in the northern and eastern part of the region) to less than one hundred millimeters per year in the Gobi desert (Fig. II.1). But, at a given site, hydrological conditions may differ drastically between years. For example, MAP at Erenhot is 158 mm yr^{-1}, but exceeds 275 mm yr^{-1} or falls below 45 mm yr^{-1} in every second year (NOAA NCDC Climate Data Online, 2007). These factors may exert strong influences on the carbon isotope composition of grassland *via* (i) effects on the relative abundance of C3 and C4 species (which differ by about 10 to 18‰

in carbon isotope discrimination; O'Leary, 1981; Farquhar *et al.*, 1989), and (ii) variation of $^{13}\Delta$ in C3 plants.
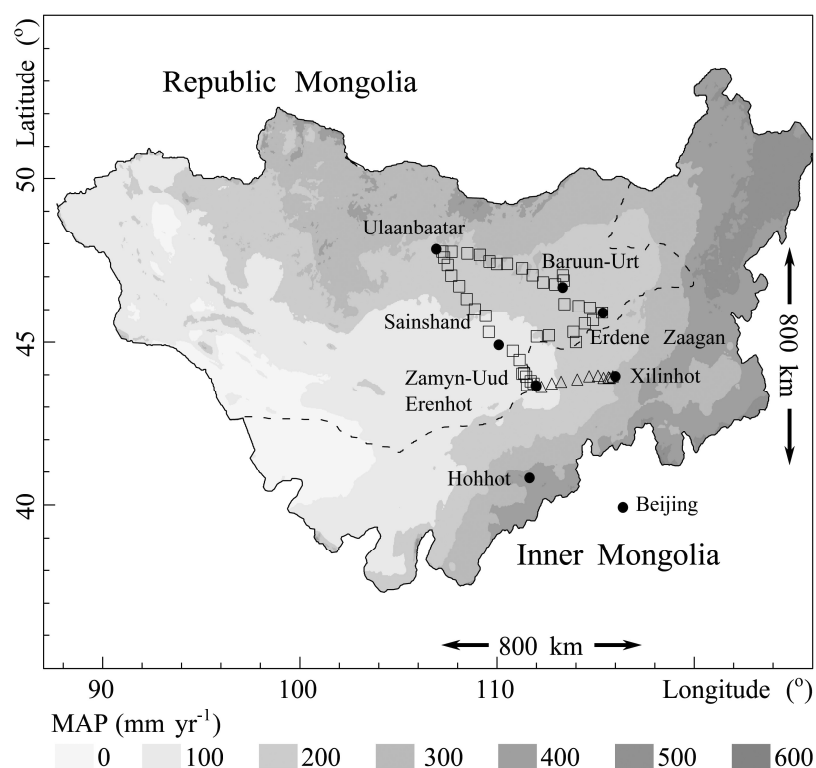
Fig. II.1 Mean Annual Precipitation (MAP, mm yr^{-1}; normal period 1961 - 90; data from Climate Source Inc.), major towns and sampling sites (triangles denote the 2005 and squares the 2006 sampling campaign).

The carbon isotope signal produced by grassland vegetation is imprinted in ecosystem carbon pools (such as soil carbon) and exchange fluxes (such as biosphere atmosphere CO_2 exchange), and is therefore potentially highly useful for biogeochemical studies and ecosystem reconstruction. However, in C3/C4 mixed grassland, the separate effects of C3/C4 abundance and variation of $^{13}\Delta$ in C3 plants on community/ecosystem $^{13}\Delta$ must be known, to allow interpretation of ecosystem or community carbon isotope signals. The present work concentrates on one aspect of this problem, namely the effect of aridity on $^{13}\Delta$ in C3 plants, and then discusses its implications for C3/C4 mixing models.

The effect of aridity on $^{13}\Delta$ in C3 plants has generally been studied in aridity-transect studies where the $^{13}\Delta$ of species was related to mean annual precipitation (MAP) at the sampling sites. The responses observed in theses studies were highly variable (e.g., Stewart *et al.*, 1995; Schulze *et al.*; 1991, 1996; Wang *et al.*, 2003a; Liu *et al.*, 2005; Zheng & Shangguan, 2007). Here, we test the hypothesis, that the variability of the $^{13}\Delta$-response of C3 plants to MAP in (semi-)arid grasslands is related to interannual variation of weather conditions. To test this

prediction, we analysed the relationship between $^{13}\Delta$ of C3 species and short-term (growing period) and long-term (climatic, 30 years) precipitation along aridity gradients in Inner Mongolia in 2005 and in the Republic of Mongolia in 2006. The data set was complemented with published and unpublished (partially own) data, thus expanding the temporal and geographic representation.

Material and Methods

Sampling area

Stipa species were sampled in the Republic of Mongolia in 2006 (total transect length of approx. 1650 km) and in Inner Mongolia, People's Republic of China in 2005 (total transect length of approx. 400 km) passing three vegetation zones, forest steppe, typical steppe and desert steppe (Wallis de Vries *et al.*, 1996), with MAP ranging from around 280 mm yr^{-1} near Ulaanbaatar to 125 mm yr^{-1} and less in the Gobi area near Sainshand and Erenhot and rising again to 280 mm yr^{-1} near Xilinhot (Fig. II.1).

The mean temperature ranges from about -23°C in winter in Ulaanbaatar to about 23°C in summer in Zamyn-Uud. The local precipitation is highly variable in space and time (Gong *et al.* 2004). Even closely vicinal climate stations show remarkable differences: although the distance between Zamyn-Uud (Republic of Mongolia) and Erenhot (Inner Mongolia) is only 10 km, the mean monthly precipitation in August is 0.52 mm day^{-1} for Zamyn-Uud and 1.29 mm day^{-1} for Erenhot (mean for last normal period 1961-1990). Similarly, the MAP is 114 mm yr^{-1} for Zamyn-Uud and 158 mm yr^{-1} for Erenhot, while mean monthly temperatures are similar for both locations (calculated from NOAA NCDC Climate Data Online, 2007).

Sampling methods and sampled species

Sampling stops were made about every 30 km. Leaves of *Stipa* (if present), and other C3 species were cut with scissors from several plants within an area of about 50 x 50 m (sometimes there were only one or two specimen of a species at a sampling site). The samples for each site were transferred to separate paper bags and dried behind the front window of the car. The current position was measured with a mobile outdoor global positioning system (Casio Satellite Navi 2 or Garmin GPS 12). In total 39 *Stipa* samples were taken in the Republic of Mongolia in 2006 and ten *Stipa* samples were taken in Inner Mongolia in 2005 (see Table A1 in appendix of the online version of this article for original data). In a few cases

specimens of *Stipa* could not be classified at the species level. The following species were collected: *Stipa krylovii*, *Stipa grandis* and *Stipa glareosa* in Republic of Mongolia, and *Stipa gobica* and *Stipa grandis* in Inner Mongolia. In addition, 61 samples of another 19 C3 species were collected in 2005; and six samples of six C3 species in 2006.

For validation, the data set was expanded with two samples of *Stipa* and 20 samples from 20 C3 species sampled in 2003 in Inner Mongolia, three bulk C3 samples collected in Mongolia in 2006 and eight C3 bulk samples collected in Inner Mongolia in 2007. Sampling procedures were the same as during the 2005 and 2006 sampling campaigns.

Inclusion of published data and normalization for altitude effect

Published data (Table II.1) were included in the validation data base with the primary aim of increasing the temporal (and secondarily the geographic) representation. These data covered a wide range of site conditions, with altitudes extending above 2000 m above sea level (especially Ivanov *et al.*, 2007). As altitude affects the δ^{13}C of C3 plants (Körner *et al.*, 1988), all data were normalized to an altitude of 1000 m above sea level, which is close to the average altitude of our sampling locations (mean: 1160 m a.s.l; SD: 180 m). The altitude effect was corrected for by 1.15‰ per 1000 m following Männel *et al.* (2007) with a mean absolute correction to $^{13}\Delta$ of 0.26‰. For publications containing no altitude data, this information was obtained with Google Earth® using the coordinates of the sample sites.

Table II.1 Sampling year, region, mean annual precipitation (MAP) and species name of literature references and own data used for validation. Top: *Stipa* data, bottom: other C3 species.

Sampling year	Region	MAP (mm yr^{-1})	Altitude (m a.s.l.)	Number of data	Species	Reference
1996	Mongolia	250	1750	1	*Stipa krylovii*	Cerling & Harris 1999
1999	Northwest China	290	1730	3	*Stipa breviflora*	Wang *et al.*, 2005
2001	Inner Mongolia	325	1267	1	*Stipa grandis*	Chen *et al.*, 2005a
2001	Mongolia	118	2170	2	*Stipa glaeosa*	Ivanov *et al.*, 2007
2002	Northwest China	240 - 670	498 – 2095	37	*Stipa bungeana*	Liu *et al.*, 2005
2003	Inner Mongolia	340	1180	2	*Stipa krylovii, Stipa* unidentified	This study
2003 –2004	Inner Mongolia	250	1200	3	*Stipa grandis, S. baicalensis, S. krylovii*	Liu *et al.*, 2004
2004	Inner Mongolia	321	1184	11	*Stipa grandis*	Gao, 2004*
2004 –2005	Inner Mongolia	323	1267	4	*Stipa grandis*	Gong *et al.*, 2008
1997	Mongolia	250	1750	4	*Carex rhynchophysa, Agrostis trinii, Leymus chinensis, Ptilogostis mongolica*	Cerling & Harris 1999
1999	Northwest China	194	1730-1850	15	*Allium polyrhizum, Sympegma regelii, Reaumuria soongorica, Ajania fruticolosa* and others	Wang *et al.*, 2005
2001	Mongolia	118	2170	5	*Allium polyrhizum, Ephedra sinica*	Ivanov *et al.*, 2007
2002	Northwest China	240 - 670	498 - 2095	31	*Lespedeza davurica, Heteropappus less*	Liu *et al.*, 2005
2003	Inner Mongolia	323	1180	20	20 different C3 species	This study
2003	Northwest China	50 - 350	395 – 1956	21	*Reaumuria soongorica*	Ma *et al.*, 2005
2004	Inner Mongolia	323	1267	4	*Carex korshinskyi, Leymus chinensis*	Gong *et al.*, 2008
2005	Inner Mongolia	323	1267	3	*Carex korshinskyi, Leymus chinensis*	Gong *et al.*, 2008
2005	Inner Mongolia	146 - 364	951 - 1204	61	*Leymus sp., Caragana sp., Agropyron sp.* and others	This study
2006	Mongolia	125 - 257	1253 - 1479	6	*Caragana microphylla, Hedysarum pumilum, Astralagus sp., Salsola laricifolia*	This study

* Gao, Y.: Yinzhi Gao, Institut für Pflanzenernährung, Chrisitan-Albrechts Universität zu Kiel, Unpublished data, 2004.

Isotope analysis

Samples were further dried in the laboratory (1 h at 95°C and 48 h at 60°C), ground with a ball mill and then combusted in an elemental analyser (NA 1110; Carlo Erba, Milan)

interfaced (ConFlo III; Finnigan MAT, Bremen) to an isotope ratio mass spectrometer (Delta Plus; Finnigan MAT). Carbon isotope data are presented as δ^{13}C relative to the international VPDB standard: δ^{13}C = ($R_{sample}/R_{standard}$) − 1; where R_{sample} and $R_{standard}$ are the ratios of ^{13}C/^{12}C in the sample and standard.

All samples were measured against laboratory working standard gases, which were previously calibrated against IAEA secondary standards (IAEA CH6 for ^{13}C, accuracy of calibration ±0.06‰ SD). Wheat flour (C/N 21:6) as solid internal laboratory standard (SILS)was calibrated against these references. One SILS was measured after every tenth sample. The precision for sample repeats was better than 0.2‰ for δ^{13}C.

C3 community-mean $^{13}\Delta$ was calculated as the biomass- or ground cover-weighted average of all C3 species in the community. The arithmetic (unweighted) mean $^{13}\Delta$ was calculated if information on ground cover or biomass fraction was missing. In this way C3 community-mean $^{13}\Delta$ was obtained from 35 data sets by Chen *et al.* (2005a), Wang *et al.* (2005), Liu *et al.* (2005), Gong *et al.* (2008) and for 10 data sets obtained by us in 2005. This was complemented by 11 C3 samples collected as bulk samples in our 2006 and 2007 sampling trips.

Carbon isotope composition of atmospheric CO$_2$

$\delta^{13}C_a$ of sampling years was estimated with a third order polynomial that was fitted to measured $\delta^{13}C_a$ data of Keeling (1979), Friedli *et al.* (1986), Conway *et al.* (1994), Francey *et al.* (1999), Gat *et al.* (2001), Allison *et al.* (2003) and the NOAA data base (NOAA NCDC Climate Data Online, 2007) for the stations Mauna Loa, Siple, Antarctica, Ulan Uul, Shetland Islands, Hegyhatsal and Ochsenkopf (n = 62), as in Geist *et al.* (2005):

$$\delta^{13}C_a = m_1 \cdot yr^3 + m_2 \cdot yr^2 + m_3 \cdot yr + m_4, \qquad\qquad (II.2)$$

where yr is the sampling year and m_1, m_2, m_3 and m_4 are constants (11.70712·10^{-6}; -69.78999·10^{-3}; 138.640104 and -91,785 valid from 1959 to 2006). The standard error of this regression was 0.09‰. $\delta^{13}C_a$ decreased from -8.12‰ in 1996 to -8.48‰ in 2007. The mean $\delta^{13}C_a$ during the growing period was then estimated from the mean $\delta^{13}C_a$ of the year (Eq. II.2) by applying a correction for the seasonal departure from the annual mean $\delta^{13}C_a$. This correction was obtained from air samples collected at Ulan Uul (Tans *et al.*, 2005), which is a long-term measuring station located near the center of the sampling area (44°27′ N, 111°05′ E). Average $\delta^{13}C_a$ was less negative by 0.25‰ between April and August as compared to the

annual average. Thus a $\delta^{13}C_a$ of -8.17‰ and -8.19‰ resulted for the growing periods in 2005 and 2006 respectively. The mean $\delta^{13}C_a$ during the growing period of other years was estimated accordingly.

Statistical methods

Linear and linearised regressions were used to evaluate the datasets. The coefficient of determination was tested with a two-sided test for significance of the regression. In addition the 95%-confidence interval for the samples and the 95%-confidence interval for the regression were calculated to allow for comparison between data sets. A pairwise comparison of means was used to test whether the species differed regarding MAP and Δ. These statistical procedures followed standard protocols (Sachs & Hedderich, 2006).

Geostatistical analyses (for theory see Nielsen & Wendroth, 2003; Webster & Oliver, 2004) were conducted with package geoR (Ribeiro & Diggle, 2001) of the software GNU R 2.6 (R Development Core Team, 2007). The semivariance of a parameter (e.g., precipitation) is defined as the half mean quadratic difference of the parameter values of points which are separated by a certain distance (called lag). For calculation of the latter, the coordinates of sampling points were transformed from geographic coordinates to UTM coordinates using the package PBSmapping (Schnute *et al.*, 2007, UTM zone = 50). Semivariances were then grouped by lag classes and semivariances and lags within a group were averaged yielding the empirical semivariogram (x axis: lag, y axis: semivariance). A theoretical semivariogram was fitted to minimize weighted least squares, with weights calculated from the ratio of pairs within a class to mean lag. This gives more weight to the classes, which are based on many data pairs and which are more important for interpolation. Maps were constructed by spatial interpolation, which was carried out for a rectangular grid by ordinary point kriging, based on the theoretical semivariogram. The quality of the predictions from the resulting maps is given as the krige standard deviation averaged for the sampling locations.

Meteorological data

The growing period in the sampling area starts in April with only one growth cycle (no regrowth after cutting or heavy grazing). Sampled plant material thus included biomass grown in the period of April to mid of July (2005) or April to end of July/beginning of August (2006). This period is termed 'growing period' in the following and indexed with 'G'. Most of the rain usually falls during this period. For 40 meteorological stations in and around the

sampling area, long-term mean precipitation from April to September correlated closely with MAP (r^2 = 0.98) and contributed 74% to MAP. Normally only little growth occurs in September, which receives only 10% of MAP in an average year. Hence, above-ground living biomass in ungrazed areas generally peaks between late July and late August (Xiao *et al.*, 1995b). The effective water availability for plants in different years and studies was estimated as the mean daily precipitation during the respective growing period (P_G; mm day^{-1}). This accounted for the fact that sampling did not occur on exactly the same dates in the different studies, but provided a common denominator for comparison of data gathered in different years.

Sampling sites, either own or from literature, were usually not located near meteorological stations. Two data sets were used to estimate meteorological data for the sampling sites. (i) The long-term averages of the last normal period (1961-1990) of precipitation data (monthly and annual means) were taken from high resolution maps obtained from The Climate Source Inc., Corvallis, Oregon. These maps have a pixel resolution of 0.02° x 0.02° (approximately 1.5 x 1.5 km², Fig. II.1), judged sufficient to locate the sampling sites. These data were created using the PRISM method (parameter-elevation regressions on independent slopes model; Daly *et al.*, 2002), which accounts for topography, rain shadows, lake effects, temperature inversions, and more. (ii) For year-specific data we assumed that the principal causes underlying the PRISM maps were also valid at the small scale but were modified by large-scale trends. Daily precipitation data at 40 climate stations, provided by the NOAA Satellite and Information Service (NOAA NCDC Climate Data Online, 2007) was compiled. These stations were situated inside or just outside the sampling area. The latter were included to improve the geostatistical interpolation at the periphery of the sampling area. To calculate P_G, the difference between the normal-period mean daily precipitation during the growing period (MPG) and P_G was calculated for each meteorological station to quantify the anomaly, dP_G. These anomalies were geostatistically interpolated for the whole sampling area and each year to represent the large-scale spatial pattern of the anomalies. This trend was then superimposed on high-resolution normal-period maps to obtain high-resolution maps for individual years; e.g., for the year 2005 P_{G05} was given by:

$$P_{G05} = MGP + dP_{G05}. \tag{II.3}$$

While annual precipitation was similar in 2005 and 2006, $P_{G05/06}$ differed considerably (Fig. II.2). In some locations anomalies in P_G between the two years and between MGP and P_G

were as large as 0.6 mm day^{-1}, which was more than one third of the mean precipitation. The best geostatistical interpolation was obtained by ordinary kriging interpolation with Gaussian models (Fig. II.2). In both years there was a trend in the anomaly, which decreased from northwest to southeast. In 2005 the trend differed somewhat in direction, strength, curvature and the position of the zero isohyet compared to 2006. In 2006 the zero isohyet was near the center of the sampling area, the south-eastern part of the sampling area being drier than average and the north-western part wetter. In 2005 the zero isohyet was near the northern border of the sampling area, and most of the area received less precipitation during the vegetation period than in a normal year (Fig. II.2).
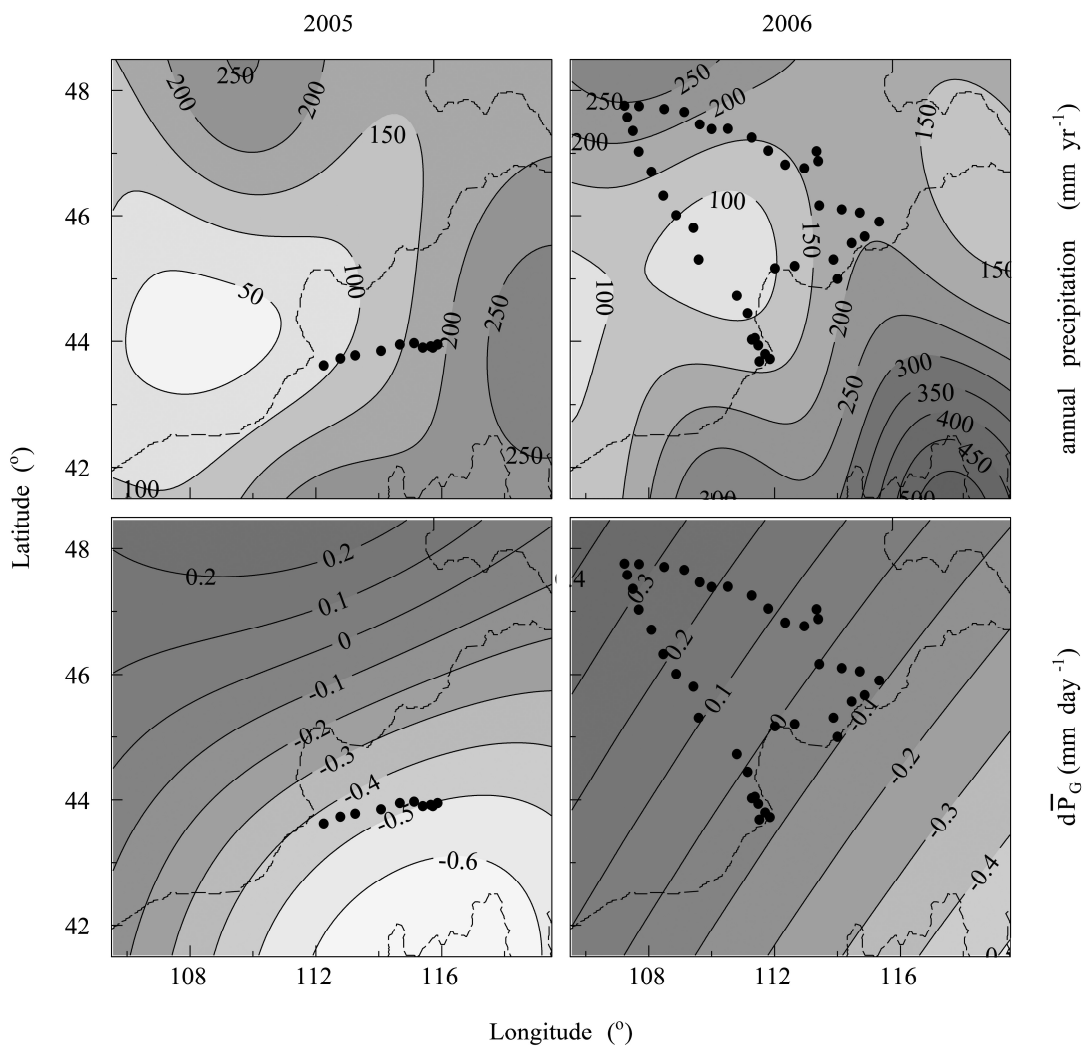
Fig. II.2 Isohyet maps for the annual precipitation in 2005 (top left) and 2006 (top right) and of the anomalies in mean daily precipitation during the growing period (dP$_G$) in 2005 (bottom left) and 2006 (bottom right). For details see text. Circles denote sampling sites; dashed lines are the border between the Republic of Mongolia and Inner Mongolia Autonomous Region of China. Mean krige standard deviation is 46 mm yr^{-1} (top left), 52 mm yr^{-1} (top right), 0.32 mm day^{-1} (bottom left) and 0.41 mm day^{-1} (bottom right) for the locations of vegetation sampling.

Results

Carbon isotope discrimination of *Stipa* as related to mean annual precipitation

The gradient of MAP covered by the sampling transects was similar in the two years, and ranged between approx. 130 and 290 mm yr^{-1} (Fig. II.3). $^{13}\Delta$ among *Stipa* increased with MAP in both years, although the increase was only significant in 2006 (P<0.01). The slope of this relationship ($^{13}\Delta$ *versus* MAP) was very similar in both years (P>0.05), but there was a significant offset between the years, so that $^{13}\Delta$ at a particular MAP was 1.1‰ higher in 2006 than in 2005. In both years, there was no relationship between $^{13}\Delta$ and the C/N ratio of samples suggesting that variation in photosynthetic capacity related to changed nitrogen concentration did not cause the variation in $^{13}\Delta$.
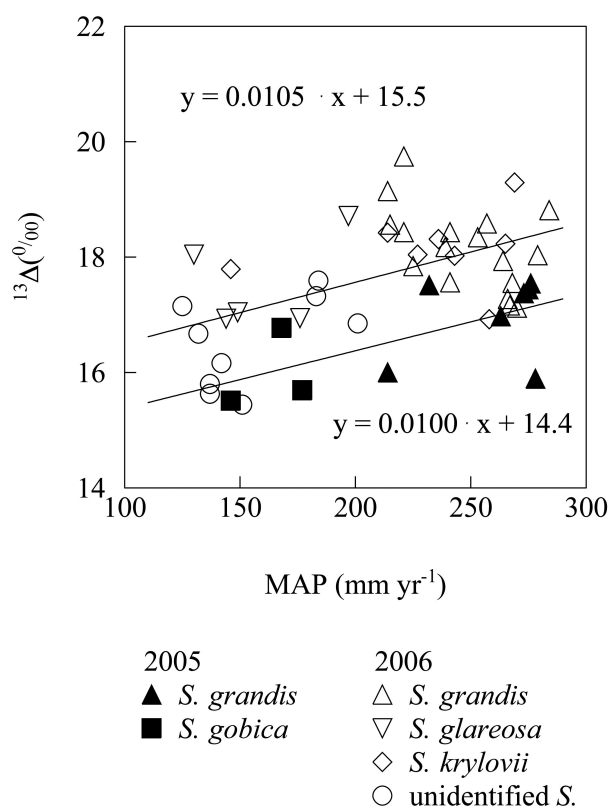
Fig. II.3 Carbon isotope discrimination ($^{13}\Delta$) of different *Stipa* species depending on mean annual precipitation (MAP) for samples collected in 2005 (r² = 0.385, P<0.1) and in 2006 (r² = 0.296, P<0.001).

In both years, the effect of MAP on $^{13}\Delta$ coincided at least partially with species replacement along the aridity gradient: *S. gobica* and *S. glareosa* were only present in the most arid part of the transects (MAP <200 mm yr^{-1}) and had lower than average $^{13}\Delta$ (Table II.2). Conversely, *S. grandis* and *S. krylovii* had higher than average $^{13}\Delta$ and mainly occurred where MAP was >210 mm yr^{-1}. This was true except for one site with MAP = 146 mm yr^{-1} where *S. krylovii*

was also found in 2006. It is not known if *S. krylovii* has a wider ecological range with respect to MAP, or if a local hydrological peculiarity, such as local groundwater or lateral water flow, accounted for its presence. The significant separation between species regarding MAP and $^{13}\Delta$ suggests that species distribution in the area was (at least partially) determined by MAP, whereas species $^{13}\Delta$ was strongly influenced by year-to-year variability in precipitation.

Table II.2 Mean annual precipitation (MAP) at sampling sites and carbon isotope discrimination ($^{13}\Delta$) of different species of *Stipa* in the 2005 and 2006 transect studies. Mean, minimum and maximum, standard deviation (SD), and significance of species separation at P<0.05.

Species	MAP (mm yr^{-1})					$^{13}\Delta$ (‰)				
	Mean	Min.	Max.	SD	Significance	Mean	Min.	Max.	SD	Significance
					2006					
S. glareosa	160	130	197	27	A	17.5	16.9	18.7	0.81	B
S. krylovii	232	146	269	40	B	18.1	16.9	19.3	0.66	B
S. grandis	246	209	284	25	B	18.2	17.1	19.7	0.71	B
					2005					
S. gobica	164	146	177	16	A	16.0	15.5	16.8	0.68	A
S. grandis	259	214	278	25	B	17.0	15.9	17.5	0.72	A

Carbon isotope discrimination of *Stipa* as related to actual precipitation

Mean daily precipitation during the growing period ranged from 0.1 to 0.7 mm day^{-1} in 2005 and from 0.5 to 1.8 mm day^{-1} in 2006. $^{13}\Delta$ of *Stipa* collected in 2005 and 2006 followed the same relationship with P_G, with $^{13}\Delta$ (‰) $= 15.0 + 2.53$ sqrt(P_G) (Fig. II.4). Thus, the difference between years (apparent when $^{13}\Delta$ was regressed against MAP) disappeared when $^{13}\Delta$ was regressed against growing period precipitation. This suggested that interannual variations in $^{13}\Delta$ were related to interannual variations in precipitation. The square root expression indicates that the effect was strong when P_G was very low and decreased with increasing precipitation.

Species did not differ in the relationship between $^{13}\Delta$ and P_G (highlighted for *S. grandis* in Fig. II.4). Thus, it seemed that species-replacement was controlled by long-term precipitation patterns, whereas the within-species response to short-term precipitation patterns controlled $^{13}\Delta$.
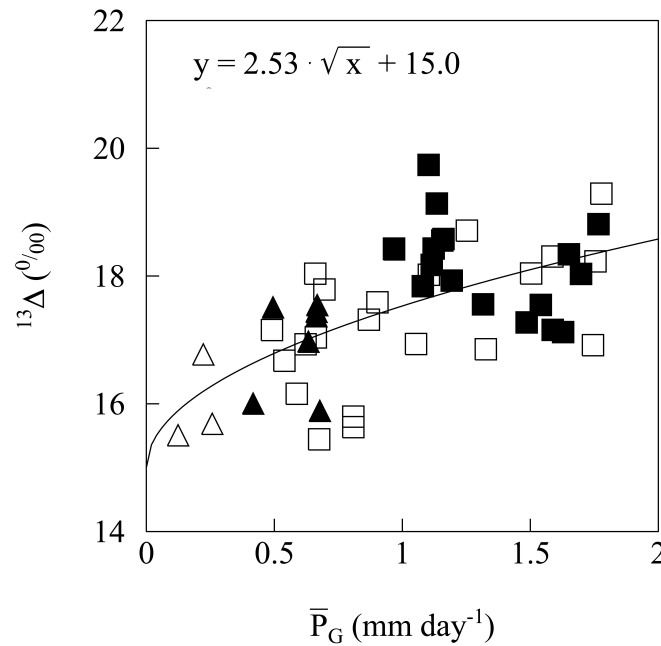
Fig. II.4 $^{13}\Delta$ and mean daily precipitation during the growing period (P_G) for *Stipa* species sampled in 2005 (triangels) and in 2006 (squares). Closed symbols: *S. grandis*. r² = 0.352, P<<0.001.

Comparison with published *Stipa* data

There were eight data sets (six published and two unpublished; see Table II.1) for which the relationship between $^{13}\Delta$ and actual precipitation could be calculated and compared with the results from our transects (see Table A2 in appendix of the online version of this articel for precipitation data). These studies reported a total of 62 data for different species of *Stipa*. The data corresponded well with the relation derived from the 2005 and 2006 samples (Fig. II.5 top). Notably, this was also true for studies in which *Stipa* experienced higher precipitation than the maximum encountered in our transect studies (Fig. II.5, hatched areas denoting P_G>2.2 mm day^{-1}). Inclusion of data of Liu *et al.* (2005) reporting $^{13}\Delta$ of *S. bungeana* along a rainfall gradient in Northwest China further expanded the range of precipitation, and also agreed with the relationship derived from the 2005 and 2006 collections of *Stipa*. Overall, these comparisons supported the notion that the relationship presented in Fig. II.4 provides a general and unbiased prediction of the $^{13}\Delta$ of *Stipa* in the grasslands of Mongolia.

The relation with P_G even explained data obtained in 2001 (Table II.1), which was an extremely dry year in Inner Mongolia. In that year more than half of the numerous lakes of the province of Qinghai (in the west of Inner Mongolia) disappeared (Reuters, 2001), and in the Republic of Mongolia the herders lost one quarter of their large-animal livestock such as horses and cattle (Retzer, 2007).
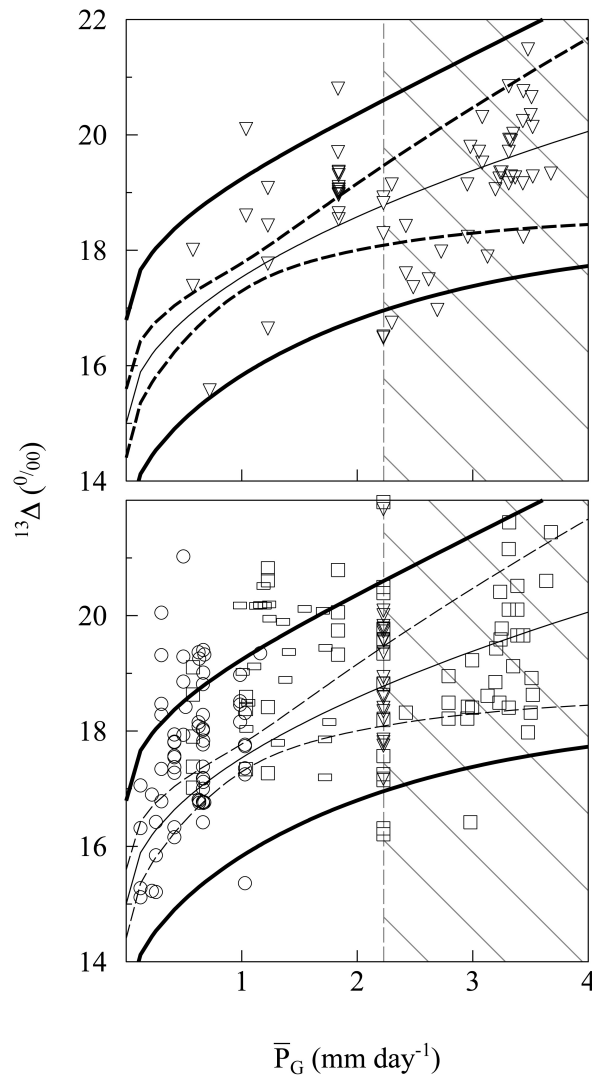
Fig. II.5 Validation of the ${}^{13}\Delta$ *versus* mean daily precipitation relationship of *Stipa* sp. shown in Fig. II.4. Top panel: *Stipa* data from nine studies (n = 64) given in Table II.1. Bottom panel: Data from other ('non-*Stipa*') C3 species as taken from literature (squares; n = 83; see Table II.1) or collected in our own 2005 and 2006 transect studies (circles; n = 87, see Table II.1). Data from *Reaumuria soongorica* (from Ma *et al.*, 2005; samples obtained in 2003) are shown as rectangles (n = 21). In both panels, the lines denote the *Stipa* regression from Fig. II.4, which was obtained from the 2005 and 2006 samples (central line), the 95% confidence intervals for the regression (dashed inner lines) and the 95% confidence intervals for the individual *Stipa* samples collected in 2005 and 2006 (thick outer lines). The hatched areas denote the precipitation range that exceeded the range covered by the 2005 and 2006 sampling transects.

The ${}^{13}\Delta$-response of C3 communities to growing period precipitation

The C3 community-mean ${}^{13}\Delta$ responded to mean daily growing period precipitation in the same way as ${}^{13}\Delta$ of *Stipa* (Fig. II.6; see Table A3 in appendix of the online version of this aricle for main species and biomass data) but with less scatter due to an averaging effect, which compressed the 95% confidence interval for the data points. The regression for the community data, calculated to obtain a robust relation over the entire range of precipitation,

was virtually the same relation as that for *Stipa*, but the confidence interval of the regression remained narrow over the entire range due to the continuous support by data.

$$y = 2.74 \cdot \sqrt{x} + 14.35$$
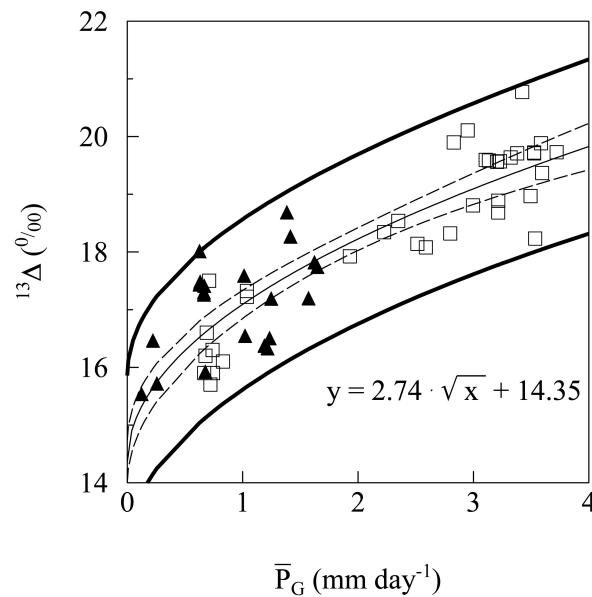
Fig. II.6 $^{13}\Delta$ and P_G for C3 communities from this study (closed triangles) in 2005 (n=10), in 2006 (n=3) and in 2007 (n=8) and from literature data (squares; n=35). All data were adjusted to a common altitude of 1000 m a.s.l. The middle line denotes the square-root regression (r^2 = 0.753, P<<0.01), dashed lines the 95%-confidence interval for the regression and outer bold lines the 95%-confidence interval for the data points.

The relationship between precipitation anomalies and $^{13}\Delta$ anomalies

The deviation of growing period precipitation from the long-term mean (precipitation anomaly) was directly related to the deviation between actual $^{13}\Delta$ and $^{13}\Delta$ predicted for mean conditions at the respective sites ($^{13}\Delta$ anomaly). This effect was manifested at the level of C3 communities (Fig. II.7), and species (data not shown). The precipitation anomalies modified the community-mean $^{13}\Delta$ by ±1‰. The response at the community level and the large spatial extension of precipitation anomalies imply that this response was also effective at larger scales.
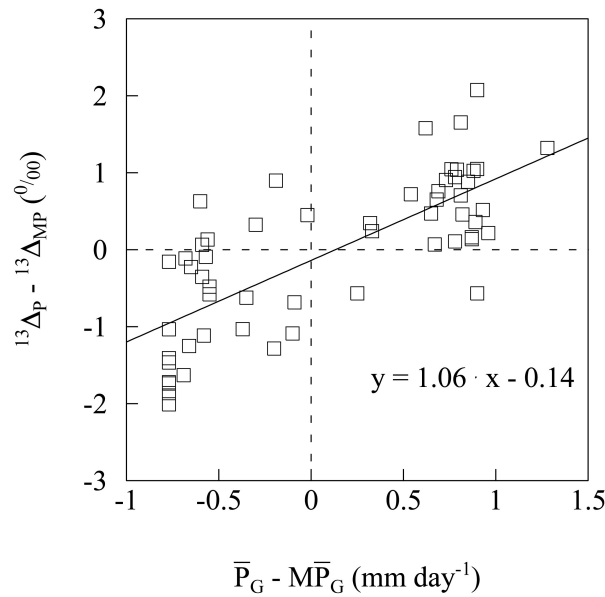
Fig. II.7 Relation of the anomalies in C3 community mean $^{13}\Delta$ and growing period precipitation (r^2 = 0.556, P<<0.001, same communities as in Fig. 6). Both anomalies are expressed as the difference between the annual and the long-term value. The long-term average discrimination, $^{13}\Delta_{MP}$, was calculated from the equation shown in Fig. II.6. Year-specific precipitation for the sites of other studies was obtained by geostatistical interpolation analogously to our own data.

Discussion

Interannual variation of $^{13}\Delta$ results from growing period precipitation anomalies

This work reveals strong effects of interannual variation in precipitation on $^{13}\Delta$ of C3 plants in the grasslands of Mongolia. This effect was particularly evident in the genus *Stipa* for which a large data set was available from collections along precipitation gradients in two different years, and published data from several years. Moreover, the effect was also apparent at the level of communities and functional groups (grasses other than *Stipa*, perennial forbs, and shrubs and semi-shrubs). An important consequence of the interannual variability of precipitation was that it caused interannual fluctuations in the relationship between (annually determined) $^{13}\Delta$ and MAP. This means, that a stable and unbiased relationship between $^{13}\Delta$ and MAP can only be obtained by relating long-term averaged $^{13}\Delta$ to MAP.

Interannual variation of $^{13}\Delta$ (or $\delta^{13}C$) has been observed before, particularly in trees (Sparks & Ehleringer, 1997; Warren *et al.*, 2001; Li *et al.*, 2007), but also in grassland taxa (Mole *et al.*, 1994; Tsialtas *et al.*, 2001), and was also explained by rainfall anomalies (Mole *et al.*, 1994; Li *et al.*, 2007) or other weather related factors (e.g., seasonal variation in water vapour deficit as reported by Sparks & Ehleringer (1997), variation in soil water content as reported by Tsialtas *et al.* (2001) and Schnyder *et al.* (2006)). However, effects of weather anomalies on

$^{13}\Delta$ have generally not been considered in aridity transect studies. It is evident from the present work, that (at least) part of the variation in $^{13}\Delta$ observed in those studies (e.g., compare studies of Gong *et al.*, 2008, or Wang *et al.*, 2005) can be explained by such deviations of growing year weather conditions from long-term mean conditions. This may also include interannual variability of soil moisture and atmospheric vapour pressure, which exert direct effects on $^{13}\Delta$ (Sparks & Ehleringer, 1997; Tsialtas *et al.*, 2001). However, since these factors are usually (but not always, see Schulze *et al.* 1996) correlated with precipitation, their effect is (at least partially) included in the relationship of $^{13}\Delta$ with year-specific growing period precipitation.

Species, functional groups and communities share the same precipitation response of $^{13}\Delta$

The $^{13}\Delta$-response (that is the slope of the relationship between $^{13}\Delta$ and P_G) of *S. grandis* was the same as that of the other species of *Stipa* in the data base. It was also very similar to the 'mean' species response, and the response of C3 communities. Even in *R. soongorica*, which had a comparatively high $^{13}\Delta$ in all rainfall conditions, the slope of the relationship between $^{13}\Delta$ and year-specific growing period precipitation was the same as that of the "mean" species. Although the relationship between $^{13}\Delta$ and growing period precipitation has not been studied in any detail, the uniformity in the $^{13}\Delta$ responses of species' or functional groups to rainfall, as seen here, was not expected: inter-specific differences in the $^{13}\Delta$-response to environmental parameters have been observed before (e.g., Handley *et al.*, 1994), and the adaptive significance of different strategies of water use have been discussed and emphasized (e.g., Golluscio & Oesterheld, 2007). Yet, it is acknowledged that, although the number of species in the data base was relatively large (55 C3 species, including 7 *Stipa* species), it was nevertheless a small fraction of the total flora of the grasslands of Mongolia. Moreover, the data base was dominated by perennial grasses (over 50% of all data). But in this respect the data base reflects the species composition of most grassland communities of Mongolia. For instance, *Stipa* accounted for more than 40% and perennial grasses (including *Stipa*) for more than 70% of total aboveground biomass in the communities sampled in 2005. Perennial grasses from arid and semi-arid temperate grasslands share great similarities in phenology, leaf structure, and root architecture and placement, which may explain the similarity in their water use (Golluscio & Oesterheld, 2007). Thus, the similarity in the precipitation response of $^{13}\Delta$ by C3 communities was related to the predominance of perennial grasses (particularly

Stipa) in these communities and the similarity in water use strategies among grasses. These similarities might also explain, why species-replacement along the aridity gradient did not affect the precipitation response of $^{13}\Delta$ (Fig. II.4).

Nevertheless, there was significant scatter in the overall relationship between $^{13}\Delta$ and growing period precipitation. Although there was no difference between functional groups, the scatter may partly be due to differences between species within groups. Differences between species have been interpreted in terms of differences in intrinsic water use efficiency (e.g., Condon *et al.*, 1990; Ehleringer *et al.*, 1992; Meinzer *et al.*, 1992) and may be related to differences in phenology (e.g., Smedley *et al.*, 1991), rooting pattern/depth (Golluscio & Oesterheld, 2007) and leaf anatomy (including leaf thickness and nitrogen content) (Farquhar *et al.*, 1989; Schulze *et al.*, 2006). Mechanisms also include special adaptations to arid conditions such as leaf shedding in response to drought as expressed in *R. soongorica* (Ma *et al.*, 2005) and phreatophyte lifestyle. This also explains why the precipitation response at the community level was associated with much less scatter than that at the species level (cf. Figs. 4 and 6): communities include species with a range of functional attributes which are related to differences in $^{13}\Delta$.

Finally, there exists large short-term, small-scale variability in precipitation in the region, which is not represented entirely when interpolating precipitation at collection sites from weather station data. For instance, the Erenhot and Zamyn-Uud weather stations are separated by approximately 10 km but differ by an average of 15 mm in the monthly precipitation during the growing period. Therefore, errors in precipitation estimates are probably substantial and explain some of the scatter in the $^{13}\Delta$ versus precipitation relationship.

A stable and unbiased relationship between $^{13}\Delta$ and precipitation for use in C3/C4 mixing models

The findings of this work are useful for estimation of the relative abundance of C3 and C4 vegetation in community biomass from community $^{13}\Delta$ ($^{13}\Delta_{community}$), which requires knowledge of the $^{13}\Delta$ of the C3 ($^{13}\Delta_3$) and C4 ($^{13}\Delta_4$) members in a two-component mixing model (e.g., Still *et al.*, 2003a). Evidently, errors in the assessment of $^{13}\Delta_3$ and $^{13}\Delta_4$ cause errors in the estimation of C3/C4 abundance. In general, neglect of variation of $^{13}\Delta_3$ or $^{13}\Delta_4$ overestimates the true variation of the fraction of C3 (and C4) vegetation in communities, because all variation of $^{13}\Delta_{community}$ is attributed to variation in the relative abundances of C3 and C4 plants. If variation of $^{13}\Delta_3$ (or $^{13}\Delta_4$) is systematic, then neglect of this variation

provokes a biased variation of the relative abundances of C3 and C4 plants. For instance, neglect of the effect of aridity on $^{13}\Delta$ of C3 plants would underestimate the relative abundance of C3 plants in the dry section of the transect. As shown above the potential variation of $^{13}\Delta_3$ is large in semi-arid and arid grassland, creating opportunities for large errors in estimation of the relative abundance of C3 and C4 plants from $^{13}\Delta_{community}$. In the present study aridity caused a variation of $^{13}\Delta_3$ of up to ~5‰ (Fig. II.6). This is about one third to one half of the difference in $^{13}\Delta$ between C3 and C4 plants in arid and semiarid grassland (Schulze *et al.*, 1996; Wang *et al.*, 2005). Accordingly, the shift from the wet to the dry end of the aridity gradient has the same effect on $^{13}\Delta_{community}$, as a 33-50% replacement by C4 plants. A possible systematic variation of $^{13}\Delta_3$ must also be considered when interpreting long-term records such as soil organic matter or sediments in terms of the C3/C4 abundance. Variation of $^{13}\Delta_4$ would have a similar effect, although, variation in $^{13}\Delta_4$ is generally smaller (e.g., Liu *et al.*, 2005; Wang *et al.*, 2005) than that of C3 plants and communities as observed here, and $^{13}\Delta_4$ responds much less to drought (Schulze *et al.*, 1996; Ghannoum *et al.*, 2002). Yet, systematic differences in $^{13}\Delta$ between C4 metabolic types exist (e.g., Schulze *et al.*, 1996; Ghannoum *et al.*, 2002) and the relative abundance of metabolic types may change with aridity (Schulze *et al.*, 1996).

Conclusions

This work demonstrates that rainfall anomalies cause large variations of the $^{13}\Delta$ *versus* MAP relationship, which are greatly reduced when $^{13}\Delta$ is related to actual rainfall during the growing period. Significantly, the $^{13}\Delta$-response to growing period rainfall was stable across years, and it was very similar for dominant taxa (*Stipa* members), functional groups (including herbaceous dicots, semi-shrubs and grasses), and C3 communities. Moreover, the relation was also valid outside the geographical and climatic range where it was developed, giving proof of its robustness. Because of its generality and stability, the relationship between $^{13}\Delta$ and growing period rainfall allows an unbiased estimation of $^{13}\Delta$ of the C3 member for use in C3/C4 mixing models based on community C isotope composition.

CHAPTER III – Do grazer hair and faeces reflect the carbon isotope composition of semi-arid C3/C4 grassland?[2]

Abstract

The carbon isotope composition ($\delta^{13}C$) of C3/C4 mixed grassland reflects the $\delta^{13}C$ of diet, hair or faeces of grazers, if ^{13}C discrimination ($^{13}\Delta$) between grassland vegetation and these tissues is known and constant. However, these relationships could be modified by selective grazing or differential digestibility of the C3 and C4 components, potentially creating a bias between grassland and grazer tissue $\delta^{13}C$. Importantly, these factors have never been studied in detail. We investigated the relation between $\delta^{13}C$ of C3/C4 grassland vegetation and that of faeces and hair of sheep in a 3-yr (2005-2007) experiment in the Inner Mongolian semi-arid steppe. The experiment employed six stocking rates (0.375 to 2.25 sheep ha^{-1} year^{-1}; four replications), which allowed for a large variation in species composition, digestibility, and diet selection. Faecal-nitrogen content, a proxy for digestibility, decreased from 1.9% to 1.5% during the grazing period due to aging of the herbage. At the same time, the C3/C4 ratio decreased due to the later growth initiation of C4 species. $^{13}\Delta$ between diet and faeces ($^{13}\Delta_{DF}$; 0.6‰) and between diet and hair ($^{13}\Delta_{DH}$; –3.9‰) were not influenced by stocking rate, period in the season or C3/C4 ratio. Moreover, faeces-hair discrimination ($^{13}\Delta_{FH}$; –4.3‰), which reflects differences between digestibility of the C3 and C4 components, did not vary along the different gradients. The $\delta^{13}C$ of grassland vegetation can be estimated from the $\delta^{13}C$ of sheep faeces and hair, provided that $^{13}\Delta$ was accounted for. This is useful for landscape- or regional-scale investigations or reconstruction of C3/C4 vegetation distribution from faeces and hair, which provide different temporal and spatial integration of grassland isotope signals.

Introduction

The carbon isotope composition of a primary consumer (herbivore) is a reflection of the photosynthetic mechanism of the primary producers on which it subsists (DeNiro & Epstein, 1978). This relationship between feed and animal isotope composition is of great value for the reconstruction of C3 and C4 vegetation patterns, since C3 and C4 plants differ strongly in carbon isotope composition ($\delta^{13}C$) (O'Leary, 1981). This isotope signal is propagated in the food web, which is based on C3 or C4 vegetation (e.g. Tieszen *et al.*, 1983; Sponheimer *et al.*, 2006; Barbosa *et al.*, 2009b). In consequence, fossil animal tissues (teeth and bone tissue) can be used as surrogates for plant remains, which may not be available. In the same way, animal tissues can also be used as an isotopic archive of contemporaneous vegetation composition and change. This is especially valuable in the case of grassland vegetation since this consists of short-lived plants. In particular, it is interesting for monitoring changes in the proportions of C3 and C4 plants in mixed grasslands.

As animals move across landscapes they feed on spatially and temporally distributed resources, ingesting isotopic information over space and time. Hair appears to be particularly useful for recording (and integrating) such isotopic information, because it is durable, chemically uniform, and grows at an approximately constant rate over an extended period of time (Schwertl *et al.*, 2003; Ayliffe *et al.*, 2004; Schnyder *et al.*, 2006). Faeces, if collected at intervals, provide better resolved temporal records of animals' diets (Codron *et al.*, 2006; Jones & Hu, 2006), with the additional advantage that animals need not be captured for sample collection.

The isotopic reconstruction of grassland vegetation from animal tissue or faeces isotope composition relies on the assumptions that there is a known constant relationship between the $\delta^{13}C$ of vegetation and that of the tissue or faeces. In the case of C3/C4 grassland this requires that (1) animals do not prefer one photosynthetic type over the other (non-selective feeding) and (2) the C3 and C4 components have similar digestibility, so that they are assimilated and excreted in similar proportions. These are important assumptions, which need testing because presently the relationships between diet, tissue and faeces are extrapolated from only a handful of experiments of mostly domestic species, and have small sample sizes. This is especially true for C3/C4 grassland systems such as the grasslands of Central Asia, the largest contiguous biome of the world, which is grazed extensively by domestic animals, particularly sheep, goats and cattle. However, even these species are thought to graze selectively, choosing a diet of better quality than the average vegetation (Prache *et al.*, 1998). This could create a significant bias between hair $\delta^{13}C$ ($\delta^{13}C_{hair}$) or faeces $\delta^{13}C$ ($\delta^{13}C_{faeces}$) and that of

vegetation, as C4 plants are believed to be less nutritious and less digestible than C3 plants (Caswell *et al.*, 1973; Barbehenn *et al.*, 2004). We are not aware of any systematic investigation of the relationship between the $\delta^{13}C$ of natural C3/C4 grassland vegetation and that of hair or faeces of bulk grazers such as sheep. In particular, it is unclear if differential selection and digestibility of C3 and C4 vegetation components affect this relationship.

Keratinous tissue (such as hair) is generally enriched in ^{13}C relative to diet by approximately 3‰ in domestic and wild ruminants fed pure C3 or C4 diets (Minson *et al.*, 1975; Auerswald *et al.*, 2009). Conversely, faeces are commonly slightly depleted (–0.6‰) relative to diet ($\delta^{13}C_{diet}$; Jones *et al.*, 1979; Coates *et al.*, 1991; Norman *et al.*, 2009). These isotopic deviations are termed discrimination ($^{13}\Delta$) and are attributed to isotope fractionation during digestion or metabolism (e.g. DeNiro & Epstein, 1978; Tieszen *et al.*, 1983). They are calculated as

$$^{13}\Delta = \frac{\delta^{13}C_{diet} - \delta^{13}C_{tissue}}{1 + \delta^{13}C_{tissue}} \qquad\qquad\qquad (III.1)$$

with $\delta^{13}C_{tissue}$ denoting either $\delta^{13}C_{hair}$ for estimation of diet-hair discrimination ($^{13}\Delta_{DH}$) or $\delta^{13}C_{faeces}$ for estimation of diet-faeces discrimination ($^{13}\Delta_{DF}$).

These discriminations are often treated as constants (e.g. Codron *et al.*, 2007; Norman *et al.*, 2009; Sponheimer *et al.*, 2003a) although there is some variation in the data. Certainly, some (and perhaps most) of the variation is due to error, such as sampling and analytical error or isotopic non-equilibrium between diet and tissue when slowly turning over body pools contribute to hair growth and defecation (Ayliffe *et al.*, 2004; Zazzo, *et al.*, 2008; Auerswald *et al.*, 2009). While the variation in discrimination may be of little relevance, if C3 diets are compared with C4 diets due to the large isotopic spacing between both, the spacing becomes small and the variation in discrimination becomes relevant if the objective is to detect a change in diet from C3/C4 from 0.8/0.2 to 0.7/0.3. In such cases it is important to know, under which conditions, discrimination is constant and when it changes as a function of animal, diet, habitat or environment.

Where ruminants graze freely in C3/C4 grassland, the isotopic composition of the herbage eaten (diet) is different from the isotopic composition of the vegetation (=herbage on offer) as it is influenced by diet selection, which is usually unknown. However, the relationship between vegetation ^{13}C and hair ^{13}C (i.e. vegetation-hair discrimination, $^{13}\Delta_{VH}$) provides a clue about selection given that digestibility is identical and isotopic scrambling in the rumen

distributes C3 and C4 derived components evenly among microbial protein: $^{13}\Delta_{VH}$ should be <–3‰, if animals prefer the C3 component, and >–3‰ if the C4 component is preferred. In the same way, vegetation-faeces discrimination ($^{13}\Delta_{VF}$) should be <0.6‰, if animals prefer C3, and >0.6‰ if C4 is preferred. Furthermore, the comparison of vegetation-hair and vegetation-faeces discrimination should provide a clue about the relative digestibilities of the C3 and C4 components of the diet. If the ingested C4 is less digestible than the C3, then the C4 will contribute a larger fraction of the total faecal output, causing a less positive diet-faeces discrimination. Consequently, the C4 contribution to hair growth would be smaller, hence causing a less negative diet-hair discrimination and both causing a two-fold effect on the faeces-hair discrimination. Therefore, selection and digestibility have different effects on diet-hair and diet-faeces discrimination, and can thus be distinguished based on ^{13}C analysis of vegetation (herbage on offer), hair and faeces. Whereas selection would influence $^{13}\Delta_{DF}$ and $^{13}\Delta_{DH}$ in the same direction, effects of differential digestibility should have opposite effects (Fig. III.1). As far as we know, these putative effects have never been explored with domestic ruminants grazing C3/C4 grassland.

This work was conducted to shed light on this issue, and in particular to explore the potential of sheep hair and faeces as archives of the carbon isotope composition of Inner Mongolia C3/C4 grassland. The study was conducted in the frame of an extensive grazing experiment, with a wide range of stocking rates which determined opportunities for selective grazing of C3 and C4 components. There was little opportunity for selection at high stocking rates (=high grazing pressure), but ample opportunity for selection at low stocking rates (=low grazing pressure). Effects of selection and digestibility were analyzed in terms of diet-hair and diet-faeces discrimination (as predicted in Fig. III.1).
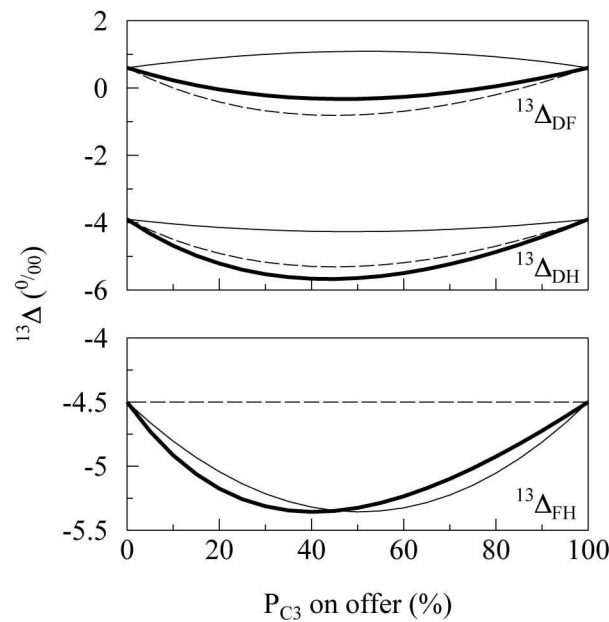
Fig. III.1 Modeled apparent diet-tissue discriminations (top box: diet-faeces discrimination, $^{13}\Delta_{DF}$, and diet-hair discrimination, $^{13}\Delta_{DH}$) and resulting faeces-hair discrimination, $^{13}\Delta_{FH}$ (bottom box) with increasing relative abundance of C3 plants on offer. In the calculations, we assumed a higher digestibility of the C3 component and a higher selection of the presumably more palatable C3 component: solid lines denote a better digestibility of the C3 component (D3 = 60% and D4 = 54%) without selection (S3 = S4 = 100%); the dashed lines denote a preference of the C3 component over the C4 component (S3 = 120% and S4 = 80%) at the same digestibility (D3 = D4 = 56%); the bold lines denote the combined effect of higher digestibility and higher selection of the C3 component (D3 = 60%, D4 = 54%, S3 = 120% and S4 = 80%). Other input parameters were taken as constants (values that are applicable to the research area; see eqns. III.4-III.11: $d\delta^{13}C$ = –12.1‰, $p^{13}\Delta_{DF}$ = 0.6‰, $p^{13}\Delta_{DH}$ = –3.9‰).

Material and Methods

Grazing Experiment

The grazing experiment was situated 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 Forschungsgemeinschaft research group 536 MAGIM (www.magim.net). The vegetation is a semi-arid steppe, dominated by perennial C3 grasses (mainly *Stipa grandis* and *Leymus chinensis*), but perennial C4 grasses (mainly *Cleistogenes squarrosa*) and annual C4 herbs (mainly *Salsola collina*) were also present.

The growing period in Inner Mongolia lasts from April/May to September/October. During this period the mean nitrogen content of aboveground biomass decreases from about 2.1% to 0.7% (Liu, 1993). Growth of C4 plants starts about 30 to 50 days later (Bai *et al.*, 2004).

The grazing experiment consisted of 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}). The sheep were about 2 years old non-pregnant and non-lactating ewes from the local Mongolian fat-tail breed.

Vegetation samples were taken at around 1st to 16th of July, August or September of 2005, 2006 and 2007. Bulk vegetation samples were obtained from the outside of three frames of 0.5 m² in pasture cages (hereafter cage) and from the inside of pastures (hereafter pasture). Thus, we determined the proportion of vegetation ingested by the animals. In both situations, vegetation was clipped at 1 cm stubble height. After clipping, the cages were moved to a previously grazed part to determine growth for the subsequent month. In total 217 vegetation samples from cages and 178 vegetation samples from pastures were analyzed. At the same time, fresh faeces were sampled in 2005 and in 2006. In 2007, faeces sampling was performed at around 20th to 30th of June, July and August. In total, we analyzed 152 faeces samples. In addition, in 2007, we sampled the leaves of the dominant C3 species (*S. grandis*, n=72) and C4 species (*C. squarrosa*, n=72) in the grazed area.

Hair samples originated from shearing at the end of the grazing period in late September of the years 2005, 2006 and 2007. This included only hair grown during the grazing period because as sheep had also been shorn just before the beginning of the grazing period (June). In total, 40 hair samples were analyzed.

Sample preparation, isotope and elemental analysis

Plant and faeces samples were dried prior to milling: plant samples for 48 hours at 60°C and faeces for 48 hours at 40°C. Plant and faeces samples were ground to homogeneity with a ball mill; 0.7-0.8 mg of plant or faeces material per tin cup. The distal first cm of the hair samples was discarded. This accounted for the stubbles left after shearing, which were marked by dye to account for the proportion of hair within the hair channel and turnover of body pools. The remaining hair (approximately 4 to 6 cm) was cleaned by the procedure of Schwertl *et al*. (2003), ground to homogeneity with a ball mill, and 0.2-0.4 mg hair material was packed into tin cups for isotope analysis.

The δ^{13}C and the nitrogen content of each sample 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). Carbon isotopic data are presented as δ^{13}C, with δ^{13}C = (R$_{sample}$/R$_{standard}$) − 1, where R is the ^{13}C/^{12}C ratio and standard is the Vienna Pee Dee Belemnite standard. Each sample was measured against a laboratory working

standard CO_2 gas, which was previously calibrated against an IAEA secondary standard (IAEA-CH6, accuracy of calibration 0.06‰ SD). After every tenth sample a solid internal lab standard (SILS) with similar C/N ratio as the sample material (fine ground wheat flour for plant and faeces material, and protein powder for hair material) was run as a blind control. The SILS were previously calibrated against an international standard (IAEA-CH6). The precision for sample repeats was better than 0.15‰ (standard deviation).

All data on $\delta^{13}C$ of vegetation, faeces and wool and the data on nitrogen content of vegetation and faeces are available in Table 1 – 3 in Appendix A in the online version of this article.

Calculation of dietary $\delta^{13}C$ and ^{13}C discrimination

The $\delta^{13}C$ of the diet ($\delta^{13}C_{diet}$) was estimated using an isotopic mass balance:

$$\delta^{13}C_{diet} = \frac{\delta^{13}C_{cage} \times DM_{cage} - \delta^{13}C_{pasture} \times DM_{pasture}}{DM_{cage} - DM_{pasture}} \qquad (III.2)$$

with $\delta^{13}C$ of aboveground biomass samples taken inside the cage ($\delta^{13}C_{cage}$) and on the pasture ($\delta^{13}C_{pasture}$) and the aboveground dry biomass of the respective samples taken inside the cage (DM_{cage}) and on the pasture ($DM_{pasture}$). The estimated $\delta^{13}C_{diet}$ may have a large error when subtrahend and minuend in the denominator are similar. This was not the case as DM_{cage} was on average 38% higher than $DM_{pasture}$.

The different ^{13}C discriminations (diet-hair, diet-faeces, faeces-hair) could then be calculated from the measured isotopic composition of the different tissues and the dietary ^{13}C according to eqn. III.1. Additionally, the proportion of C3 plants (P_{C3}) and C4 plants ($P_{C4} = 1-P_{C3}$) at each site was estimated from the isotopic composition of the aboveground biomass following Auerswald *et al.* (2009).

Faecal nitrogen content, digestibility and relative feed intake

Faecal nitrogen content ($\%N_{faeces}$) can be used as a proxy for digestibility (Boval *et al.*, 2003; Wang *et al.*, 2009). This relation is based on the decreasing amount of diet and increasing relative amount of undigested ruminal microbial protein excretion in faeces as dietary digestibility increases. Mean digestibility of organic matter was estimated using the equation by Wang *et al.* (2009), which has been derived in the same environment. Relative feed intake was calculated as:

$$\text{relative feed intake} = \frac{DM_{cage} - DM_{pasture}}{DM_{cage}} \qquad \text{(III.3)}$$

Statistical methods and outliers

Linear regressions and multiple non-linear regressions (weighted least-squares estimations) were used to evaluate the datasets. The coefficients of determination were tested with a two-sided test for significance of the regressions. Regression coefficients were tested on parity with Student's t-test. Hypothesis testing on equal means of groups or on parity of the mean of the population and a specified value were carried out using Student's t-test (two-sided). This was generally performed against a 95% confidence interval, preceded by a test for normal distribution and if necessary by an F-test on parity of variances. Mean values are presented with 95% confidence intervals (CI) prefixed by a plus-minus sign. Significance thresholds of $P \geq 0.05$ (n.s.), $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***) were used. All procedures followed standard protocols (Sachs & Hedderich, 2006) and were carried out using GNU R 2.9.0 (R Development Core Team, 2009). Two $\delta^{13}C$ outliers in the vegetation data and seven $\%N_{faeces}$ outliers were removed from the data set.

Modelling of apparent diet-tissue and tissue-tissue discriminations

The apparent diet-tissue and tissue-tissue discriminations were modeled with the following equations:

$$S4 = 2 - S3 \qquad \text{(III.4)}$$

$$sC4 = \frac{S4 \times (1 - P_{C3})}{S4 \times (1 - P_{C3}) + S3 \times P_{C3}} \qquad \text{(III.5)}$$

$$sC3 = 1 - sC4 \qquad \text{(III.6)}$$

$$dC4 = \frac{D4 \times sC4}{D4 \times sC4 + D3 \times sC3} \qquad \text{(III.7)}$$

$$rC4 = \frac{(1 - D4) \times sC4}{(1 - D4) \times sC4 + (1 - D3) \times sC3} \qquad \text{(III.8)}$$

$$^{13}\Delta_{DF} = p^{13}\Delta_{DF} + (1 - P_{C3} - rC4) \times d\delta^{13}C \qquad \text{(III.9)}$$

$$^{13}\Delta_{DH} = p^{13}\Delta_{DH} + (1 - P_{C3} - dC4) \times d\delta^{13}C \qquad \text{(III.10)}$$

$$^{13}\Delta_{FH} = \frac{1 + {}^{13}\Delta_{DF}}{1 + {}^{13}\Delta_{DH}} - 1 \qquad \text{(III.11)}$$

where S4 denotes the relative selectivity of C4 biomass, S3 the relative selectivity of C3 biomass, P_{C3} the relative contribution of C3 species to aboveground biomass, sC3 the selected C3 biomass, sC4 the selected C4 biomass, dC4 the digested proportion of C4 biomass, D4 the digestibility of the ingested C4 biomass, D3 the digestibility of the ingested C3 biomass, rC4 the residual proportion of C4 biomass in the faeces, $p^{13}\Delta_{DF}$ the 'pure' diet-faeces discrimination, $p^{13}\Delta_{DH}$ the 'pure' diet-hair discrimination, $d\delta^{13}C$ the difference of $\delta^{13}C$ between C3 and C4 species, and $^{13}\Delta_{FH}$ the faeces-hair discrimination. Here, digestibility is defined as the relative fraction of the either C3 or C4 derived biomass in faeces to the ingested respective biomass. Relative selectivity is defined as the ratio of ingested C3 or C4 derived mass to the respective mass in the vegetation and 'pure' discrimination as the $^{13}\Delta$ arising for an infinite feeding of one component (i.e. not influenced of either the digestibility or selectivity).

Results

Seasonal change in abundance and leaf nitrogen content of C3 and C4 plants

P_{C3} on offer, calculated from $\delta^{13}C$ of bulk vegetation from the cages, decreased significantly during the growing period (P<<0.001; not shown) due to the later growth initiation of C4 plants (Bai *et al*., 2004). On the 170th day of the year (mid of June) approximately 95% of aboveground biomass consisted of C3 plants, while by the 270th day of the year (end of September) P_{C3} had dropped to approximately 80%.

On average, the leaf nitrogen content of the C3 component (%N_{C3}) was 0.35% less than that of the C4 component (%N_{C4}) (P<<0.001; Table III.1). This was true until September (P<0.01) when C3 and C4 biomass had the same digestibility. The nitrogen content of herbage on offer (bulk vegetation from the cages) decreased from 1.8±0.1% (CI) in July to 1.7±0.1% (CI) in August and finally to 1.5±0.1% (CI) in September (see Appendix in online version of this article).

Table III.1 Mean differences between the carbon isotopic composition of vegetation from inside of pasture cages and from the pasture ($\delta^{13}C_{cage}$–$\delta^{13}C_{pasture}$), mean diet-faeces discrimination ($^{13}\Delta_{DF}$) and mean difference of nitrogen content between the C4 and the C3 component (%N_{C4}–%N_{C3}). The 95% confidence intervals (CI) and error probabilities (P) for July, August, September and the grazing period mean are given. P_1 denotes the significance of a mean larger than 0. P_2 denotes the significance of the $^{13}\Delta_{DF}$ deviating from 0.6‰, the average $^{13}\Delta_{DF}$ compiled from literature.

Month	$\delta^{13}C_{cage}$–$\delta^{13}C_{pasture}$ (‰)		$^{13}\Delta_{DF}$ (‰)			%N_{C4}–%N_{C3} (%)	
	Mean ± CI	P_1	Mean ± CI	P_1	P_2	Mean ± CI	P_1
July	0.31 ± 0.21	0.005	0.39 ± 0.87	0.360	0.646	0.20 ± 0.08	0.002
August	0.46 ± 0.16	<<0.001	0.48 ± 0.55	0.084	0.659	0.61 ± 0.10	<<0.001
September	0.23 ± 0.26	0.08	0.96 ± 0.78	0.017	0.355	0.24 ± 0.10	0.006
Mean	0.34 ± 0.10	<<0.001	0.61 ± 0.41	0.004	0.995	0.35 ± 0.04	<<0.001

Seasonal change in %N_{faeces}

The %N_{faeces} decreased from 1.9% (July) to 1.5% (September) during the grazing period (P<<0.001; not shown). Following Wang *et al*. (2009), this corresponds to a digestibility of organic matter of approximately 57% in July and 52% in September. Multiple non-linear regression analysis exhibited no significant influence of P_{C3} (P>0.5) on %N_{faeces}. The day of year was the only significant (P<<0.001) variable explaining %N_{faeces}.

Apparent selective grazing of sheep

Preferential grazing of one photosynthetic type is reflected in a difference between $\delta^{13}C_{cage}$ and $\delta^{13}C_{pasture}$. The mean $\delta^{13}C_{cage}$–$\delta^{13}C_{pasture}$ was significantly higher than 0, for July, August and for the grazing period mean (all P<0.05; Table III.1; Fig. III.2) but not for September (P>0.05; Table III.1; Fig. III.2). This might indicate a preferential grazing of the C4 component in the early grazing period. The highest mean $\delta^{13}C_{cage}$–$\delta^{13}C_{pasture}$ of 0.5±0.2‰ (CI) occurred in August, coinciding with C4 peak biomass. Grazing period mean $\delta^{13}C_{cage}$–$\delta^{13}C_{pasture}$ was 0.3±0.1‰ (CI) (Table III.1). Given a 12.1‰ difference in $\delta^{13}C$ between C3 and C4 species (unpublished data of >300 samples), this effect corresponded to a preference of C4 biomass of 4% in August and 2% throughout the grazing period. There was no significant difference, however, in mean $\delta^{13}C_{cage}$–$\delta^{13}C_{pasture}$ between the stocking rates (all P>0.05, Fig. III.3) although $\delta^{13}C_{cage}$–$\delta^{13}C_{pasture}$ was significantly larger than 0 only for 0.75, 1.5 and 1.875 sheep ha^{-1} year^{-1}.
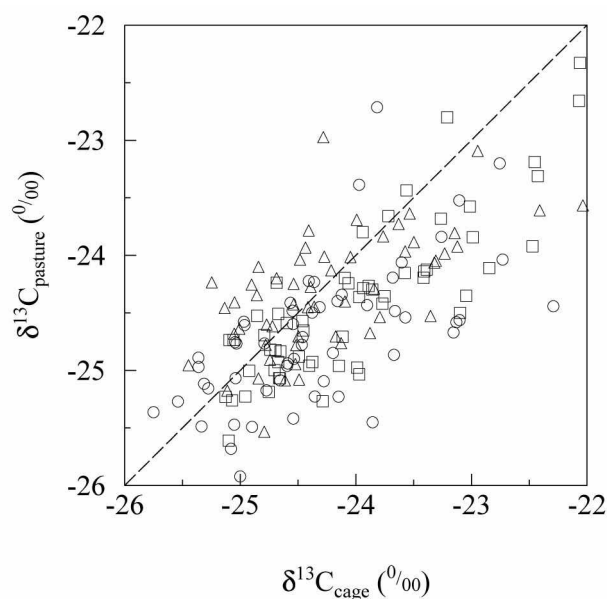
Fig. III.2 Relationship between the carbon isotopic composition of vegetation from inside of pasture cages ($\delta^{13}C_{cage}$) and from the pasture ($\delta^{13}C_{pasture}$) for July ($\circ$), August ($\square$) and September (Δ). Dashed line denotes the 1:1 line.

One might expect a stronger relationship of $\delta^{13}C_{cage}$–$\delta^{13}C_{pasture}$ with relative feed intake than with stocking rate, as relative feed intake reflects the real opportunity for selection. When relative feed intake varied from 5% to 80%, there was no clear effect on $\delta^{13}C_{cage}$–$\delta^{13}C_{pasture}$ (Fig. III.4). This meant that C4 may have been slightly preferred but this effect did not vary with feed intake.
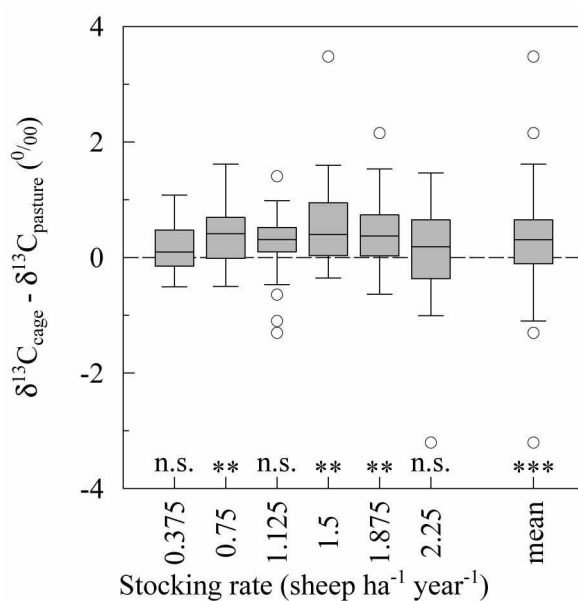
Fig. III.3 Box plot for the difference between the isotopic composition of vegetation from the cage ($\delta^{13}C_{cage}$) and from the pasture ($\delta^{13}C_{pasture}$) for all stocking rates and the overall mean. Boxes show the 75% percentile, the median and the 25% percentile; whiskers denote 1.5 times the interquartile range; outliers bigger than that are denoted as circles. The bottom line denotes the significance of the mean deviating from 0.

Within the experiment, relative feed intake covered a wide range. The common stocking rate in the region is slightly above 1 sheep ha^{-1} year^{-1}. For this stocking rate, feed intake ranged between 20% and 60% while $\delta^{13}C_{cage}$–$\delta^{13}C_{pasture}$ varied from –1.3‰ to +1.0‰. Multiple non-linear regression analysis indicated no significant effect of relative feed intake or day of year (P>0.5) on $\delta^{13}C_{cage}$–$\delta^{13}C_{pasture}$.
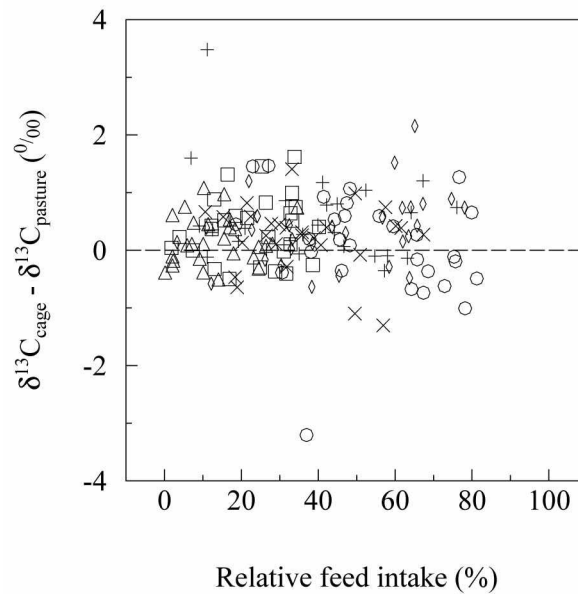
Fig. III.4 Relationship between relative feed intake (%) and difference between the isotopic composition of vegetation from the cage ($\delta^{13}C_{cage}$) and from the pasture ($\delta^{13}C_{pasture}$) for all stocking rates: 0.375 (△), 0.75 (□), 1.125 (×), 1.5 (+), 1.875 (◇) and 2.25 (○) sheep ha^{-1} year^{-1}. The relationships of the individual stocking rates were all not significant (P>0.1).

Seasonal variation in diet-faeces discrimination

The $^{13}\Delta_{DF}$ did not differ among stocking rates (all P>0.1; not shown). The faeces were slightly less depleted in July (0.4±0.9‰ (CI)) compared to diet than in September (1.0±0.8‰ (CI)). Overall the difference between months was 0.6‰. In no month, did $^{13}\Delta_{DF}$ differ significantly from the mean 0.6‰ that was observed in a literature compilation of $^{13}\Delta_{DF}$ for pure and mixed C3 and C4 diets (Jones *et al*., 1979; Coates *et al*., 1991; Norman *et al.,* 2009). In July and August, the $^{13}\Delta_{DF}$ was not significantly different from 0, but in September and for the grazing period mean the $^{13}\Delta_{DF}$ differed significantly from 0 (Table III.1).

Relation between $\delta^{13}C$ of faeces and diet compared to hair

The change in $\delta^{13}C_{faeces}$ or $\delta^{13}C_{diet}$ was not significantly different from the respective change in $\delta^{13}C_{hair}$ (both P>0.1; Fig. III.5). This indicated constant faeces-hair ($^{13}\Delta_{FH}$) and diet-hair ($^{13}\Delta_{DH}$) discriminations that were independent of the C3/C4 ratio of ingested biomass. The average $^{13}\Delta_{FH}$ was $-4.3\pm0.2‰$ (CI) and the average $^{13}\Delta_{DH}$ was $-3.9\pm0.3‰$ (CI) (Fig. III.5). The latter was not significantly different from an average $^{13}\Delta_{DH}$ of $-3.3‰$ for sheep from literature (P>0.1).
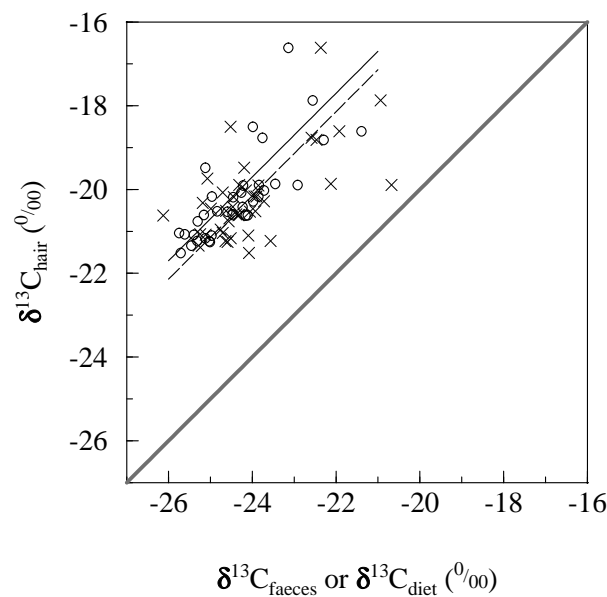
Fig. III.5 $\delta^{13}C$ of hair and grazing period mean $\delta^{13}C$ of faeces ($\circ$, solid line) or diet ($\times$, dashed line). The lines denote a constant $^{13}\Delta_{FH}$ of $-4.3‰$ and a constant $^{13}\Delta_{DH}$ of $-3.9‰$ respectively. The bold grey line denotes the 1:1 line.

Discussion

Influence of grazing pressure on apparent selection

Our experimental design provided a broad range of stocking rates and yielded little evidence for effects of stocking rate (and related grazing pressure and relative feed intake) on C3 versus C4 selection. This is not an intuitive finding: one might expect that grazers (such as sheep, goat and cattle) select for a certain component when herbage is available in ample supply. Conversely, if competition for feed is high (i.e. when stocking rates increase) then the opportunity for selection is low (e.g. Edwards *et al.*, 1994; Mobæk *et al.*, 2009). Potential causes for selection are multiple: selection for certain parts of plants driven by taste and odour (Hülber *et al.*, 2005), species composition (Jones & Hu, 2006; Kausrud *et al.*, 2006; Mobæk

et al., 2009), site productivity (Kausrud *et al*., 2006; Mobæk *et al*., 2009) or dietary quality (Garcia *et al*., 2003). However, we found no effect of stocking rate and relative feed intake on the relationship between the ^{13}C of ingested feed, meaning that variable opportunities for selection had actually no effect on selection for C3 and C4. Hence, the contrast in attributes that drive selection between C3 or C4 species was apparently too small to cause an effect in this typical Inner Mongolian steppe. This finding agrees with those of Hodgkinson *et al*. (1995) who examined the defoliation pattern of two contrasting C3 and C4 grasses in semi-arid and arid Australian woodland and found no clear effect of grazing pressure on selection. Further, Schiborra *et al*. (2009) compared the organic matter digestibility of herbage on offer and herbage ingested in the same experiment and found no evidence of an effect of grazing intensity on selection.

The small difference between $\delta^{13}C_{cage}$ and $\delta^{13}C_{pasture}$ (+0.3±0.1‰ CI) was barely significant owing to the large number of data points (*n*=177) and such a small effect could be due to a number of reasons. Most likely the effect is an artefact of the sampling, e.g. when clipping height did not exactly match the biting height (Davies *et al.*, 1950; Griffiths *et al.*, 2003). Importantly, the lack of any correlation to parameters that indicate the opportunity of selection like stocking rate or relative feed intake indicates that the difference does not originate from selection.

Influence of growth initiation and digestibility

Several studies have reported an inferior digestibility of C4 species compared to C3 species (e.g. Barbehenn *et al*., 2004; Norman *et al*., 2009). But digestibility also decreases strongly with plant age (Wilson & Hacker, 1987; Buxton, 1996). As the C4 species of the Inner Mongolian grassland initiate growth about 30 to 50 days later than C3 plants (Bai *et al*., 2004), the C4 species likely have a relatively high digestibility (see Milchunas *et al.*, 1995). This agrees with the present results in that the average nitrogen content of the C4 component was actually higher than that of the C3 component and that %N of herbage on offer decreased significantly from August to September (P<<0.001). In the same experiment, Schiborra *et al.* (2009) analysed *in vitro* digestibility of organic matter and found the same effects.

However, a significant influence of differential digestibility on $^{13}\Delta_{DF}$ was not evident. The $^{13}\Delta_{DF}$ was not significantly different from the mean $^{13}\Delta_{DF}$ of 0.6‰ which was observed when sheep were fed pure and mixed C3 or C4 diets (Jones *et al.*, 1979; Coates *et al.*, 1991; Norman *et al*., 2009). Also, $^{13}\Delta_{DF}$ was near-constant during the grazing period.

Differences in digestibility between C3 and C4 components should also influence $^{13}\Delta_{DH}$. As digestibility influences $^{13}\Delta_{DF}$ and $^{13}\Delta_{DH}$ in opposite directions (Fig. III.1), the effect of differential digestibility on $^{13}\Delta_{FH}$ should be even more pronounced ($^{13}\Delta_{FH}$ is equal to the difference between $^{13}\Delta_{DF}$ and $^{13}\Delta_{DH}$). $^{13}\Delta_{FH}$ was –4.3‰, close to the expected value (–3.9‰) that was derived from published estimates of $^{13}\Delta_{DF}$ (mean –0.6‰) and $^{13}\Delta_{DH}$ (mean –3.3‰). Furthermore, there was no systematic variation in the $^{13}\Delta_{FH}$ related to an increase in the proportion of C4. Thus there was no evidence for effects of differential digestibility on $^{13}\Delta_{DF}$ or $^{13}\Delta_{DH}$.

δ¹³C of faeces and hair as a tool to estimate the C3/C4 ratio of vegetation

The above findings are important for the estimation/reconstruction of C3/C4 ratios of grassland from the $\delta^{13}C$ of sheep faeces and wool. To a close approximation, sheep grazed C3 and C4 vegetation non-selectively, and both components had similar digestibility, yielding approximately constant $^{13}\Delta_{DF}$ and $^{13}\Delta_{DH}$. Thus, the $\delta^{13}C$ of grassland vegetation could be predicted from that of faeces or wool by applying a constant discrimination, which was related to constant animal properties. Notably, the integration over time (and, hence, space if animals range unconfined) usually differs between faeces (a few days) and hair (a few months to one year). This can be used to estimate C3/C4 ratios at different scales from $\delta^{13}C$ by analyzing the appropriate tissue. Faeces integrate the diet of less than one week (Raymond, 1948), which corresponds to the area grazed in less than one week, while hair integrates the isotopic signal of the diet ingested between two shearings. Thus, hair provides a much higher degree of spatio-temporal integration (or averaging) and is more suitable for the assessment of yearly mean C3/C4 composition, when the latter fluctuates seasonally or spatially within the grazing area of a flock of sheep. Evidently, a time series of hair samples would be useful for the reconstruction of long-term changes in the C3/C4.

Conclusions

The C3/C4 ratio of the Inner Mongolian grassland as indicated by community $\delta^{13}C$ varied in space and time due to the differential growth initiation of C3 and C4 plant species. Instantaneous sampling of vegetation did not reflect the C3/C4 ratio of annual growth under these conditions. The $\delta^{13}C$ of faeces and hair integrated over the grazing area and period, with integration times differing for hair and faeces. Neither faeces nor hair were influenced by selective grazing of sheep within a reasonable degree of uncertainty. The mean $^{13}\Delta_{DF}$ of

$0.6\pm0.4‰$ (CI) and the mean $^{13}\Delta_{DH}$ of $-3.9\pm0.3‰$ (CI) in this study were close to published estimates obtained with C3 or C4 feeding. $^{13}\Delta_{FH}$ of $-4.3\pm0.2‰$ (CI) was near-constant, although this parameter should respond strongly to selective grazing and differences in digestibility between the C3- and C4-component. This indicated that discrimination was not influenced by differential digestibility of C3 and C4 species within uncertainty. Our findings demonstrate that $\delta^{13}C_{faeces}$ and $\delta^{13}C_{hair}$ are suitable proxies for the C3/C4 ratio of Inner Mongolia grassland.

CHAPTER IV – Stocking rate of sheep does not affect nitrogen isotope fractionation between grazed vegetation, soil, and faeces and wool

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 multi-year (2005−2007) experiment with different stocking rates (0.375 – 2.25 sheep ha^{-1} year^{-1}) in semi-arid Inner Mongolia grassland. Bulk vegetation nitrogen concentration varied between 0.9% and 3.7% of dry matter and increased with increasing stocking rate. Yet, we found no effect of stocking rate on δ^{15}N of vegetation, soil, and faeces and wool of sheep. Furthermore, we found no difference between δ^{15}N of vegetation and diet. Hence, ^{15}N fractionation between vegetation and faeces ($\varepsilon_{veg-faeces}$), vegetation and wool ($\varepsilon_{veg-wool}$), faeces and soil ($\varepsilon_{faeces-soil}$) and soil and vegetation ($\varepsilon_{soil-veg}$) was constant with $\varepsilon_{veg-faeces}$ = 3.0‰ (±0.1‰, 95% confidence interval), $\varepsilon_{veg-wool}$ = 5.3‰ (±0.1‰), $\varepsilon_{faeces-soil}$ = 1.1‰ (±0.4‰) and $\varepsilon_{soil-veg}$ = -4.1‰ (±0.3‰). This finding is useful for vegetation ^{15}N reconstruction from grazer tissues, particularly since selective grazing and grazing pressure are often unknown.

Introduction

Stable isotopes are increasingly used for studying ecosystem processes because they allow tracking the flow of elements and thus fluxes and processes associated with them. In the study of grassland vegetation, grazer tissues like wool (Hobson, 1999; Schnyder *et al.*, 2006; Auerswald *et al.*, 2009), horn (e.g. Barbosa *et al.*, 2009a), bone (e.g. Richards *et al.*, 2000), teeth (Cerling & Harris, 1999) and faeces (Sponheimer *et al.*, 2003b) have become favourite objects of study as variation in the isotopic composition of the grazed vegetation imprints the grazer's tissue with an isotopic signal (De Niro & Epstein, 1981). In turn, the isotopic composition of plants may reflect soil factors, such as nutrient cycling and losses (Högberg, 1997). For example, nitrogen isotope analysis of cattle hair shed light on farm-scale nitrogen balances (Schwertl *et al.*, 2005) and or allows retrospective view on (ingested) former grassland vegetation (e.g. Barbosa *et al.*, 2009a; Cerling *et al.*, 2009). The different tissues of animals have different turnover rates and so provide isotopic records with different temporal integration: bones the lifetime, wool the time between successive shearings, and faeces a period of less than one week (Raymond, 1948).

When using grazer tissue as a proxy for (grazed) vegetation, the isotopic offset between grazer tissue and vegetation must be known. This offset was often expressed as the diet-tissue shift $\Delta' = \delta_P - \delta_S$ (e.g. Männel *et al.*, 2007), where δ_P and δ_S denote the isotopic composition of the product and the source in common δ notation. Theoretical considerations show (Mariotti *et al.*, 1981; Auerswald *et al.*, 2010) that it should be expressed in terms of isotopic fractionation. This can either be 'enrichment' $\varepsilon_{S-P} = (\delta_P - \delta_S) / (1 + \delta_S)$, which is often used in the case of nitrogen (Mariotti *et al.*, 1981) and will be used by us in the following or 'discrimination' $\Delta_{P-S} = (\delta_S - \delta_P) / (1 + \delta_P)$ in the case of carbon (Auerswald *et al.*, 2009; Barbosa *et al.*, 2009a). For carbon the discrimination between diet and hair or faeces of sheep grazing Inner Mongolia pasture seemed to be constant over a wide range of conditions (Wittmer *et al.*, 2010). For nitrogen, it is known that enrichment within various animals (e.g. mammals, fish and insects) tends to decrease with increasing N concentration (or increasing dietary value or decreasing C:N ratio) of the diet (Adams & Sterner, 2000; Pearson *et al.*, 2003; Vanderklift & Ponsard, 2003; Robbins *et al.*, 2005). These relationships have – to our knowledge – not yet been verified for sheep grazing natural grassland. Thus, it is not clear in how far grazer tissues reflect the nitrogen isotopic composition of the grassland.

We see two main reasons why one may expect variable diet-tissue ^{15}N enrichment of grazers on natural grassland:

First, the grazer may select for the N-rich plants and plant parts, which are usually more digestible. Selection is particularly advantageous where the vegetation has a low digestibility, as is often the case in semi-arid grasslands. Opportunities for selection arise if plants species differ in their stage of development. Such a systematic difference is to be expected in C3/C4 mixed grassland, such the vast Central Asia steppe. C4 plants start growth at higher temperature, and thus later in the season, than C3 plants (Ehleringer *et al.*, 1997). So, C4 plants 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 more mature, less palatable and digestible plants. Such a situation likely offers opportunities and motivation for selective grazing. The grazer ^{15}N composition would then not reflect the total vegetation, but rather that of the selected diet, causing the apparent vegetation-tissue enrichment to deviate from that obtained with non-selective grazing

Second, the grazer causes a feedback mechanism. As it removes leaves, it initiates re-growth and reduces leaf age. Young leaves usually have a higher N concentration than old leaves (Field, 1983). Thus, grazing changes the N concentration of the vegetation. As grazing pressure is usually not controlled in regional studies, a variation in grazing pressure may lead to variation in N concentration of grazed vegetation and an ensuing change in vegetation-tissue enrichment.

The grazer also changes the N return into the soil. The organic N pool of the soil is either fed by senescent plant parts, which is associated with the vegetation-soil enrichment, or by faeces, which is associated with the faeces-soil enrichment. Under ungrazed conditions senescence dominates while under heavily grazed conditions almost all above-ground biomass-N is returned to the soil as excrements (Baron *et al.*, 2002), which in turn could influence the apparent enrichment between soil and vegetation (Högberg, 1997).

Our first hypotheses is that differences in nitrogen content and δ^{15}N exist within grazed plots, which can affect apparent enrichments between vegetation and grazer tissues due to selection. The second hypothesis is that the nitrogen concentration of the bulk vegetation increases with grazing pressure and that this causes a decreasing diet-tissue enrichment.

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 Central

Asian grassland and where typically grazing range from almost ungrazed areas where drinking water is not available to heavily grazed areas.

Material & Methods

Grazing experiment and sampling procedure

The grazing experiment was situated 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 Forschungsgemeinschaft research group 536 MAGIM (www.magim.net). The vegetation is a semi-arid steppe, 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, although present (*Oxytropis myriophylla*, *Melilotoides ruthenica, Astragalus galactites*, *Lespedeza davurica*) accounted for less than 1% of total biomass. The soils were classified as Calcic Chernozems (Wiesmeier *et al*., 2009).

The growing period in Inner Mongolia lasts from April/May to September/October. During this period, the mean nitrogen concentration in aboveground 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. IV.1). The experiment covered a total area of approximately 2 km². The sheep (*Ovis aries*) were about 2 years old, non-pregnant, and non-lactating ewes from the local Mongolian fat-tail breed. The sheep grazed the pasture from beginning of June until September. Previous to the experiment the whole area was moderately grazed by local semi-nomadic herders until 2003. Then the grass sward was left to recover for two years prior to the beginning of the experiment in 2005.
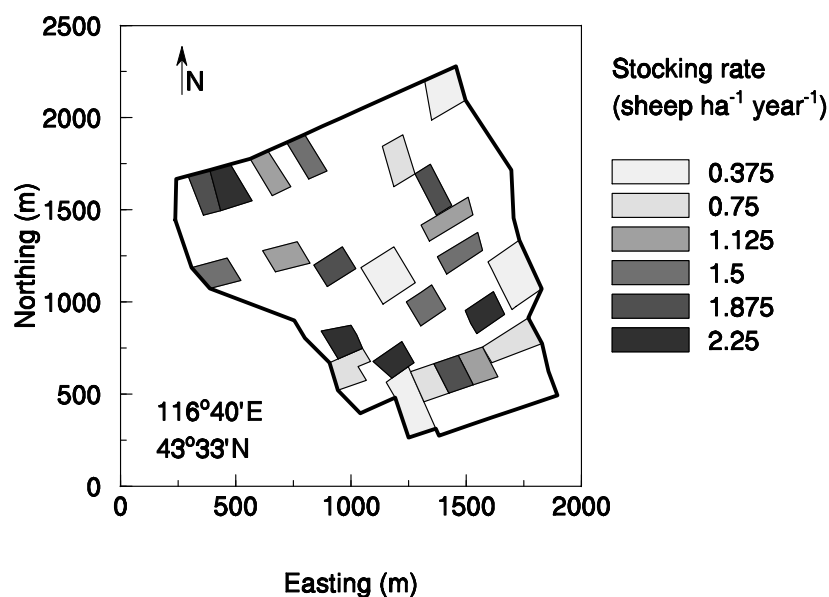
Fig. IV.1 Layout of the grazing experiment in Xilingol League in the Autonomus Region of Inner Mongolia, China. Six stocking rates were replicated four times.

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 situations, vegetation was clipped at 1 cm stubble height. After clipping, the cages were moved to a previously grazed part to determine growth for the subsequent month. In total, 217 vegetation samples from cages and 212 vegetation samples from the continuously grazed pastures were analyzed. Additionally, in 2007 we sampled the leaves of the three dominant species (*S. grandis*; n=72, *L. chinensis*; n=48, *C. squarrosa*; n=72), to determine eventual difference in isotopic composition of these species. These three species together accounted for about 75% of standing biomass. The selection for other species would have had little effect as *A. cristatum* and *C. korshinskyi* accounted for only 10% and the others for less than 3% of the standing biomass.

Fresh faeces were sampled at the time of vegetation sampling in 2005 and in 2006. In 2007, faeces sampling was performed at around the 20th to the 30th of June, July, and August. In total, we analyzed 152 faeces samples. Hair samples originated from shearing at the end of the grazing period in late September of the years 2005, 2006, and 2007. This included only hair grown during the grazing period because sheep had also been shorn just before the grazing period (June). In total, 40 wool samples were analyzed.

Top soil samples (0–10 cm) were taken in September 2007. In total, 24 soil samples were analyzed. Further information on the grazing experiment and the sampling can be found elsewhere (Schönbach *et al*., 2009; Wittmer *et al*., 2010).

Nitrogen isotope and elemental composition

Plant, faeces, and soil samples were dried prior to milling: plant and soil samples for 48 hours at 60°C and faeces for 48 hours at 40°C. Plant and faeces samples were ground to homogeneity with a ball mill and 0.7–0.8 mg of plant or faeces material per tin cup were analyzed. The distal first cm of the wool samples was discarded. This accounted for the stubble left after shearing, which was marked with dye, to account for the proportion of wool within the hair channel and the turnover of body pools. The remaining wool (approximately 4 to 6 cm) 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 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 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 is the air nitrogen standard. 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‰ SD). After every tenth sample, a solid internal lab 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‰ (standard deviation).

Nitrogen isotope enrichments between a certain source and a certain product were calculated. The δ^{15}N of the diet however could not be measured directly but was calculated from the mass and isotopic composition of the vegetation in the cage and on the pasture. Though 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.

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 statistical spread is reported as 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 & Hedderich, 2006). We excluded one outlier (vegetation data from pasture) from a total of 837 samples.

Results

The nitrogen concentration (%N in dry biomass) within a grazed plot varied by about a factor of three and the largest contrast occurred between different organs of a plant. 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 phytomers ($r^2 = 0.66$, n =35). Also, species differed in %N: *C. squarrosa,* which was similar to *L. chinensis* (2.71±0.10%, n=48), had consistently higher %N (mean 2.61±0.08%, n=72) than *S. grandis* (mean 2.26±0.06%, n=72) This difference was especially large in July when the species differed in %N by 0.6%.

Moreover there were large species- and phytomer-level differences in δ^{15}N. *C. squarrosa* had a consistently lower δ^{15}N (mean 0.29±0.13‰, n = 72), than *S. grandis* (mean 1.33±0.16‰, n=72) and *L. chinensis* (mean 1.19±0.21‰, n=48). Larger differences were evident between leaves of different age. For instance, the leaves of different phytomers of *C. squarrosa* differed by up to 8.0‰ with differences as large as 6.0‰ between successive phytomers. However, these differences within a plant were inconsistent (e.g. correlation with leaf nodal positions yielded $r^2=0.009$), perhaps due to (spatio-)temporal variation in the δ^{15}N of the soil nitrogen source.

Considerable differences also occurred between plots. The %N in bulk vegetation ranged from 1.3 to 2.4%. In general %N was lowest in September (1.52±0.07%) and highest in July (1.74±0.09%). 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. IV.2 top). Similarly, the %N of bulk vegetation increased with stocking rate (Fig. IV.2 bottom). A pair-wise t-test per stocking rate showed that %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.
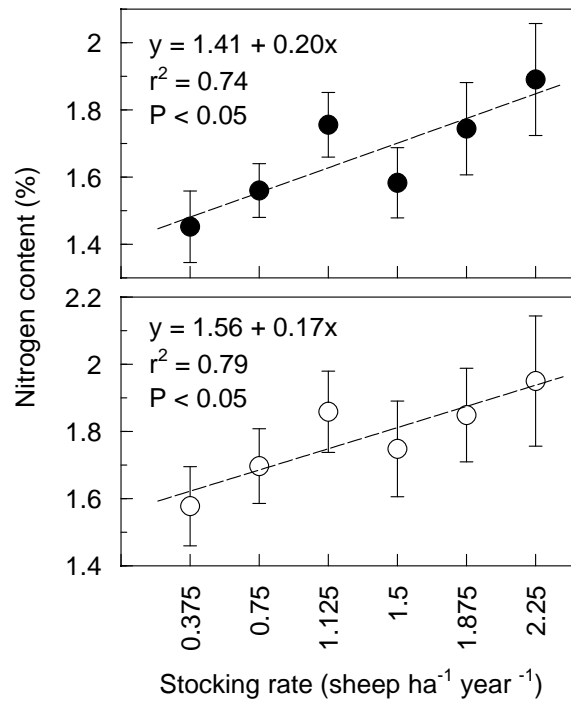
Fig. IV.2 Nitrogen concentration in bulk vegetation from the cages (●, top) and from the pasture (○, bottom) as related to stocking rate. Error bars denote the 95% confidence interval of the mean. Lines denote linear regressions.

The δ^{15}N varied over a range of 0.5‰ without a clear pattern: thus, there were no relationships between stocking rate and δ^{15}N from the cages, pasture, soil, faeces or wool (δ^{15}N$_{cage}$, δ^{15}N$_{pasture}$, δ^{15}N$_{soil}$, δ^{15}N$_{faeces}$ or δ^{15}N$_{wool}$, respectively) (all P>0.05, Fig. IV.3). The means averaged over all stocking rates were 0.56±0.09‰ for δ^{15}N$_{cage}$, 0.64±0.09‰ for δ^{15}N$_{pasture}$, 4.61±0.32‰ for δ^{15}N$_{soil}$, 3.61±0.12‰ for δ^{15}N$_{faeces}$, and 5.94±0.22‰ for δ^{15}N$_{wool}$ (Fig. IV.3). The means of the species were 1.33±0.16‰ for *S. grandis*, 1.19±0.20‰ for *L. chinensis* and -0.29±0.13‰ for *C. squarrosa*. Further, a pair-wise t-test per stocking rate showed that δ^{15}N$_{cage}$ was not significantly different from δ^{15}N$_{pasture}$ (all P>0.05). No net effects were evident that would relate to selection and thus would change with stocking rates despite a difference of about 1.5‰ between the dominant species, which together accounted for more than 75% of aboveground biomass. Consequently, the δ^{15}N of the diet was similar to δ^{15}N of the vegetation and there was no evidence of any ^{15}N enrichment between vegetation and diet. Accordingly, we did not distinguish between diet and vegetation in the following. The δ^{15}N of vegetation (δ^{15}N$_{veg}$) was calculated as the mean of δ^{15}N$_{cage}$ and δ^{15}N$_{pasture}$. The overall mean δ^{15}N$_{veg}$ was 0.60±0.06‰.
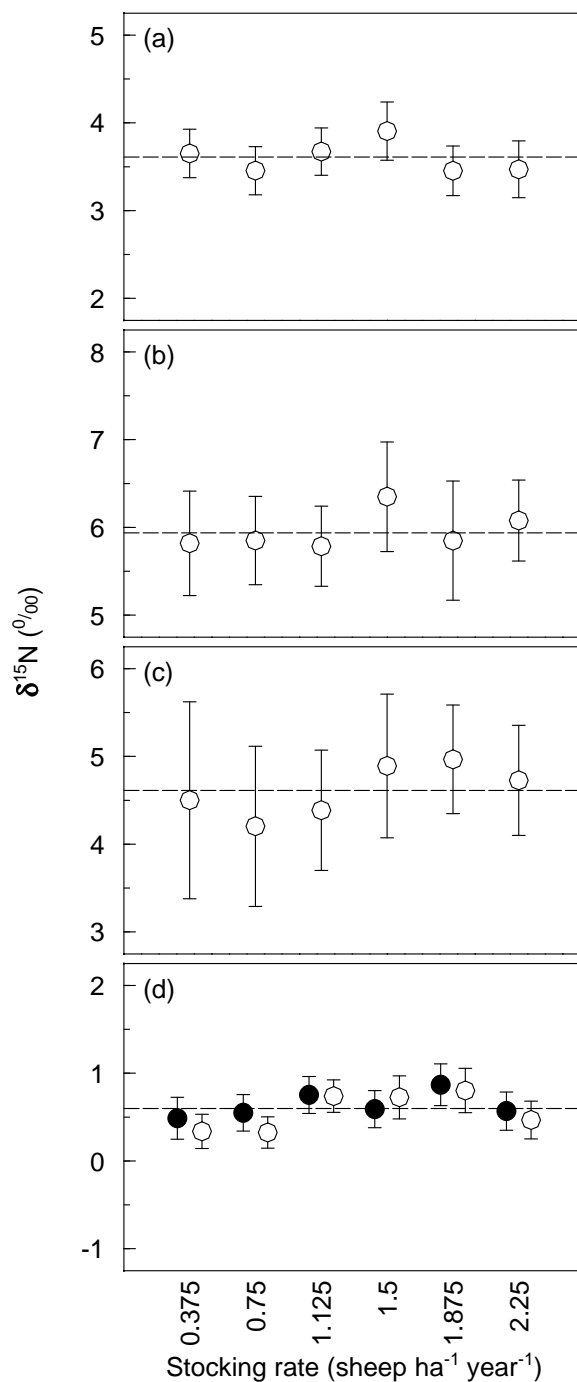
Fig. IV.3 Nitrogen isotope composition (δ^{15}N) of faeces (a) and wool (b) of sheep, soil (c) and vegetation (d) from the pasture (●) and the cages (○) in relation to stocking rate. Error bars denote the 95% confidence interval of the mean. Dashed lines denote the mean over all respective samples. Total *n* is 152, 40, 24, 211 and 217 for faeces, wool, soil, pasture and cage samples. To allow for comparison, the y-axis spans always 3.0‰.

The ^{15}N enrichment between individual components of the plant-soil-animal N cycle and in wool production were then calculated (Fig. IV.4). The largest enrichment within the cycle occurred between vegetation and soil ($\varepsilon_{\text{veg-soil}} = 4.1$‰). The enrichment between vegetation

and faeces was also large ($\varepsilon_{veg\text{-}faeces}$ = 3.0‰), but that between faeces and soil was small ($\varepsilon_{faeces\text{-}soil}$ = 1.1‰). ^{15}N enrichment in wool production was very large ($\varepsilon_{veg\text{-}wool}$ = 5.3‰).
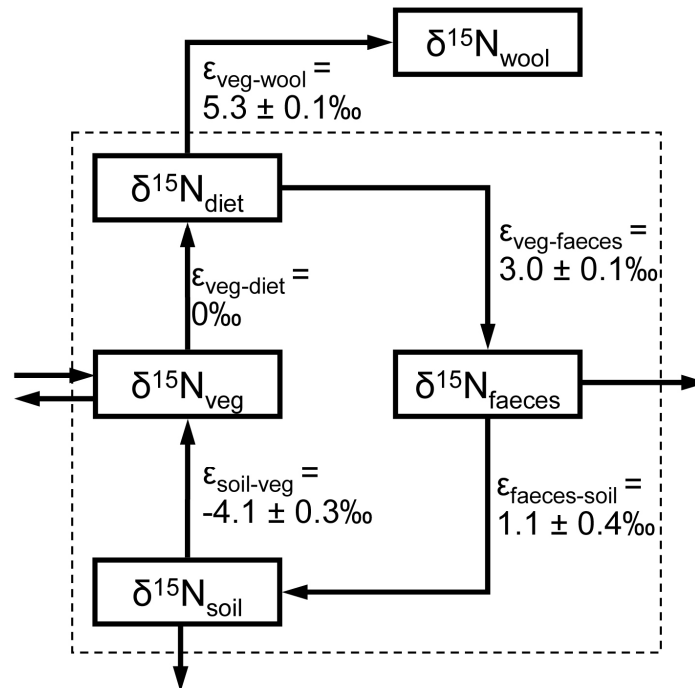
Fig. IV.4 Conceptual model of nitrogen isotope enrichment between vegetation, faeces, soil and wool. The dashed line denotes the plant-soil-animal system. Exports and imports are denoted by arrows out or in of the dashed box. Enrichments are reported together with the 95% confidence interval of the mean.

Discussion

No effect of selective grazing

In agreement with our first hypothesis there was considerable variation in %N within a grazed plot. %N was higher in young than in old leaves, consistent with many studies (e.g. Field 1983 and citations therein). Also, *C. squarrosa*, which started to grow later than the C3 species, had higher %N and additionally within *C. squarrosa* %N was higher in younger parts. Hence there was an opportunity for selective grazing of an N-rich diet, which could have caused a ^{15}N enrichment between whole vegetation and diet. Furthermore there was a 1.0‰ difference between *C. squarrosa* and the main C3 species from which an apparent enrichment due to the selection of a component of lower δ^{15}N would result. Both, the expected true enrichment due to the higher %N and the apparent enrichment due to the lower δ^{15}N of *C. squarrosa* act in the same direction and should cause a large effect if selection happens consistently. However, we did not find a difference between the δ^{15}N of the cage and

the pasture, meaning that selective grazing did not occur or that it was too small to cause an effect. This finding is consistent with data from carbon isotope analysis which presented no evidence for selection of *C. squarrosa* (Wittmer *et al.*, 2010).

The lack of any significant effect of %N$_{veg}$ on $\varepsilon_{veg\text{-}fec}$ or $\varepsilon_{veg\text{-}wool}$ is in contrast with results from controlled feeding experiments (e.g. Adams & Sterner, 2000; Pearson *et al.*, 2003; Robbins *et al.*, 2005) but it does not invalidate those results. That is because the effect was not different from zero but at the same time it was also not different from the weak effect reported from the controlled feeding experiments. In the study by Robbins *et al.* (2005) dietary %N ranged from 2.5 to 12%. This is much larger than the range of 0.9 to 3.7% in %N$_{veg}$, which is reported here and which is typical for the semi-arid grassland of Inner Mongolia (Liu, 1993; Chen *et al.*, 2007). Following the regression by Robbins *et al.* (2005), the present difference in %N$_{veg}$ caused by grazing (Fig. IV.2) would only cause a change in enrichment of less than 0.1‰, which is too small to be identified. Even the total range of individual samples (0.9–3.7%) would only cause a variation in enrichment of 0.3‰. This cannot be detected from body tissues of free-ranging grazers especially in consideration of the fact that the contrast of 0.9-3.7% only applies if different months are compared and full selection is assumed. The difference between months, however, is attenuated due to the relatively long nitrogen turnover times (e.g. more than eight weeks for new hair growth reported by Sponheimer *et al.*, 2003c) and full selection does not seem to occur.

Furthermore, the higher %N on the intensively grazed plots, where selection was less possible, might have been attenuated by selection of N-rich parts on the plots of low stocking rates, as ungulates tend to maximize their N uptake (Arnold, 1960; Garcia *et al.*, 2003). Hence, effects of grazing intensity on selection and isotopic enrichment were not apparent and if applicable they are possibly counteracting.

Enrichments between N compartments of grazed ecosystems

The $\varepsilon_{veg\text{-}wool}$ of 5.3‰ is similar to that reported by Sponheimer *et al.* (2003c) for goats fed on a pure *Medicago* diet (5.0‰) and to that of 5.2‰ established for several free ranging domestic animals (e.g. sheep, goat, yak) in Mongolia (Kohzu *et al.*, 2009). However, it slightly differs from the $\varepsilon_{veg\text{-}wool}$ of 3.6‰ for sheep grazing pure C3 vegetation reported by Männel *et al.* (2007), which is presumably caused by onetime sampling of Männel *et al.* (2007) where a seasonal variation in δ^{15}N in the vegetation would not be captured but contribute to the isotopic composition of the wool. In our experiment $\varepsilon_{veg\text{-}faeces}$ was 3.0‰, which was also found for sheep fed on a pure *Medicago* diet (Sutoh *et al.*, 1993). For goats,

Sutoh *et al.* (1987) reported slightly higher $\varepsilon_{veg\text{-}faeces}$ of about 3.6‰. For llama, $\varepsilon_{veg\text{-}faeces}$ was about 3.0‰ for animals fed either a pure *Medicago* or *Cynodon* diet (Sponheimer *et al.*, 2003b). This close similarity of most of the enrichments over a wide range of environmental and feed conditions indicates that both have little impact on the physiological process causing enrichment. Faecal δ^{15}N however could be altered after deposition, e.g. by gaseous losses like ammonia volatilization, which could be associated with pronounced enrichments if incomplete (Högberg, 1997). However, faeces mainly consist of protein, lignin and other indigestible components (van Soest, 1994). Gaseous losses from faeces and hence exogenous enrichments are small (Holst *et al.*, 2007; Ma *et al.*, 2007). Volatile compounds are mainly produced by microbial conversion of non-utilized nutrients and gastrointestinal products under anaerobic conditions (Le *et al.*, 2005; Shabtay *et al.*, 2009), while faeces dry fast under semi-arid conditions and become aerated. Furthermore, isotope effects can only result from a partial loss of volatile compounds, which is unlikely under semi-arid conditions and excluded by drying prior to analysis. This is supported by the tight 95% confidence interval of only 0.1‰ (Fig. IV.3) associated with $\varepsilon_{veg\text{-}faeces}$.

The $\varepsilon_{veg\text{-}soil}$ of 4.1‰ is similar to that reported by Cheng *et al.* (2009) of approximately 4.5‰ established along a transect in Inner Mongolia, and also similar to that predicted by Amundson *et al.* (2003) for the semi-arid Inner Mongolian grassland. Further, a meta-analysis of the data (inclduding grasslands, forests and woodlands) presented by Amundson *et al.* (2003) in the form of supporting material, exhibited no influence of mean annual precipitation on globally established $\varepsilon_{soil\text{-}veg}$ but a mean of -4.8‰, which is close to our $\varepsilon_{veg\text{-}soil}$. Watzka *et al.* (2006) reported a constant $\varepsilon_{soil\text{-}veg}$ of about -3.7‰ established for various fertilization regimes (e.g. no fertilization, mineral fertilizer, cattle manure, slurry or urine) of grasslands. Thus it appears that $\varepsilon_{veg\text{-}soil}$ is more or less constant along various environmental conditions (e.g. forest, natural grassland, managed grassland), which may indicate that the pathway of nitrogen cycling in the plant-soil system (e.g. directly *via* senescence or by grazer faeces) does not affect $\varepsilon_{soil\text{-}veg}$. This also follows from the present finding that $\varepsilon_{veg\text{-}soil}$ was constant in a wide range of grazing intensities differing in the consumption to senescence ratio. However, further investigations are needed to verify this assertion, especially with regard to the heterogeneity of soils and the associated uncertainties in estimating $\varepsilon_{soil\text{-}veg}$.

A precondition for the determination of enrichments is that the system has had enough time to equilibrate isotopically. This is usually difficult to achieve in controlled experiments, as this requires an isotopically uniform diet for the animal throughout most of its lifetime. We could not identify any effect of the experiment on δ^{15}N of vegetation or animal-related enrichments.

The experiment covered also the conditions of pastures on which the animals were kept prior to the experiment. Hence, the animals had received similar feed throughout their lifetime after weaning and thus should have equilibrated.

Isotopic equilibration to determine the enrichment of the top soil even requires that land use and nitrogen cycling has not changed at least during the period reflected by the mean age of the nitrogen pool in the soil. We sampled only top soil, for which the mean age of the organic matter is in the order of 50 years in this area (Steffens *et al.*, 2009). So we would need a period with constant conditions for at least this period of time to determine $\varepsilon_{veg\text{-}soil}$. The Inner Mongolian grassland has traditionally been used for grazing for centuries (Sneath, 1998) with no measures to improve the pastures (e.g., fertilization, sowing). For the area where the grazing experiment was located, this can be confirmed since 1979, when the nearby experimental station was established. Hence, the system was probably in equilibrium at the beginning of the grazing experiment. All enrichments determined in this study thus seem to reflect near equilibrium conditions and true enrichments.

Conclusions

This work demonstrated considerable differences in %N and δ^{15}N of vegetation in grazed paddocks. However, grazing pressure – and hence opportunities for selection – did not cause changes in ^{15}N enrichment between vegetation and grazer tissues. This suggests, that eventual selection did not entail ^{15}N enrichment during grazing. Also, our second hypothesis – that increasing %N in herbage leads to increased ^{15}N enrichment in animals – was not apparent in the data. Accordingly, it does seem that isotopic offsets between vegetation and animal tissues are near constant irrespective of grazing pressure. Thus, knowledge of grazing pressure or stocking rate is not required, to estimate vegetation ^{15}N composition from grazer tissues. This means that grazer tissues are valid proxies for reconstruction of vegetation ^{15}N composition in regional or historic studies of vegetation ^{15}N variation.

CHAPTER V – Large regional scale variation C3/C4 distribution pattern of Inner Mongolia steppe is revealed by grazer wool carbon isotope composition[3]

Abstract

This work explored the spatial variation of C3/C4 distribution in the Inner Mongolia, P.R. China, steppe by geostatistical analysis of carbon isotope data of vegetation and sheep wool. Standing community biomass (n=118) and sheep wool (n=146) were sampled in a ~0.2 Mio km^2 area. Samples from ten consecutive years (1998-2007) were obtained. Community biomass samples represented the carbon isotopic composition of standing vegetation on about 1000 m² ('community-scale'), whereas the spatio-temporal scale of wool reflected the isotope composition of the entire area grazed by the herd during a 1-yr period (~5-10 km^2, 'farm-scale'). Pair wise sampling of wool and vegetation revealed a ^{13}C-enrichment of 2.7±0.7‰ (95% confidence interval) in wool relative to vegetation, but this shift exhibited no apparent relationships with environmental parameters or stocking rate. The proportion of C4 plants in above-ground biomass (P_{C4}, %) was estimated with a two-member mixing model of ^{13}C discrimination by C3 and C4 vegetation ($^{13}\Delta_3$ and $^{13}\Delta_4$, respectively), in accounting for the effects of changing ^{13}C in atmospheric CO_2 on sample isotope composition, and of altitude and aridity on $^{13}\Delta_3$. P_{C4} averaged 19%, but the variation was enormous: full-scale (0% to 100%) at community-scale, and 0% to 85% at farm-scale. The farm-scale variation of P_{C4} exhibited a clear regional pattern over a range of ~250 km. Importantly P_{C4} was significantly higher above the 22°C isotherm of the warmest month, which was obtained from annual high-resolution maps and averaged over the different sampling years. This is consistent with predictions from C3/C4 crossover temperature of quantum yield or light use efficiency in C3 and C4 plants. Still, temperature gradients accounted for only 10% of the farm-scale variation of P_{C4}, indicating that additional factors control P_{C4} on this scale.

Introduction

The carbon isotope composition (δ^{13}C) of terrestrial ecosystems holds important information on photosynthetic pathways, carbon fluxes and linked biogeochemical cycles (Schimel, 1995; Ehleringer *et al.*, 2000). In grassland, the ^{13}C signal can vary considerably, which is primarily related to the presence of variable proportions of C3 and C4 photosynthetic types (Tieszen *et al.*, 1997; Bird & Pousai, 1997; Collatz *et al.*, 1998) and the large difference in carbon isotope discrimination ($^{13}\Delta$) between them (Farquhar *et al.*, 1989). Variation in the C3/C4 ratio has wide biogeochemical and land use implications: it affects the magnitude and seasonal distribution of biomass production, soil carbon storage, water use and nutrient cycling (Tieszen *et al.*, 1997; Bird & Pousai, 1997; Epstein *et al.*, 1998; Sage & Kubien, 2003; Semmartin *et al.*, 2004). Hence, as it indicates the C3/C4 ratio, δ^{13}C is a useful proxy of vital functions of grassland. However there exist very few regional-scale investigations on δ^{13}C of C3/C4 mixed grassland.

There is abundant evidence that the current distribution of C4 plants is primarily controlled by growing season temperature (Ehleringer *et al.*, 1997; Collatz *et al.*, 1998) and that this is related to the higher effective quantum yield of CO_2 fixation (Ehleringer & Bjorkman, 1977) or higher maximum photosynthetic rate of C4 plants at high temperature (Sage & Kubien, 2003, Still *et al.*, 2003b). Central East Asia has experienced one of the largest temperature increases on earth during the last decades (Chase *et al.*, 2000; Yu *et al.*, 2003), which should promote C4 plants. On the other hand, rising atmospheric CO_2 stimulates C3 photosynthesis more than C4 (e.g., Ehleringer *et al.*, 1997). Nutrient limitation due to increased photosynthesis may again favour C4 species (Sage & Kubien, 2003) leading to a complex web of interactions, which makes it difficult to predict the overall effect. Furthermore, the different influences act on different spatio-temporal scales. While CO_2 concentration changes globally, temperature varies regionally and nutrient limitation may differ down to the scale of urine patches. The effects may thus vary regionally or even locally and can only be assessed by taking scales into account. Furthermore, the seasonal distribution of precipitation (Murphy & Bowman, 2007), aridity, and disturbance (for instance by overgrazing) may exert secondary, modifying effects. E.g., predominance of summer rainfalls typically benefits C4 more than C3 (Hattersley 1983; Paruelo & Lauenroth, 1996). C4 dicots predominate in hot arid, saline or highly disturbed habitats (e.g., Ehleringer *et al.*, 1997). Presumably such secondary controls have their strongest effect on C3/C4 abundance in those regions which have a growing season mean temperature that is near the C3/C4 crossover temperature of light

use efficiency (Ehleringer *et al.*, 1997; Sage & Kubien, 2003; Still *et al.*, 2003b). In this respect, the grassland of Inner Mongolia is of particular interest, as the average temperature during the summer months (especially July) when most of the annual precipitation falls is close to the C3/C4 crossover temperature. There are several reports indicating increases in the number and abundance of C4 species with aridity in (Inner) Mongolia grassland (Pyankov *et al.*, 2000; Wang, 2004) and desertification (Wang, 2002b), although conflicting evidence has also been presented (Wang, 2003; Ni, 2003).

Although the C3/C4 ratio is the main factor affecting $\delta^{13}C$ of grassland, variation in $^{13}\Delta$ of C3 plants that follow regional patterns may also contribute, as the $\delta^{13}C$ of C3 plants decreases with precipitation, moisture availability (e.g., Schulze *et al.*, 1998; Liu *et al.*, 2005; Wittmer *et al.*, 2008) and altitude (Körner *et al.*, 1991, Männel *et al.*, 2007).Variation in the $\delta^{13}C$ of C3 plants thus has to be taken into account when estimating the C3/C4 ratio from carbon isotope composition. Further, $^{13}\Delta$ of C4 plants can also vary along environmental gradients (Schulze *et al.*, 1996, Chen *et al.*, 2002) and must also be considered.

Another difficulty in regional-scale studies of the ^{13}C signal of grassland is a potentially high spatio-temporal variation: the life-span of the aboveground parts of grassland plants is short, reflecting growing conditions prevailing in a period of weeks to a few months before sampling. This is aggravated in C3/C4 grassland due to the asynchronous development of C3 and C4 components resulting from their different thermal preferences. One possibility to overcome (integrate) these spatio-temporal variations is to collect representative bulk samples over a large area and to sample several times during the vegetation period. An alternative to this is to take advantage of the 'sampling' activity of livestock which are grazing the grassland. Provided that they do not select between photosynthetic types, and eventual post-ingestion fractionation (possibly resulting from differential digestibility of C3 and C4 plants) of carbon isotopes is known, the $\delta^{13}C$ of vegetation can be inferred from wool or other animal tissue (e.g., Jones *et al.*, 1981; Schnyder *et al.*, 2006; Norman *et al.*, 2009). Geostatistical analysis can then be used to separate between the regional pattern and the noise of the individual samples, e.g. caused by small-scale peculiarities of individual animals, farms or years.

The aim of this work was to answer the following questions by geostatistical analysis of $\delta^{13}C$ derived from wool samples by taking into account the diet-wool shift and environmental influences on the C3 and C4 end-members:

(i) How are C3 and C4 grasses spatially distributed in the grassland of Inner Mongolia?

(ii) How does this pattern relate to driver patterns like (growing period) temperature and/or precipitation?

Materials and Methods

Study area

The study area was situated between 111°38' and 117°49'E (approximately 500 km) and 41°48' and 45°46'N (approximately 450 km) in the Autonomous Region of Inner Mongolia in the Peoples Republic of China (Fig. V.1). Sampled altitudes ranged from 800-1700 m above sea level (a.s.l.). Mean annual precipitation (MAP, mm yr^{-1}) increases from 100 mm yr^{-1} in the western part to 400 mm yr^{-1} in the eastern part of the study area. Most of the precipitation (approximately 75%) falls during the growing period (April-September). The spatial variation of temperature follows altitudinal and latitudinal trends. Mean annual temperature and mean temperature of the growing period vary between 0 - 6 °C and 14 - 19 °C, respectively. Almost the whole area is used for small ruminant livestock production (mainly sheep and cashmere goats) with little agriculture, mainly cultivation of maize. In general, animals graze from spring till winter depending on snow cover and availability of herbage. In overgrazed areas grazing is suspended during winter and/or early spring to allow for recovery of vegetation. Animals do not receive supplements on pasture. Pen fodder in the winter and/or spring consists of hay from meadows, supplemented in some cases with small amounts (<10% of the ration) of maize (information obtained from interviewing the local herders).
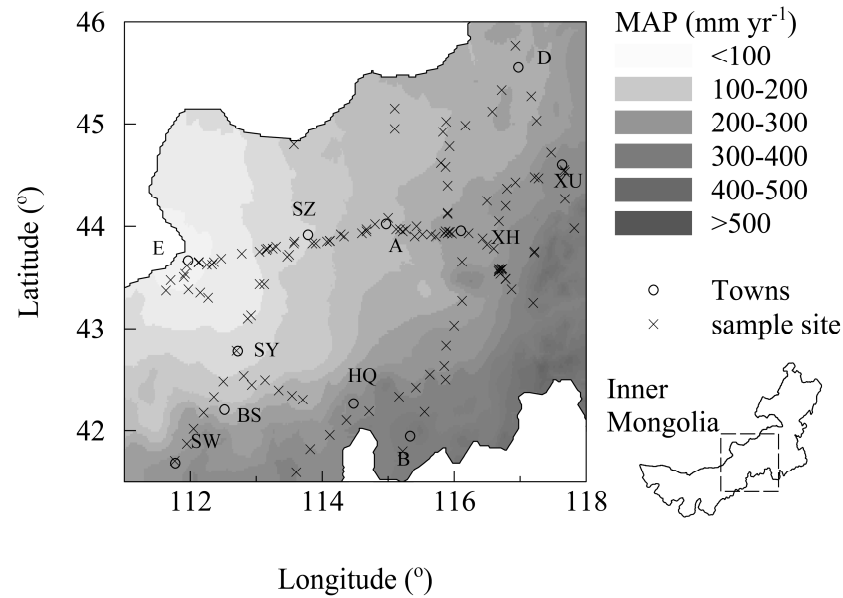
Fig. V.1 Mean annual precipitation (MAP, mm yr^{-1}, normal period 1961-90) and sampling sites within the study area in Inner Mongolia, People's Republic of China. Towns are A = Abag Qi, B = Baochang, BS = Bayan Sum, D = Dong Ujimqin Qi, E = Erenhot, HQ = Huang Qi, SW = Siziwang, SY = Sonid Youqi, SZ = Sonid Zuoqi, XH = Xilinhot, XU = Xi Ujimqin Qi.

Sampling

Samples were collected in August/September 2003, August/September 2004, July 2005, July 2006 and June/July 2007. The sampling area (116°12' to 118°54' E and 40°36' to 47°42' N) exceeded the study (target) area. This was done to evade boundary effects at the periphery of the study area during spatial analysis and interpolation. Otherwise the number of samples available for estimation close to the boundary would decrease and be unfavorably distributed in space, and both these factors would increase the error variance (Ayyub & McCuen, 1990). The current sampling position and altitude was measured with a mobile outdoor global positioning system. Bulk leaf samples representing total standing biomass were collected within approximately 1000 m² ('community-scale' samples) on 112 sites within the study area and on 6 sites outside the periphery. At some sites (n=52), encompassing the entire aridity gradient in the sampling area, the leaves of the dominant and in parts the co-dominant species (contributing individually >5% to total aboveground biomass) were sampled separately (>3 different plants per sample), to obtain separate estimates of the $\delta^{13}C$ of the C3 and C4 components of plant communities.

The wool samples resulted from the last shearing and therefore reflected the period in between two consecutive shearing (mostly one year), which generally take place in June, and the grazing grounds of one herder ('farm-scale' samples). Wool from 146 sheep dating from

1998 – 2007 was sampled on 82 sites (2003, 2004 and 2007) within the study area and on ten sites beyond the periphery. Most wool samples were provided by local herders and originated from the recent shearing.

Additional samples were taken between late June and mid of September in 2005 and 2006 from a long term grazing experiment at 116°40'E, 43°34'N. The grazing experiment allowed comparing vegetation and wool directly and to assess selective grazing because it covered a large gradient of six stocking densities between 1.5 sheep per hectare, which allowed for selection, and 9.0 sheep per hectare, where the total aboveground biomass was grazed leaving bare soil. This experimental set up, with four replicates per stocking density in different landscape positions, also allowed for a variation in the proportion of C4 biomass to total aboveground biomass. Vegetation samples were obtained from inside exclosures (pasture cages) which were redistributed in the pasture at monthly intervals. In addition, we obtained the wool from 90 sheep, which were shorn before they were moved to the pasture (mid of July 2005 and 2006 respectively) and at the end of pasturing (mid of September 2005 and 2006 respectively).

Sample preparation

The plant samples for each site were held in separate paper bags and dried behind the front window of the car. In addition plant samples were dried for 48 hours at 60°C and then ground to homogeneity with a ball mill. 0.7-0.8 mg plant material in tin cups was analyzed for their isotopic composition. The wool samples were cleaned by the procedure of Schwertl *et al.* (2003) and 0.2-0.4 mg wool was packed into tin cups for isotope analysis. For the wool originating from the years 2003, 2004 and 2005 an additional segmentation was carried out: the wool was cut into 1 cm-long pieces, which were analyzed separately. These segments could be assigned to a time axis (Schwertl *et al.*, 2003) with the proximal segment grown immediately before the last shearing and the distal segment immediately after the previous shearing. This allowed to quantify an isotopic shift between the summer wool segments and summer vegetation (n=62 for repeated regional samplings and n=14 for the grazing experiment).

Isotope and elemental analysis

The carbon isotope composition and carbon and nitrogen content of each sample was 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). Carbon isotopic data are presented as $\delta^{13}C$, with $\delta^{13}C = (R_{sample}/R_{standard}) - 1$, where R is the $^{13}C/^{12}C$ ratio and standard is the Vienna Pee Dee Belemnite standard. Each sample was measured against a laboratory working standard CO_2 gas, which was previously calibrated against an IAEA secondary standard (IAEA-CH6, accuracy of calibration 0.06‰ SD). After every tenth sample a solid internal lab standard (SILS) with similar C/N ratio as the sample material (fine ground wheat flour for plant samples; protein powder for wool) was run as a blind control. The SILS were previously calibrated against an international standard (IAEA-CH6). The precision for sample repeats (SD) was 0.06‰ for plant samples and 0.11‰ for wool samples.

Carbon isotope discrimination of plants

The $\delta^{13}C$ of plants ($\delta^{13}C_P$) differs from the $\delta^{13}C$ of air ($\delta^{13}C_a$) depending on the discrimination $^{13}\Delta$ (Farquhar *et al.*, 1989):

$$^{13}\Delta = \frac{\delta^{13}C_a - \delta^{13}C_P}{1 + \delta^{13}C_P} \qquad (V.1)$$

with $\delta^{13}C_a$ continuously decreasing over time mainly due to fossil fuel burning (see citations in Wittmer *et al.*, 2008). Mean annual $\delta^{13}C_a$ was predicted by applying a third order polynomial and corrected for seasonal variation in $\delta^{13}C_a$ as described by Wittmer *et al.* (2008).

Meteorological data

For year-specific data on precipitation and temperature we followed the procedure by Wittmer *et al.* (2008) and corrected long-term average high resolution (2 km x 2 km) maps (The Climate Source LLC, 2002) obtained by the PRISM method (parameter-elevation regressions on independent slopes model; Daly *et al.*, 2002) for the deviation of an individual year, which was geostatistically interpolated between meteorological stations. Daily precipitation and temperature data of 46 climate stations, provided by the NOAA Satellite and Information Service (NOAA NCDC Climate Data Online 2008) were used. The annual maps were then averaged for the period of the investigation (1998-2007).

Estimation of C4 fraction

The relative contribution of C4 plants (P_{C4}) to aboveground biomass at each site was estimated from $^{13}\Delta$ of the community using a two-member mixing model:

$$P_{C4} = \frac{^{13}\Delta_c - {}^{13}\Delta_3}{^{13}\Delta_3 - {}^{13}\Delta_4} \qquad\qquad (V.2)$$

where $^{13}\Delta_c$ denotes community, and $^{13}\Delta_3$ and $^{13}\Delta_4$ are the end-members of the mixing model (local $^{13}\Delta$ of pure C3 and C4 vegetation communities, respectively). Regionalized estimates of $^{13}\Delta_4$ (taken as a constant, see Results) were obtained from $\delta^{13}C$ of C4 vegetation samples, whereas $^{13}\Delta_3$ was derived from study-area specific data accounting for precipitation during the growing period as reported by Wittmer *et al.* (2008 and citations therein) and for altitude following Männel *et al.* (2007). Combining the equations of both studies yielded an estimate for $^{13}\Delta_3$:

$$^{13}\Delta_3 = 14.4\text{‰} + 1.1\text{‰} \cdot \frac{A - 1000}{1000} + 2.7\text{‰} \cdot \sqrt{P} \qquad\qquad (V.3)$$

with A denoting altitude (m a.s.l.) and P denoting the mean precipitation (mm day^{-1}) during the growing period. The constant 14.4‰ is the minimal reference $^{13}\Delta_3$ as reported by Wittmer *et al.* (2008) for 1000 m a.s.l.

In principle, recent P_{C4} can be estimated from wool considering the isotopic offset with respect to vegetation: in studies with pure or mixed C3 or C4 diets the $\delta^{13}C$ of hair was 2 to 3‰ enriched with ^{13}C relative to diet (Jones *et al.*, 1981; Sponheimer *et al.*, 2003a; Männel *et al.*, 2007). This offset is termed 'vegetation-wool shift', S_{VW} ($S_{VW} = \delta^{13}C_{wool} - \delta^{13}C_{vegetation}$), and results from ^{13}C fractionation during digestion or metabolism of animals (De Niro and Epstein, 1978). Hence, the $^{13}\Delta$ of the community can directly be estimated from Eq. V.1 with $\delta^{13}C_c = \delta^{13}C_{wool} - S_{VW}$. So far published estimates of the shift for wool for mixed C3/C4 diets are missing as the shift of sheep wool from Männel *et al.* (2007) was established for pure C3 grassland, which exhibits higher digestibility than that in Inner Mongolia (Wang *et al.*, 2009). In principle, such factors could affect S_{VW}. Therefore, we assessed S_{VW} by paired sampling across the study area and in the grazing experiment. We compared the $\delta^{13}C$ of vegetation with that of the most-recently grown 1 cm-long wool segment from sheep which actually grazed the vegetation. From the grazing experiment, the whole wool was used, which was grown while a

sheep was grazing on an individual plot. In this case, the first cm was discarded to avoid effects of the metabolic turnover of body carbon originating from prior feeding.

Statistical methods and geostatistical analysis

Linear regressions were used to evaluate the datasets. The coefficient of determination was tested with a two-sided test for significance of the regression. Hypothesis testing on equal means of groups were carried out using Student's t-test, customarily performed against a 95% confidence interval if not stated otherwise. Kernel densities (Silverman, 1986) were calculated using Gaussian kernels to yield estimates of the density distribution. Bandwidth of density estimation of different sized data sets was defined *via* Silverman's 'rule of thumb'. To allow for comparison of differently sized data sets integral density was adjusted to unity. Statistical spread is denoted as standard deviation (SD) or 95% confidence interval ($CI_{95\%}$). All these procedures followed standard protocols (Sachs & Hedderich, 2006) and were carried out using GNU R 2.7.2 (R Development Core Team, 2008).

Geostatistics quantify the nature of spatial dependence of a property. This allows to separate the data uncertainty from the spatial pattern, to interpret the pattern, and to estimate the property at unrecorded positions (see Rossi *et al.*, 1992, and citations therein). Geostatistical analyses were conducted with the auxiliary packages geoR (Ribeiro & Diggle, 2001) and gstat (Pebesma, 2004). The semivariance (γ) equals the variance for values at points which are separated by a certain distance called lag (Bachmaier and Backes, 2008). The semivariances for classes of different lags yields the empirical semivariogram (x axis: mean lag, y axis: mean semivariance). A theoretical semivariogram was fitted to minimize weighted least squares, with weights calculated from the ratio of pairs within a class to mean lag. This gives more weight to those classes, which are based on many data pairs and which are more important for interpolation (Wittmer *et al.*, 2008). The theoretical semivariogram delivers three parameters: the nugget effect, the sill and the range. The nugget effect quantifies the small-scale variation including data uncertainty. The sill quantifies the total variation caused by the nugget effect and the variation due to the spatial pattern. The nugget to sill ratio reflects the ratio of random (unexplained) to total variation. The range quantifies the distance of autocorrelation caused by the extension of the pattern elements. The quality of the fit between the experimental and the theoretical semivariogram is expressed as root mean squared error (RMSE). For calculation of the lag, the coordinates of sampling points were

transformed from geographic coordinates to UTM coordinates with the package PBSmapping (Schnute *et al.*, 2008; UTM zone = 50).

Spatial interpolation to construct maps was then carried out for a uniform rectangular grid (200 × 200 nodes) by ordinary block kriging for 5 × 5 km² blocks, using the theoretical semivariogram. Samples beyond the periphery of the study area were included because this greatly reduced the error at the margins of the study area. The quality of the predictions from the resulting maps is given as the (block) krige standard deviation averaged for the study area.

Results

The $\delta^{13}C$ of vegetation and hair

The isotopic composition of carbon pools varied greatly in the sampling area. Furthermore, the range differed strongly between types of pools: the δ^{13}C of whole vegetation varied between -12.5‰ and -28.4‰ (Fig. V.2a), whereas the site-means for wool ranged between -13.1‰ and -23.7‰ (Fig. V.2b). The δ^{13}C of the C3 components varied between -23.3‰ and -28.5‰ (mean: -25.0‰), the δ^{13}C of the C4 components of vegetation between -12.5‰ and -15.8‰ (mean: -14.2‰). The δ^{13}C of the C3 and C4 components exhibited variations three to four times smaller than that of whole vegetation (Fig. V.2a) meaning that the C3/C4 ratio must have varied strongly in the region (see below).
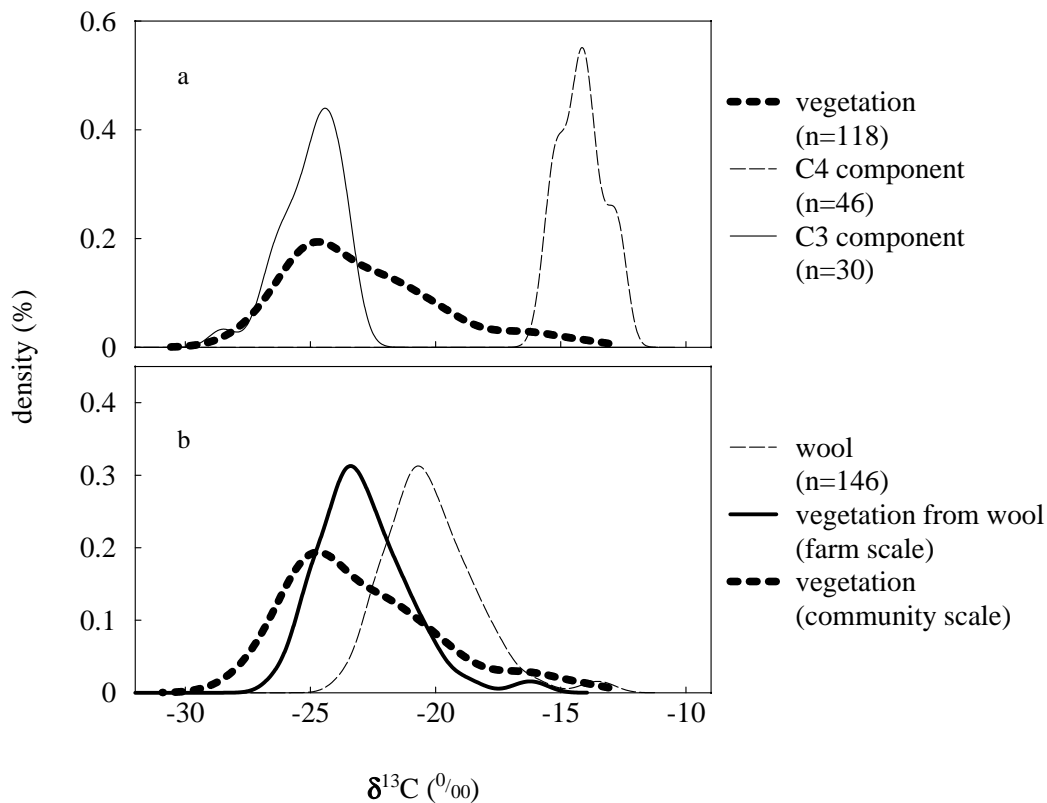
Fig. V.2 Density distribution of carbon isotope composition ($\delta^{13}C$) of carbon pools in Inner Mongolia grassland: (a) standing vegetation, C4 component and C3 component; (b) site mean of wool, whole vegetation and wool with applied shift (data from the grazing experiment are not included).

Bulk vegetation and wool differed markedly in their distributions of $\delta^{13}C$ (Fig. V.2a, b). These differences corresponded almost exactly to the isotopic shift, which was observed by paired sampling: on average, wool was enriched by 2.7‰ (n=76, $CI_{95\%}$=0.7‰) relative to vegetation sampled at the same location. There was no indication of preferential grazing in the grazing experiment, when the shift was regressed either against stocking densities or against P_{C4} derived from vegetation analysis (P>0.05 and P>0.75 respectively). Correcting for this independently derived shift led to similar density distributions for vegetation derived from either vegetation itself or from wool (Fig. V.2b). The main difference between the density distributions then was that the extremes were lost when using wool, reflecting the larger temporal and spatial integration in wool samples. The density peak for $\delta^{13}C$ of vegetation derived from wool (-23.4‰) was close to the median (-23.6‰) and the mean (-22.8‰) of $\delta^{13}C$ from bulk vegetation.

The relationship between $\delta^{13}C$ and environmental gradients

The response of $\delta^{13}C$ to the environmental variables was the same for vegetation and wool with no significant differences in slope of the regression, which were very highly significant for mean July temperature, MAP and altitude (Table V.1) with similar strength. However, the

correlations were rather weak and effects were not independent, since there were also close correlations between the mean July temperature and MAP (r^2=0.75, P<<0.001) as well as between mean July temperature and altitude (r^2=0.41, P<<0.001), and MAP and altitude (r^2=0.26, P<<0.001). As a result, the effects of environmental variables on $\delta^{13}C$ of vegetation could not be separated, and each response function of $\delta^{13}C$ to an environmental variable included direct and indirect effects.

Table V.1 Effect of the environmental variables mean annual precipitation (MAP), mean precipitation during the growing period of the actual year (P), mean annual temperature (MAT), mean July temperature (MJulT) and mean July temperature 1998 – 2007 (MJulT$_{98-07}$) on $\delta^{13}C$ of Inner Mongolia grassland quantified by linear regressions; climate means apply for the last normal period 1961-1990 if not stated otherwise; data from vegetation and wool were combined after applying the appropriate shift for wool.

Parameter	Range	$\delta^{13}C$ response	SE	r^2	n
Altitude	840 – 1692 m a.s.l.	-2.4‰ / 1000 m a.s.l.	± 0.001	0.02*	264
MAP	136 – 389 mm yr^{-1}	-1.3‰ / 100 mm yr^{-1}	± 0.002	0.10***	264
P	0.4 – 2.3 mm day^{-1}	0.6‰ / 1 mm day^{-1}	± 0.403	0.01 n.s.	264
MAT	1.1 – 7.9 °C	0.3‰ / 1 °C	± 0.098	0.03**	264
MJulT	18.5 – 25.2 °C	0.5‰ / 1 °C	± 0.108	0.09***	264
MJulT$_{98-07}$	20.5 – 25.0 °C	0.8‰ / 1 °C	± 0.151	0.10***	264

*, **, *** denote error probability smaller than 0.05, 0.01 and 0.001 respectively

$^{13}\Delta$ of C4 vegetation and C4 abundance

Mean $^{13}\Delta_4$ was 6.0‰ (n=46; SD ±0.9‰). Within the C4 plants there was a highly significant difference of 1.1‰ (P<<0.0001, α=0.01) between *Cleistogenes squarrosa* (mean 6.6‰; n=19), a common perennial xerophytic C4 grass, and the other C4 species (mean 5.5‰; n=27; see also the two shoulders in the density distribution of $\delta^{13}C$ of C4 component in Fig. V.2a). However, we found no significant relationship between $^{13}\Delta_4$ and normal-period (P>0.60) or annual precipitation (P>0.75), normal-period (P>0.80) or annual temperature (P>0.10), or plant available soil water (P>0.23, calculated after Allen *et al.*, 1998 and Schnyder *et al.*, 2006). As no indication for a relevant regional variation was evident, the mean $^{13}\Delta_4$ was used to calculate P_{C4} from ^{13}C of wool samples considering the isotopic shift between vegetation and wool.

C4 plants accounted for 19% of aboveground plant biomass when averaged over all wool samples, but P_{C4} varied widely between sites (0 to 85%). C4 abundance increased by 6% with

an increase of 1°C of mean July temperature (normal period, P<<0.001), decreased by 6% for each 100 mm yr^{-1} increase in MAP (P<0.05). Although all these relationships were significant, the scatter was large in all of them (r² between 0.04 and 0.10).

Geographic variation of C4 abundance

The theoretical semivariogram followed a spherical model for P_{C4} (Fig. V.3). The range was approximately 260 km and the nugget-to-sill ratio was 0.4. Hence the nugget effect corresponds to an uncertainty of approximately 16% for P_{C4}. Furthermore, the nugget/sill ratio indicates that 60% of the variation was caused by a spatial pattern (Table V.2). This is a much bigger effect than was suggested by regression analysis (Table V.1) which assumed linear relationships with isolated environmental variables.
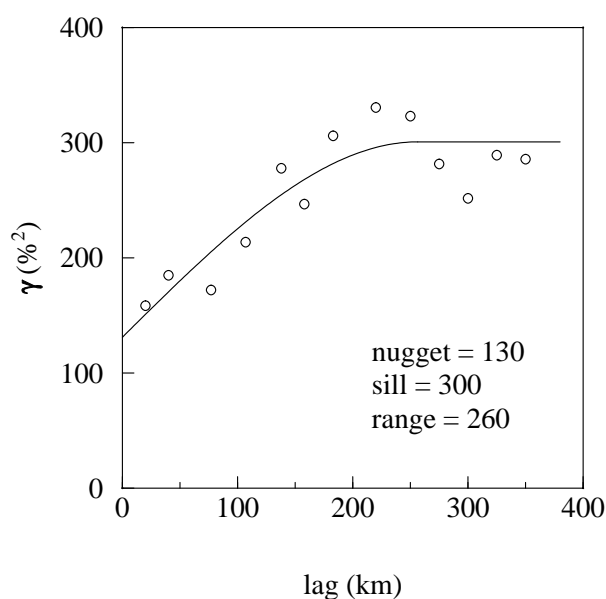
Fig. V.3 Empirical (circles) and theoretical (line) semivariogram of percent C4 in above-ground biomass (in %) calculated from isotopic signatures of wool.

Table V.2 Semivariogram parameters for spherical models of P_{C4} derived from $\delta^{13}C$ of wool or vegetation. Nugget uncertainty of P_{C4} is the square root of twice the nugget.

Semivariogram parameter	Wool semivariogram	Vegetation semivariogram
nugget (%²)	130	690
sill (%²)	300	950
range (km)	260	305
nugget/sill ratio (%)	43	73
nugget uncertainty (%)	16	37
RMSE (%²)	24.9	86.5

The map of P_{C4}, as reflected in $\delta^{13}C$ of wool generated by ordinary block kriging, ranged only between 0 and 40% because block kriging separates the local variation (noise) from the pattern (regional variation). The map (Fig. V.4) characterized an increase of P_{C4} from 0% in the south-eastern part to around 40% in the western part of the study area near the Gobi desert and also a 'C3 favoring valley' with $P_{C4}<20\%$ which extended from south-east to north-east. From Collatz *et al.* (1998) a crossover temperature for C3/C4 distribution of 22°C for the warmest month followed for a CO_2 partial pressure of 37 Pa. This theoretical threshold agreed well with our estimated distribution of C4 plants, which followed the 22°C July isotherm averaged for the years 1998 - 2007 ($MJulT_{98-07}$). Separated into two areas, $MJulT_{98-07}$ below and above 22°C, P_{C4} differed significantly ($P<0.001$, $\alpha=0.01$) by approximately 13% with sample site mean $P_{C4}=23\%$ above 22°C (mean $MJulT_{98-07}=22.9°C$) and sample site mean $P_{C4}=10\%$ below 22°C (mean $MJulT_{98-07}=21.5°C$). This indicates a substantial change in a narrow range of temperature.
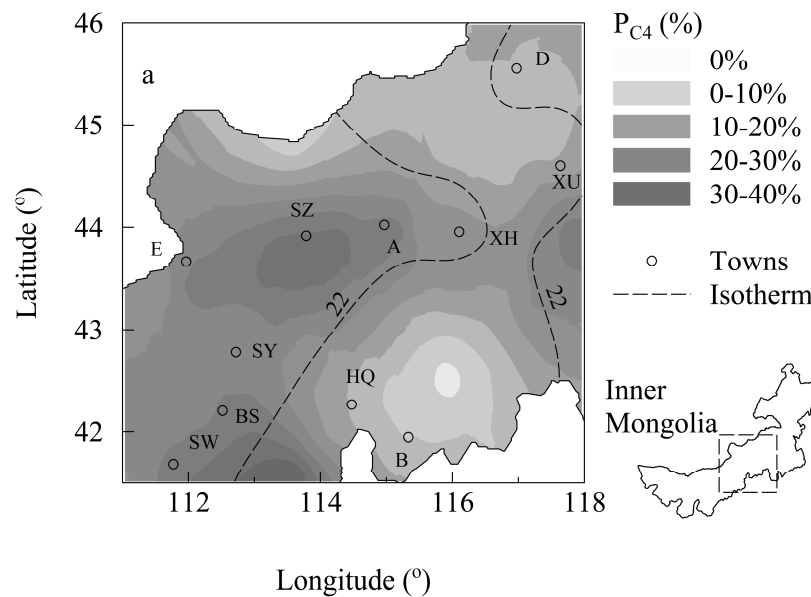
Fig. V.4 Regional P_{C4} developed *via* kriging, estimated from wool originating from 1998-2007. Mean krige standard deviation for the study area is 9.4%. Towns are the same as in Fig. V.1. The 22°C isotherm reflects meteorological conditions during the period 1998-2007 and it is equivalent to the predicted crossover temperature for the CO_2 concentration during this period.

Discussion

Variation of $\delta^{13}C$ of Inner Mongolia grassland at differing spatio-temporal scales

Individual vegetation samples give the mean isotopic composition of aboveground biomass grown in a period of weeks to months in an area of approximately 1000 m². The short temporal integration of vegetation samples likely leads to a bias of the results by the time of sampling as C4 develops considerably later than C3 in this area (Bai *et al.*, 2004). This causes a high variability in $\delta^{13}C$ of vegetation samples leading to a semivariance about five times as high as for wool samples (nugget: 690%² *versus* 130%²) and a semivariogram being almost a complete nugget (nugget/sill ratio of 73%) with a large RMSE (Table V.2), which does not allow to retrieve a spatial pattern from vegetation analyses. Whole wool, however, integrates the isotopic signal of feed ingested between two successive shearing (one year) on an area that encompasses the grazing grounds and the haymaking areas of an entire farm (several km²).

Despite the large spatio-temporal integration of wool its inter-annual variation may be large. Inter-annual weather variability (e.g., dry years *versus* wet years; Wittmer *et al.*, 2008) and a semi-arid situation, where moisture availability is almost entirely driven by precipitation but not by the spatially varying storage capacity of soil, certainly can alter the spatial patterns between years. Theoretically, inter-annual variations can be excluded by pooling the data (Schuurmans *et al.*, 2007) by years prior to the calculation of the empirical semivariogram. However, this procedure neither improved the semivariogram (actually the nugget and the nugget/sill ratio were higher) nor the kriging prediction (all not shown). The main random variability hence mainly resulted from the intra-annual variation of the C3/C4 ratio and not from the inter-annual variation which was excluded by using wool instead of vegetation.

Isotopic shift between vegetation and wool

Preferential grazing is one mechanism by which grazers can modify vegetation composition (Caswell *et al.*, 1973), and hence carbon pools, fluxes and their isotopic composition. Yet, the present data indicated no preferential grazing of one photosynthetic type (C3 relative to C4, or *vice versa*). This was suggested by the fact that the observed vegetation-wool shift (+2.7‰) was similar to that of pure C3 or C4 diets (average shift: +2.6‰ ± 0.6‰ SD for hairs of different animal species in the studies of Minson *et al.*, 1975; Jones *et al.*, 1981; Sponheimer *et al.*, 2003a; Ayliffe *et al.*, 2004; Schwertl *et al.*, 2005). If animals had grazed preferentially

one component of vegetation, then the apparent shift should deviate. A shift <2.7‰ would indicate preferential grazing of the C3 component, and a shift >2.7‰ preferential grazing of the C4 component. Preferential grazing would also result in an either right (= more negative; C3 preferred) or left (= less negative; C4 preferred) skewed density distribution of wool compared to vegetation. This was not the case as the density distributions for wool and vegetation were centered (Fig. V.2b). In the density distribution of wool, however, the extremes on both sides of the vegetation density distribution were missing due to the spatio-temporal integration by the animals. And finally, preferential grazing should cause a correlation between the shift and either sheep stocking densities or P_{C4}, which both determine the likelihood for selective grazing. Such relations were not found in the grazing experiment. Furthermore, if selection would happen opportunities would be small on an annual basis with only one growth cycle due to the high grazing pressure caused by the high stocking rate in the study area (Sneath, 1998).

Differences in digestibility between C3 and C4 species can displace the signal in the wool and thus influence the vegetation-wool shift. Several studies reported that the nutritional quality and/or digestibility of C4 species is inferior to that of C3 species (e.g., Sponheimer *et al.*, 2003d; Barbehenn *et al.*, 2004) although the opposite was also reported (Scheirs *et al.*, 2000). Digestibility decreases strongly with age of plant material (Fick *et al.*, 1994). An alternating ranking of the digestibility can thus be expected from the asynchronous growth of both photosynthetic types where the C4 species initiate growth when the aboveground parts of the C3 species are already about two months old. Certainly, further investigations on individual and combined digestibility of C3 and C4 species are needed. However, in the present study, this had only a minor influence on our results, as the reported differences in digestibility between C3 and C4 species rarely exceeded 10-15% (e.g., Sponheimer *et al.*, 2003d; Norman *et al.*, 2009) and the overall C4 abundance in the study area was not higher than 40%. Postulating a direct effect of digestibility on the pattern of C4 abundance, the variation in digestibility reported in other studies would cause a maximum error in the estimation of 6% but it would not distinctly alter the C4 abundance pattern derived from wool.

Variation in C4 end-member ($^{13}\Delta_4$) of C3/C4 mixing model

Several studies reported variations of $^{13}\Delta_4$ along environmental gradients e.g., a decrease in $^{13}\Delta_4$ with increasing precipitation (Schulze *et al.*, 1996) or, in contrast, an increase of $^{13}\Delta_4$ with increasing soil water content (Chen *et al.*, 2002). The absence of any relationship with

individual climatic gradients (temperature, precipitation and soil water parameters) may thus be caused by compensating effects. Furthermore, C4 species of different metabolic types (see Pyankov *et al.*, 2000) can differ significantly in $^{13}\Delta$ (e.g., Schulze *et al.*, 1996). This was apparent in our data, as *C. squarrosa* (NAD-ME metabolic type, Pyankov *et al.*, 2000) had a 1.1‰ higher $^{13}\Delta$ than other metabolic types (mainly NADP-ME). Several studies reported that the abundances of different metabolic types are related to precipitation gradients with abundance of NADP-ME plants increasing with increasing precipitation at the expense of NAD-ME plants (Taub, 2000; Wang *et al.*, 2003; Cabido *et al.*, 2008; Osborne, 2008). This should lead to a decrease of $^{13}\Delta_4$ with precipitation. However, this effect was not observed in our data as *C. squarrosa* was a component of the C4 community throughout the sampling area. Additionally, there was no apparent pattern in the occurrence of the different metabolic types (data not shown) with the exception of *Chloris virgata*, an annual grass of the PEP-CK metabolic type, which was restricted to the drier (western) part of the sampling area. However, *C. virgata* was never a dominant component the C4 community. Importantly, the present estimates of P_{C4} did not change very much when we varied the C4 end-member value of the C3-C4 mixing equation. If $^{13}\Delta_4$ was set to 5.5‰ (100% NADP-ME species in C4 community), then the resulting mean P_{C4} was 18.2%. Conversely, if $^{13}\Delta_4$ was set to 6.5‰ (assuming that the C4 community consisted entirely of NAD-ME species, such as *C. squarrosa*), then the mean P_{C4} was 19.8%. So, any likely error in estimation of $^{13}\Delta_4$ had a negligible effect on the spatial variation of P_{C4} (Fig.V.4).

Regional variation in C3/C4 composition is related to temperature gradients

Competition between C3 and C4 photosynthetic types is controlled by ambient CO_2 concentration and temperature during the growing period (Ehleringer *et al.*, 1997, Sage & Kubien, 2003; Still *et al.*, 2003b) with high CO_2 concentrations favoring C3 plants and high temperatures favoring C4 plants. The latter agrees with the conclusions of Pyankov *et al.* (2000) regarding the temperature-dependent distribution of C4 grasses in (Republic of) Mongolia. Due to the simultaneous increase in temperature and precipitation during the growing season (Xiao *et al.* 1995b) the temperature of the warmest month is effective for this competition. The warmest-month isotherm for the predicted crossover temperature following Collatz *et al.* (1998) essentially explained the spatial pattern. This was only true, however, if the isotherm was derived from the conditions during the study period because the study area has undergone major changes, which must be accounted for. A pronounced warming of +2°C

in mean annual temperature has occured in the last 30 years (NOAA NCDC Climate Data Online, 2008). Thus, the 22°C isotherm has shifted by about 250 km to the east. The 22°C isotherm for 1998-2007 matches the C3/C4 pattern of the same period while the 22°C of the last normal period does not. On the other hand, for the lower CO_2 concentration 30 years ago the crossover temperature was about 2°C lower according to Collatz *et al.* (1998). Although on average both effects seem to compensate each other numerically, this was not the case for the spatial pattern. The 20°C crossover isotherm of the last normal period did not differ between areas of significantly different P_{C4} (means 19% vs. 14%) while the mean difference in P_{C4} between the areas above and below the present crossover isotherm of 22°C was 13% (23% vs. 10%) and highly significant. Hence, the C4 abundance followed the crossover temperature isotherm calculated for the conditions during the sampling period. The good agreement even despite a flat gradient in summer temperature indicates the dominant influence of differences in light use efficiency on the regional pattern while the large variation found on smaller scales are hence likely to be caused by controls acting on smaller scales. To our knowledge, this is the first proof on a regional scale for the C3/C4 distribution predicted from crossover temperature of quantum yield by Collatz *et al.* (1998). It shows that the present community reflects present conditions despite large changes in CO_2 concentration and temperature in the past.

Conclusions

This work demonstrates that wool is qualified for assessing the abundance of C4 plants in the Inner Mongolia grassland on the regional scale. This is advantageous because it allows covering the whole growth cycle, many years and large areas within short sampling campaigns. A high variation on P_{C4} occurred despite the integration by wool over a 1-yr period and the grazing area. The regional pattern of P_{C4} followed predictions from C3/C4 crossover temperature of quantum yield in C3 and C4 plants (Collatz *et al.*, 1998).

CHAPTER VI – Changes in the abundance of C3/C4 species of Inner Mongolia grassland: evidence from isotopic composition of soil and vegetation[4]

Abstract

Global warming, increasing CO_2 concentration, and environmental disturbances affect grassland communities throughout the world. Here, we report on variations in the C3/C4 pattern of Inner Mongolian grassland derived from soil and vegetation. Soil samples from 149 sites covering an area of approximately 250,000 km² within Inner Mongolia, People's Republic of China were analyzed for the isotopic composition (δ^{13}C) of soil organic carbon (SOC). The contrast in δ^{13}C between C3 and C4 plants allowed for calculation of the C3/C4 ratio from δ^{13}C of SOC with a two-member mixing model, which accounted for influences of aridity and altitude on δ^{13}C of the C3 end-member and for changes in δ^{13}C of atmospheric CO_2. Maps were created geostatistically, and showed a substantially lower C4 abundance in soil than in recent vegetation (–10%). The difference between soil and vegetation varied regionally and was most pronounced within an E–W belt along 44°N and in a mountainous area, suggesting a spread of C4 plants towards northern latitudes (about 1°) and higher altitudes. The areas of high C4 abundance for present vegetation and SOC were well delineated by the isotherms of crossover temperature based on the climatic conditions of the respective time periods. Our study indicates that change in the patterns of C3/C4 composition in the Inner Mongolia grassland was mainly triggered by increasing temperature, which overrode the antagonistic effect of rising CO_2 concentrations.

Introduction

The carbon isotope composition (δ^{13}C) of plants and soil organic carbon (SOC) yields important information regarding carbon fluxes and linked biogeochemical cycles (Schimel, 1995; Ehleringer *et al.,* 2000). In grassland, the ^{13}C signal can vary considerably. This is related primarily to the presence of variable proportions of C3 and C4 photosynthetic types (Tieszen *et al.,* 1997; Bird & Pousai, 1997; Collatz *et al.,* 1998) and the large difference in carbon isotope discrimination ($^{13}\Delta$) between them (Farquhar *et al.,* 1989). Variation in the C3/C4 ratio has wide biogeochemical and land use implications: it affects the magnitude and seasonal distribution of biomass production, soil carbon storage, water use, and nutrient cycling (Tieszen *et al.,* 1997; Bird & Pousai, 1997; Epstein *et al.,* 1998; Sage & Kubien, 2003; Semmartin *et al.,* 2004). Hence, because it indicates the C3/C4 ratio, δ^{13}C is a useful proxy of vital functions of grassland. However, only a few regional-scale investigations of δ^{13}C of C3/C4 mixed grassland have been undertaken (von Fischer *et al.,* 2008).

Soil is one of the most important terrestrial carbon reservoirs, storing more than twice as much carbon as the atmosphere (Trumbore, 2000). The SOC input to this pool is mainly composed of carbon from vegetation, and the output of carbon is by soil respiration (Schimel, 1995). With increasing soil depth, the soil carbon age increases greatly (e.g. as derived from radiocarbon dating) (Rumpel *et al.,* 2002; Lopez-Capel *et al.*, 2008; Dümig *et al.,* 2008). Hence, SOC is affected by past vegetation, and therefore allows for the reconstruction of changes in the isotopically distinct C3/C4 ratio (Boutton *et al.*, 1998; Ehleringer *et al.*, 2000; Krull *et al.*, 2005; Krull *et al.,* 2007, von Fischer *et al.,* 2008), while current vegetation is strongly influenced by current environmental conditions.

Modeling has revealed that the Chinese grassland is among the most sensitive ecosystems with regard to climatic changes (Xiao *et al.,* 1995a; Gao *et al.,* 2000). This grassland has experienced extensive changes in land use and environmental conditions during the past 50 years: (1) Land use changed from nomadic to sedentary grazing in the 1960s; this change was accelerated following the stimulation of the Chinese economy in the 1990s. (2) The area experienced a temperature increase of approximately 2°C during the growing period (NOAA NCDC Climate Data Online, 2008), which is one of the largest increases on earth (Yu *et al.,* 2003). (3) Over the last century, the volumetric CO_2 concentration increased by more than 50 ppm.

There is abundant evidence that the current distribution of C4 plants is primarily controlled by growing season temperature (Ehleringer *et al.,* 1997; Collatz *et al.,* 1998) and that this is

related to the higher effective quantum yield of CO_2 fixation (Ehleringer & Bjorkman, 1977) and a higher maximum photosynthetic rate of C4 plants at high temperatures (Sage & Kubien, 2003). C4 dicots predominate in hot, arid, saline, or highly disturbed habitats (Ehleringer *et al.*, 1997). Hence, increasing temperature and land use/disturbance should promote the growth of C4 plants. On the other hand, rising atmospheric CO_2 should favor C3 plants (Collatz *et al.* 1998). Furthermore, the different influences act on different scales. Land use can vary over a few square kilometers, while temperature varies regionally and CO_2 concentration changes globally. Moreover, the seasonal distribution of precipitation and aridity may exert secondary, modifying effects. For example, a predominance of summer rainfalls typically benefits C4 plants more than C3 plants (Hattersley, 1983; Paruelo & Lauenroth, 1996). Presumably, such secondary controls have their strongest effect on C3/C4 abundance in those regions that have a growing season mean temperature that is near the C3/C4 crossover temperature (i.e. the temperature above which the light use efficiency of C4 plants is higher than that of C3 plants; see Ehleringer *et al.,* 1997, Still *et al.,* 2003b). In this respect, the grassland of Inner Mongolia is of particular interest, because the average temperature during the summer months is close to the crossover temperature when most of the annual precipitation falls (especially July). There are several reports indicating increases in the number and abundance of C4 species with increasing aridity in (Inner) Mongolia grassland (Pyankov *et al.,* 2000; Wang, 2004) and desertification (Wang, 2002b), although conflicting evidence has also been presented (Wang, 2003; Ni, 2003). However, a quantitative, regionalized assessment in terms of biomass is absent.

The spatial variation of C3/C4 species contributing to biomass was determined for recent vegetation by Auerswald *et al.* (2009), who made use of the "sampling" activity of livestock. By grazing the grassland, livestock integrate the carbon isotope information of the grazed vegetation over the entire growing period and the entire grazed area depositing isotopic information from present-day vegetation in wool growth. The soil carbon pool, due to its slow turnover, should contain information of former vegetation. Hence, the aim of this work was to compare the isotopic records of soil and vegetation in a large area of Inner Mongolia using geostatistical tools. Specifically, we address the following questions: (1) Which C3/C4 pattern is reflected by the δ^{13}C of SOC? (2) How do present-day vegetation patterns match with those of SOC? (3) Are differences between former and present-day C3/C4 patterns related to environmental patterns?

Material and Methods

Study area

The study area was situated between 111°38′ and 117°49′E (approximately 500 km) and 41°30′ and 45°46′N (approximately 450 km) in the Inner Mongolia Autonomous Region in the Peoples Republic of China (Fig. VI.1). Sampled altitudes ranged from 800–1700 m above sea level (ASL). Mean annual precipitation (MAP) increases from 100 mm yr^{-1} in the western region near the Gobi desert to 400 mm yr^{-1} in the eastern region. Most of the precipitation (approximately 75%) falls during the growing period (April–September). Mean annual temperature and mean temperature (MAT) of the growing period vary from 0–6°C and 14–19°C, respectively. The soils in the eastern region are mostly Haplic Kastanozems and Chernozems on loesslike substrates, whereas Calcisols and Cambisols are more common in the western region (Li *et al.,* 1978). Almost the entire area is used for small ruminant livestock production (mainly sheep and cashmere goats) with little agriculture, which consists mainly of vegetables and other crops to supplement the human diet, and some maize.
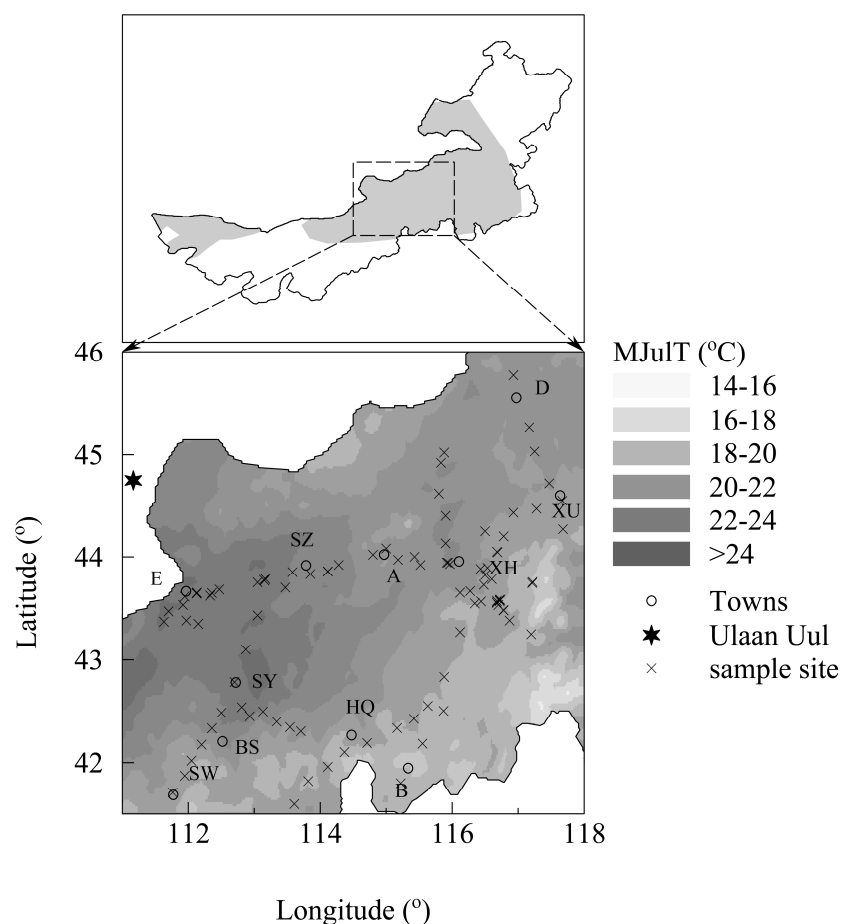
Fig. VI.1 Top Study area (rectangle) within the grassland (shaded area) of Inner Mongolia, P.R. China (contour). **Bottom** Mean July temperature (MJulT, normal period 1961–90) and sampling sites. The star denotes the NOAA/CMDL station in Ulaan Uul. Towns are A = Abag Qi, B = Baochang, BS = Bayan Sum, D = Dong Ujimqin Qi, E = Erenhot, HQ = Huang Qi, SW = Siziwang, SY = Sonid Youqi, SZ = Sonid Zuoqi, XH = Xilinhot, XU = Xi Ujimqin Qi.

Sampling, sample preparation and analysis

The steps from sampling the soil to the interpolated maps, which are necessary to derive C3/C4 pattern from soils, are illustrated as a flow chart in Figure 2. Samples were collected in August/September 2003, August/September 2004, and June/July 2007. The sampling area (106°12′ to 118°54′E and 40°36′ to 46°37′N) exceeded the study area to avoid boundary effects at the periphery of the study area during spatial analysis and interpolation. Sampling positions and altitudes were measured with a mobile GPS. Soil was sampled to a depth of approximately 10 cm within an area of approximately 20 × 20 cm². Altogether 149 samples were taken, ten of them were beyond the periphery of the study area to enhance the spatial interpolation and reduce the error at the boundaries.
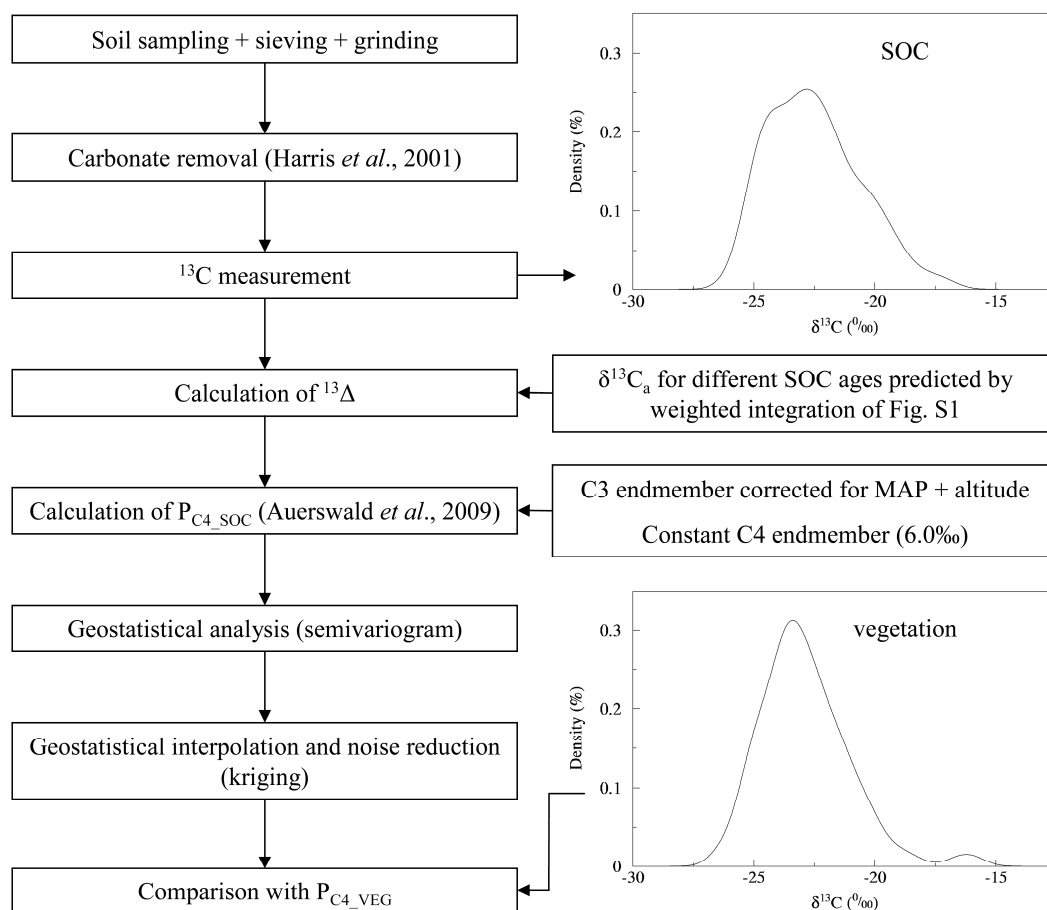
Fig. VI.2 Flow chart of the individual steps from sampling the soil to the P_{C4} map. Graphs present the density estimation of δ^{13}C of SOC (top) and δ^{13}C of vegetation (bottom).

Stones, roots, and litter were removed; the soil was sieved (1 mm), and subsamples were ground to homogeneity with a ball mill after being dried for 48 hours at 40°C. Samples were weighed into silver cups and moistened with 15 µl of de-ionized water. Carbonates were then removed by acid fumigation above a 12 N HCl solution for 24 h (modified after Harris *et al.,* 2001). Afterwards samples were dried at 60°C for 12 h. Depending on SOC content, 7–15 mg of soil in silver cups coated with tin cups were analyzed for their isotopic composition.

The carbon isotope composition was 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). Carbon isotopic data are presented as δ^{13}C, with δ^{13}C = ($R_{sample}/R_{standard}$) − 1, where R is the ^{13}C/^{12}C ratio and standard is the Vienna Pee Dee Belemnite standard. Each sample was measured against a laboratory working standard CO_2 gas, which was previously calibrated against an IAEA secondary standard (IAEA-CH6, accuracy of calibration 0.06‰ SD). After every tenth sample a solid internal lab standard (SILS) with similar C/N ratio as the sample material (fine ground wheat flour for plant samples; protein powder for wool) was run as a blind control. The SILS were previously

calibrated against an international standard (IAEA-CH6). The precision for sample repeats was 0.22‰ (standard deviation).

Carbon isotope discrimination of plants and retrieval from SOC

The δ^{13}C of plants (δ^{13}C$_p$) differs from the δ^{13}C of air (δ^{13}C$_a$) depending on the discrimination $^{13}\Delta_p$ (Farquhar *et al.,* 1989):

$$^{13}\Delta_p = \frac{\delta^{13}C_a - \delta^{13}C_p}{1 + \delta^{13}C_p},$$ (VI.1)

with δ^{13}C$_a$ continuously decreasing over time as a consequence of the CO$_2$ accumulation in the atmosphere (i.e. Suess-effect). Assuming that SOC originates entirely from plant material, $^{13}\Delta_p$ can be calculated from the δ^{13}C of SOC (δ^{13}C$_{SOC}$). However, an isotopic shift between plant and soil carbon must be considered. This varies with soil depth and, hence, integration time, and is termed inherent fractionation (Krull *et al.*, 2005). The inherent fractionation can be attributed to several processes, such as preferential degradation of some plant materials, microbiological and invertebrate activity, Suess effect, and adsorption processes (see discussions by Ehleringer *et al.*, 2000; Santuckova *et al.*, 2000; Krull *et al.*, 2005). The inherent fractionation usually ranges between 1‰–3‰ enrichment (Krull *et al.*, 2005). However, it is difficult to find a fixed value for the fractionation from paired samples of soil and plant material due to the large difference in integration time between both pools and the multitude of influences, which may differ in importance between sites. This is especially true when a change in the C3/C4 ratio affects the pools. In mixed C3/C4 grassland, the δ^{13}C$_{SOC}$ of the A horizon is enriched by approximately 1‰ compared with the present standing biomass (Krull *et al.,* 2005; von Fischer *et al.*, 2008). Because we only sampled the A horizon and we explicitly accounted for the Suess effect (see below), which corresponds to approximately 1.2‰ (see Supporting Information in the online version of this article), we assumed no further inherent fractionation. Yet, we discuss related phenomena.

Atmospheric isotope composition, CO$_2$ concentration, and crossover temperature

When determining the C3/C4 vegetation pattern from SOC, one must address the fact that SOC comprises plant material in varying proportions from different years. As δ^{13}C$_a$ changes over time (Suess effect) so does the respective δ^{13}C$_p$ (Zhao *et al.*, 2001). Hence, one must

know how $\delta^{13}C_a$ has changed, and during approximately which years the SOC originated. $\delta^{13}C_a$ and CO_2 concentrations are known from ice-core studies (Friedli *et al.*, 1986; Francey *et al.*, 1999) and from atmospheric monitoring (Keeling, 1979; Friedli *et al.*, 1986; Conway *et al.*, 1994; Francey *et al.*, 1999; Gat *et al.*, 2001; Allison *et al.*, 2003; NOAA ESRL, 2008; *n* = 62). Because the changes do not follow a general trend, three time periods with overlapping cubic functions were fitted to the $\delta^{13}C_a$ data (see Supporting Information in the online version of this article), yielding a continuous estimate of mean annual values for each year back to 1700. The root mean squared error (RMSE) for the predicted $\delta^{13}C_a$ was 0.08‰. Further we accounted for a 0.14‰ less negative $\delta^{13}C_a$ during the growing period compared to the annual mean (Wittmer *et al.*, 2008), as derived from air samples at Ulan Uul, a long-term monitoring station in the Republic of Mongolia less than 50 km northwest of the study area (Tans & Conway, 2005) (Fig. VI.1).

Because SOC cannot be assigned to a particular year, we accounted for the overall integration by assuming an exponential decay function (Wynn & Bird, 2007):

$$D = \exp(-k \cdot t_{SOC}), \quad\quad\quad (VI.2)$$

with D denoting the fraction contribution of a certain year t_{soc} (year before present, yr b.p.) to SOC. We assumed a maximum SOC age of 700 years and (numerically) varied the decay constant k between 0.02 and 0.003 to obtain mean SOC ages between 50 and 300 years. For example, a decay constant of 0.02 corresponded to a 'fast' decay and thus a relatively recent mean SOC age of 50 years. A weighted mean $\delta^{13}C_a$ was then calculated based on the respective decay function. For further calculations we assumed a mean SOC age of 100 yr, and we discuss the implications of different SOC ages (Table VI.1).

Table VI.1 Influence of mean SOC age b.p. on decay-weighted mean volumetric CO_2 concentration in the atmosphere, crossover temperature (i.e. the temperature above which the quantum yield of C4 plants is higher than that of C3 plants), $\delta^{13}C_a$ during the growing period, and the corresponding C4 abundance calculated from SOC when applying the modeled $\delta^{13}C_a$ and averaged over all sampling sites. The weighted means take into account the change of a variable over time at varying proportions depending on mean SOC age and a first order decay function (Eq. VI.2).

Mean SOC age (yr)	Volumetric CO_2 concentration (ppm)	Range for crossover temperature (°C)	Decay-weighted $\delta^{13}C_a$ (‰)	Site-mean P_{C4} (%)
50	321	16.3 - 21.5	-7.23	12.9
75	306	15.5 - 20.6	-7.04	11.1
100	298	15.0 - 20.2	-6.88	9.7
150	290	14.6 - 19.7	-6.78	8.8
200	286	14.3 - 19.4	-6.67	7.9
300	276	13.7 - 18.8	-6.52	6.5

The crossover temperature for quantum yield of CO_2 uptake is a proxy for the physiological mechanisms explaining the C3/C4 distribution along temperature and CO_2 concentration gradients according to Collatz *et al.* (1998) (see equations 1 and A1 to A4 in Collatz *et al*, 1998). This procedure also accounts for effects of CO_2 concentration on quantum yield. As the latter increased over time, the crossover temperature also increased (see Supporting Information in the online version of this article).

Meteorological data

The long-term averages of the last normal period (1961–1990) of precipitation and temperature (monthly and annual means) were taken from high-resolution maps obtained from The Climate Source LLC (2002). These maps have a pixel resolution of $0.02° \times 0.02°$ (approximately 1.5×1.5 km²), which was judged sufficient to locate the sampling sites. The maps were created using the PRISM method (parameter-elevation regressions on independent slopes model; Daly *et al.,* 2002).

Estimation of C4 fraction from SOC and present vegetation

At each site, the C4 fraction (P_{C4}) from SOC (P_{C4_SOC}) was estimated from the $^{13}\Delta_{SOC}$ using a two-member mixing model:

$$P_{C4\_SOC} = \frac{^{13}\Delta_{SOC} - {^{13}\Delta_3}}{^{13}\Delta_4 - {^{13}\Delta_3}},$$
(VI.3)

where $^{13}\Delta_3$ and $^{13}\Delta_4$ denote the end-members of the mixing model (local $^{13}\Delta$ of pure C3 and C4 vegetation communities, respectively). $^{13}\Delta_4$ was taken as 6.0‰ (Auerswald *et al.*, 2009). Regionalized estimates of $^{13}\Delta_3$ were obtained following Auerswald *et al.* (2009):

$$^{13}\Delta_3 = 14.4‰ + 1.1‰ \cdot \frac{A - 1000}{1000} + 2.7‰ \cdot \sqrt{P_G},$$
(VI.4)

accounting for precipitation during the growing period (P_G; Wittmer *et al.,* 2008) and for altitude (A; Männel *et al.,* 2007). P_{C4} for present vegetation (P_{C4_VEG}) covering the period 1998–2007 was taken from Auerswald *et al.* (2009). The latter was estimated from δ^{13}C of woolen samples obtained from sheep grazing within the study area. This approach is

advantegeous, as sheep wool integrates over the entire growing period and the grazing area. To obtain δ^{13}C of the vegetation (Fig. VI.2), a tissue specific discrimination in δ^{13}C between wool and vegetation was taken into account. This approach was discussed and justified in detail by Auerswald *et al.* (2009).

Statistical methods and geostatistical analysis

Linear and multiple regressions were used to evaluate the datasets. The coefficient of determination was tested with a two-sided test for significance of the regression. Hypothesis testing on equal means of groups were carried out using Student's t-test. Significance thresholds of P<0.05, P<0.01, and P<0.001 were used. Statistical spread is denoted as standard deviation (SD), standard error (SE), or 95% confidence interval (CI). Kernel densities (Silverman, 1986) were calculated using Gaussian kernels to yield estimates of the density distribution. Bandwidth of density estimation of different sized data sets was defined *via* Silverman's 'rule of thumb'. To allow for comparison of differently sized data sets integral density was adjusted to unity. All of these procedures followed standard protocols (Sachs & Hedderich, 2006), and were carried out using GNU R 2.7.2 (R Development Core Team, 2008).

Geostatistical analyses (see Rossi *et al.* 1992 and citations therein) were conducted with the auxiliary packages geoR (Ribeiro & Diggle, 2001), gstat (Pebesma, 2004), and PBSmapping (Schnute *et al.*, 2008). The semivariance (γ) equals the variance for values at points, which are separated by a certain distance called lag (Bachmaier & Backes, 2008). The semivariances for classes of different lags yielded the empirical semivariogram (x axis: mean lag, y axis: mean semivariance). A theoretical semivariogram was fitted to minimize weighted least squares, with weights calculated from the ratio of pairs within a class to mean lag (Wittmer *et al.*, 2008). The theoretical semivariogram delivers three parameters: the nugget effect, the sill, and the range. The nugget effect quantifies the small-scale variation including data uncertainty. The sill quantifies the total variation caused by the nugget effect and the variation due to a spatial pattern. The nugget/sill ratio hence reflects the ratio of random (unexplained by a pattern)-to-total variation. The range quantifies the distance of autocorrelation caused by the distance between pattern elements. The quality of the fit between the theoretical and the empirical semivariogram was expressed as root mean squared error (RMSE).

Maps were then constructed for a uniform rectangular grid, slightly exceeding the study area by ordinary block kriging (for 5×5 km² grid cells) using the theoretical semivariogram and all measured data. Sites outside the periphery of the study area were included in the

calculation of the map, otherwise the number of samples available for estimations close to the periphery would decrease and be unfavorably distributed in space, both increasing the error variance (Ayyub & McCuen, 1990). The quality of the predictions from the resulting maps was given as the (block) krige standard deviation, which is a measure of the prediction error of an individual block. The krige standard deviations were averaged for the study area. The difference between the P_{C4_SOC} and the P_{C4_VEG} map could then be calculated for each block.

Results

δ^{13}C of SOC and its relationship to environmental parameters

The $\delta^{13}C_{SOC}$ ranged between $-17.4‰$ and $-26.3‰$, with a mean of $-22.7\pm1.8‰$ (SD) and a median of $-23.0‰$ (Fig. VI.2). The $\delta^{13}C_{SOC}$ responded significantly to mean July temperature (MJulT), MAP, MAT, and altitude (Table VI.2). All of the correlations were highly significant (P<<0.001), but altitude explained only 7% of the variation, while the climate parameters explained 30%–35%. However, the correlations were not independent, because there were also close correlations between the environmental variables (all P<<0.001); e.g. MJulT and MAP ($r^2 = 0.88$) or MJulT and altitude ($r^2 = 0.46$). As a result, the effects of environmental variables on $\delta^{13}C_{SOC}$ could not be separated, and each response function of $\delta^{13}C_{SOC}$ to an environmental variable included direct and indirect effects. A multiple regression (MAT, MJulT) explained 37% of the variation, but only the contribution of MJulT was highly significant (P<<0.001), while that of MAT was only significant (P<0.01).

Table VI.2 Effect of the environmental variables altitude, mean annual precipitation (MAP), mean annual temperature (MAT) and mean July temperature (MJulT) on $\delta^{13}C_{SOC}$ of Inner Mongolia grassland soils quantified by linear regressions; climate means apply for the last normal period 1961–1990, n = 149.

Parameter	Range	$\delta^{13}C_{SOC}$ response	SE	r^2	P
Altitude	875—1692 m ASL	-2.8‰ / 1000 m	± 0.001	0.07	<< 0.001
MAP	139—386 mm yr⁻¹	-1.5‰ / 100 mm yr⁻¹	± 0.002	0.33	<< 0.001
MAT	-0.6—7.5°C	0.7‰ / 1°C	± 0.084	0.31	<< 0.001
MJulT	18.5—23.2°C	0.7‰ / 1°C	± 0.080	0.35	<< 0.001

Geographic variation of C4 abundance

The mean P_{C4_SOC} was $9.7\pm2.5\%$ (CI) with a median of 7.3% for the study area. The theoretical semivariogram followed a spherical model for P_{C4_SOC} (Fig. VI.3). The range was

approximately 385 km. The nugget effect corresponded to an uncertainty of approximately 14% for P_{C4_SOC} at the sampling locations. The nugget/sill ratio was 0.35 and indicated that 65% of the variation was caused by a spatial pattern. This was far greater than what was explained with regression analysis of $\delta^{13}C_{SOC}$ (Table VI.2), which assumed (multiple) linear relationships with isolated environmental variables. However, the linear relations of P_{C4_SOC} with altitude, MAP, MAT, and MJulT were narrower than those with $\delta^{13}C_{SOC}$. This can be attributed to the removal of the uncertainty at the sampling locations and the short-range noise by kriging 5 × 5 km² blocks. MAP, MAT, and MJulT each explained about 40% of the block-average P_{C4_SOC}, while only 10% was explained by altitude (Table VI.3). A multiple regression (MJulT, MAT) explained 45% of the variation of block averages.

Table VI.3 Effect of the environmental variables altitude, mean annual precipitation (MAP), mean annual temperature (MAT) and mean July temperature (MJulT) on kriged P_{C4} of 25 km² blocks around the sample sites of Inner Mongolia grassland soils (n = 149) quantified by linear regressions; climate means apply for the last normal period 1961–1990.

Parameter	Range	Kriged P_{C4} response	SE	r²	P
Altitude	875—1692 m ASL	-16% / 1000 m	± 4	0.10	<< 0.001
MAP	139—386 mm yr^{-1}	-8.4% / 100 mm yr^{-1}	± 0.8	0.41	<< 0.001
MAT	-0.6—7.5°C	3.8% / 1°C yr^{-1}	± 0.4	0.38	<< 0.001
MJulT	18.5—23.2°C	3.8%/ 1°C	± 0.4	0.42	<< 0.001
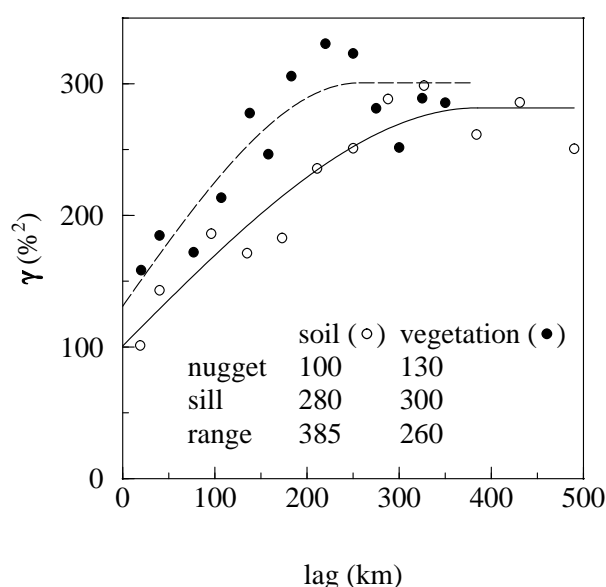
Fig. VI.3 Empirical (circles) and theoretical (line) semivariograms of percent C4 derived from soil and present (1998–2007) vegetation (the latter semivariogram was taken from Auerswald *et al.*, 2009). RMSEs are 20%² and 25%², respectively.

The map of P_{C4_SOC} ranged between 0% and 24% for the individually interpolated 5×5 km² blocks, with a mean block krige standard deviation of 7% for the study area. The map (Fig. VI.4a) was characterized by an increase in P_{C4_SOC} from 0% in the southeastern region to around 24% in the western region of the study area near the Gobi desert, and also by a C3-favoring lobe with a P_{C4_SOC} of approximately 1% in the southeast. Collatz *et al.* (1998) obtained an upper limit for the C3/C4 crossover temperature of 20°C for the warmest month at a volumetric CO_2 concentration of 300 ppm (Table VI.1). The isotherm of this theoretical crossover temperature essentially separated areas of high and low P_{C4_SOC}. Separated into two areas, MJulT below and above the crossover temperature, P_{C4_SOC} differed very highly significantly (Table VI.4), by about 15% between the sample sites above the crossover temperature (mean MJulT = 21.6°C) and the sample sites below (mean MJulT = 19.2°C), despite the flat temperature gradient. This also agreed with the C3 lobe. This extended to the town Abag Qi ("A" in Fig. VI.4a), which was situated in a small temperature depression (~70 $\times$ 70 km²) where MJulT was below the crossover temperature. This clear separation by the isotherm of the predicted crossover temperature indicated a substantial change within a narrow temperature range, and a spatial dependence on temperature.

Table VI.4 Mean contribution of C4 plants (P_{C4}) to vegetation biomass and soil organic carbon for areas of Inner Mongolia separated by the isotherm given by the upper limit of the crossover temperature (c/o) calculated for 1900 and 2000. P gives statistical probability of identical means within a row.

	P_{C4} below c/o	P_{C4} above c/o	P
1900 crossover isotherm			
Soil	1.0%	15.9%	<< 0.001
Vegetation	14.3%	19.1%	> 0.1
2000 crossover isotherm			
Soil	5.2%	11.5%	< 0.05
Vegetation	10.5%	23.3%	<< 0.001
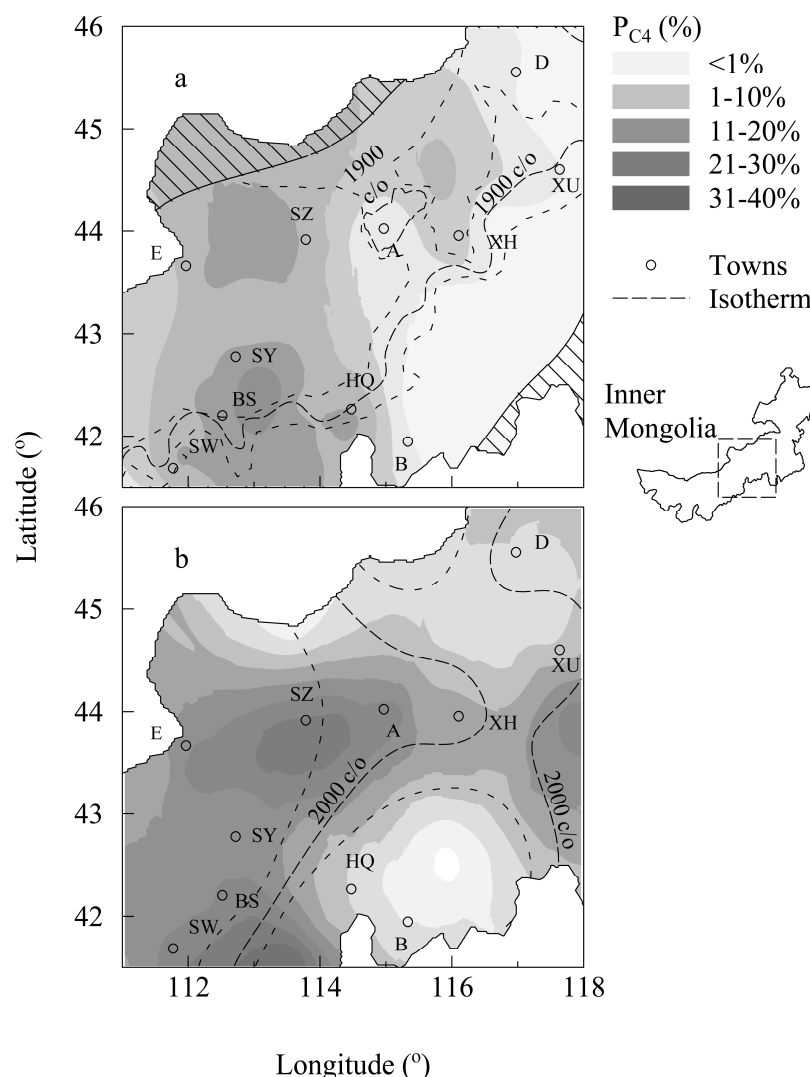
Fig. VI.4 (a) Regional P_{C4} estimated from SOC; mean krige standard deviation for the study area is 7%. **(b)** Regional P_{C4} estimated for present vegetation taken from Auerswald *et al.* (2009); mean krige standard deviation for the study area is 9%. Towns are the same as in Fig. VI.1. The long-dashed lines reflect the isotherms of upper limit for the crossover temperature predicted for the meteorological conditions during the regarded period (1900 *vs.* 2000), while the short-dashed lines give a range of +/- 0.5°C around the mean isotherm. Isotherms are not shown for areas were the krige standard deviation exceeded one class (hatched areas, krige standard deviation >10%).

Comparison of C4 abundance estimated from SOC with present vegetation

The theoretical semivariogram for P_{C4_VEG} (Auerswald *et al.* 2009) also followed a spherical model (Fig. VI.3), but had a range that was 125 km less than the range for SOC. The nugget uncertainty was 2% larger than that for SOC, and the nugget/sill ratio indicated that the spatial pattern was less pronounced by approximately 8%. All of the parameters indicated a substantial difference between both patterns. This was confirmed by the maps (Fig. VI.4), which deviated considerably. P_{C4_VEG} was approximately 10% higher (mean 19.1%) than

P_{C4_SOC} (mean 9.7%). This difference was highly significant (P<<0.001, α=0.001). Again, the structure of the vegetation pattern was related very significantly to the crossover temperature, but only when this was derived for present-day conditions. In contrast, the line of former crossover temperature, which successfully separated high and low P_{C4_SOC}, was a poor predictor for present 10-yr average vegetation (Table VI.4). Analogously, the line of the present crossover temperature poorly separated areas of differing P_{C4_SOC} (Table VI.4).

The map showing the difference between P_{C4_VEG} and P_{C4_SOC} (Fig. VI.5) demonstrated that the increase in P_{C4} mainly occurred along an east–west transect from Erenhot ("E" in Fig. VI.5) to Xilinhot at a latitude of about 44° and in the mountainous area (Da Hinggan Ling mountain ridge) east of Xilinhot ("XH"). Importantly, Fig. VI.5 was not based on any assumption regarding underlying mechanisms, but it indicated that the latitudinal and altitudinal range of C4 occurrence has expanded. A strong increase took place close to Abag Qi ("A"), where a rather small area previously below the former crossover temperature is now well above the crossover temperature. Almost no change occurred in the north and south of the study area, except for a decrease around Huang Qi ("HQ").
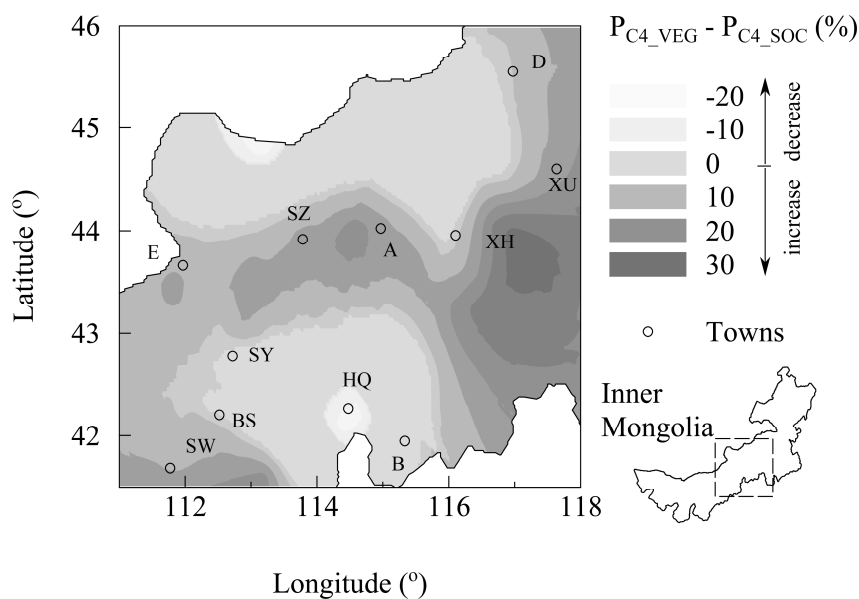
Fig. VI.5 Difference between P_{C4} of present vegetation (P_{C4_VEG}) and P_{C4} derived from soil (P_{C4_SOC}). Towns are the same as in Fig. VI.1. To account for uncertainties (absolute) differences smaller than half the maximum krige standard deviation (=7.5%) were set to zero.

Discussion

Methodological aspects of deriving C3/C4 patterns from SOC

There are two vital and related problems that must be addressed when retrieving P_{C4} from SOC. These are the unknown ^{13}C fractionation during SOC decomposition (Wedin *et al.,* 1995), the quantification of soil age, and the resulting isotopic shift between vegetation and SOC caused by the the Suess effect. With increasing soil depth, the soil ages (Rumpel *et al.*, 2002; Dümig *et al.,* 2008) and the isotopic shift compared to present vegetation increases (e.g. Torn *et al.,* 2002). Following Wynn and Bird's reasoning (2008), we restricted our analysis to the top soil, where both of these influences were smallest and where recent changes in vegetation could be determined. At shallow soil depths, both above-ground and root biomass contribute to SOC, and all of the important species have most of their roots there (Chen *et al.*, 2001) due to the semi-arid climate, in which rainwater during the vegetation period is stored within the first few decimeters of soil.

Apart from the Suess effect, we assumed that no inherent fractionation of $\delta^{13}C_{SOC}$ occurred. Yet we acknowledge that further fractionation-associated uncertainties may exist (von Fischer *et al.*, 2008) and that this may lead to further relative enrichment of $\delta^{13}C_{SOC}$. However it appears that further fractionations (e.g, due to respiration by invertebrates or microbes) are negligible during early stages of decomposition (Boutton *et al.*, 1998 and citation therein).

The assumed soil age defines the $\delta^{13}C_a$ and the atmospheric CO_2 concentration, and – in turn – influences the crossover temperature. Assuming soil ages >100 yr had only a small influence on the crossover temperature; hence, deviating soil ages would bias our calculated crossover temperature only marginally. The influence of atmospheric signature was greater, and substantially changed average P_{C4_SOC} values. In any case, P_{C4_SOC} (6.5%–12.9%, Table VI.1) was lower than average P_{C4_VEG} (19.1%, Auerswald *et al.,* 2009). While the difference between P_{C4_SOC} and P_{C4_VEG} became smaller with decreasing soil age, P_{C4_VEG} was still higher. With decreasing soil age, the contribution of present vegetation to SOC increased exponentially. Therefore, an even larger contrast with former vegetation would result if decreasing soil age caused a lower P_{C4_SOC} than in vegetation. For a difference of 6.5% between P_{C4_SOC} and P_{C4_VEG} at a mean SOC age of 50 yr, the contrast between mean P_{C4} in vegetation from the last ten years and the previous fourty years would have to be 8%. As a consequence, the uncertainty caused by the unknown SOC age is not whether P_{C4} has increased, but only whether this increase had occurred more recently and was more rapid (8%

within 50 yr), or whether the change had occurred over a longer period of time (13% within 300 yr).

SOC age should not have an influence on the regional pattern as long as the soil age did not vary spatially. However, there is abundant evidence that SOC turnover is primarily controlled by temperature (references see Wynn & Bird, 2008) e.g. leading to latitudinal gradients in turnover time (Bird *et al.,* 2002). The magnitude of the Suess effect observed in the SOC pool should then also increase with MAT or MJulT. In our analysis we applied a regionally constant Suess effect; this should relatively underestimate P_{C4} at higher temperatures. Because the primary observed effect was an increase in P_{C4} with temperature, this increase could not be caused by regionally varying decomposition rates, but may be even somewhat larger than our estimate.

Land use change and the spatiotemporal variation in C3/C4 composition

Three drivers are likely to cause changes in P_{C4}; namely, land-use change, change in CO_2 concentration, and temperature change. Land use was mainly influenced by the transition from nomadic to sedentary life style in the 1960s (Humphrey & Sneath, 1995), and by the fast economic rise in the 1990s (e.g. Xu & Zhang, 2007). Although these drivers were active within the entire study area, their effect may have differed between herders, because of differences in attitude and access to markets. Hence, land use changes would most likely result in small-scale changes, which should increase the nugget effect and decrease the range in the semivariogram (Rossi *et al.*, 1992). Both of these modifications were observed. The nugget effect of present vegetation was larger than that of soil, despite the method of retrieving the vegetation pattern from wool, which eliminated the small-scale variation within the grazing ground of a flock of sheep, and thus lowered the nugget effect. Given the patchiness of the vegetation, the nugget effect would have been much larger if the vegetation had been sampled on the same spatial scale (20 × 20 cm²) as the soil, because of the decreasing so-called 'support' (i.e. the spatial extent for which the measured value for the property is valid) increasing the nugget effect (Webster, 1991). Sampling wool, however, conserved the variation between vicinal herds, thus contributing to the nugget effect. Further, the decrease in the range of the present vegetation compared with soil supported land use as a main driver. However, the pronounced regional pattern in the change of P_{C4} (Fig. VI.5) was likely not related to these land use changes.

CO$_2$ concentration change and the spatiotemporal variation in C3/C4 composition

Changes in CO$_2$ concentration should have decreased P$_{C4}$ in the entire area, thus reducing the competitiveness of C4 plants (Ehleringer *et al.,* 1997). The present data contrast with this prediction. P$_{C4}$ increased, and this occurred in a pronounced pattern. Moreover, this increase was independent of P$_{C4\_SOC}$ (Table VI.1). Very clearly, the increase in CO$_2$ concentration was unlikely to have caused the change in the P$_{C4}$ pattern. Yet, it may have attenuated the increase in P$_{C4}$, and it may explain why, in the southwestern part of the study area, P$_{C4}$ actually decreased.

Temperature change and the spatiotemporal variation in C3/C4 composition

Changes in temperature should also cause large-scale changes, but because temperature change varies regionally, this effect was perhaps not homogenous within the area. The regional variation in temperature change became obvious when comparing the line of the present crossover temperature on the vegetation map with the line of the former crossover temperature on the soil map (Fig. VI.4). Although the present crossover temperature is expected to be 2°C higher due to the increase in CO$_2$ concentration, both isotherms should still be near parallel if the temperature pattern has not changed. However, this was not the case. While the former crossover temperature isotherm essentially extended SW-NE, the present crossover temperature extended SSW-NNE and crossed the former one, indicating a substantial change in the temperature pattern. Both P$_{C4}$ patterns basically followed the predicted crossover temperatures of the respective periods. The 'true' crossover temperature may have differed because species/metabolic groups may differ in quantum yield (Ehleringer *et al.,* 1997). Moreover, MJulT is only a proxy for leaf temperature (Teeri, 1988; Wynn & Bird, 2008), which is the direct control of photosynthesis in C3 and C4 plants (Ehleringer & Björkmann, 1977). Also, the true crossover temperature for SOC may deviate from that calculated for the last normal period (1961-1990) depending on the temporal integration of SOC. This potential bias should be small as no distinct warm or cold period occurred between 1700 and 1970 (Liu *et al.*, 2009) and most of the SOC was formed after 1960. E.g. approximately 40% of total SOC was formed after 1960 assuming a SOC age of 100 years (Eq. VI.2). Hence, it can still be expected that the true lines were near parallels of the most likely crossover temperatures, as shown in Figure 4, and thus would also explain the pattern.

The effect of even a constant temperature increase would differ depending on the initial conditions. A temperature increase should be most effective in areas close to the crossover temperature, while it becomes marginal at temperatures well above or below. The largest increase in P_{C4} (Fig. VI.5) occurred within a latitudinal belt along 44°N and in the only mountainous area in the east, indicating that the increase in temperature has enlarged the latitudinal and altitudinal range in which C4 successfully competed with C3. This is in agreement with a recent finding by von Fischer *et al.* (2008) showing that the competition between C3 and C4 in North American grasslands was particularily sensitive to changes in summer temperature.

Hence, temperature changes appeared to be the main driver for the change in P_{C4}, while the change in CO_2 concentration likely attenuated this effect. Land use changes may have added local variation. The interaction of many drivers in creating the pattern was also evident from the poor performance of the linear and multiple regressions in explaining the variation despite the pronounced pattern that was evident from the nugget/sill ratio and the maps. Geostatistical analysis does not depend upon predefined variables and types of relation, e.g. linear, polynomic or exponential. Instead, it quantifies the autocorrelation depending on distance, revealing the response of vegetation in a complex web of interactions. This is necessary because estimates of the terrestrial ^{13}C discrimination must take into account ecophysiological processes on the leaf and plant scale, translate these processes into regional or global scales (Lloyd & Farquhar, 1994; Still *et al.*, 2003b; Wynn & Bird, 2008), and enable quantification of the change and range of underlying patterns.

Conclusions

This work indicates that P_{C4} of Inner Mongolia grassland has increased by approximately 10% in the past decades. Although there are uncertainties regarding inherent fractionation during SOC formation, turnover and age, these appear to be much smaller than the change revealed by the isotope data. In particular, these uncertainties would not account for the distinct relationships of these changes with weather factors. The main driver for the increased P_{C4} appeared to be regional warming, which increased the latitudinal and altitudinal range of C4 plants. Both patterns of P_{C4}, in recent vegetation and in SOC, followed the predictions derived from crossover temperature of quantum yield for CO_2 fixation in modern time and in the period of SOC formation.

CHAPTER VII – Crossing the Mongolian border: does the C3/C4 distribution follow changes in grazing or environmental conditions – evidence from $\delta^{13}C$ of woollen artefacts

Abstract

Aims The study was carried out to investigate whether grazing conditions cause differences in the relative proportion of C4 biomass in total aboveground biomass (P_{C4}) in mixed semi-arid C3/C4 grasslands with different socio-economic backgrounds, but similar environmental conditions.

Location Mongolian-Manchurian grassland (northern China, Republic of Mongolia) between 106°28' and 117°49'E (approximately 850 km) and 41°34' and 47°50'N (approximately 800 km).

Methods We derived P_{C4} from the carbon isotope composition ($\delta^{13}C$) of sheep wool (n = 298) originating from different years (1996 – 2007). In doings so, we accounted for $\delta^{13}C$ fractionation between vegetation and wool, and effects of aridity and altitude on the C3 end-member. We used a geostatistical approach to create a map of P_{C4} for the Mongolian-Manchurian grassland and related the observed spatial pattern to temperature gradients and to the livestock density of the respective country.

Results We found no differences in P_{C4} between the grasslands in Inner Mongolia and Republic of Mongolia close to the border. The spatial pattern of P_{C4} greatly agreed with the isotherms of the mean July temperature (MJulT) of the respective period 1996 – 2007. MJulT explained 71% of the pattern of P_{C4} at the sampling sites. P_{C4} showed no significant relationship to livestock density, despite a distinct variation of a factor of about three between Inner Mongolia and Republic of Mongolia and transhumant *versus* sedentary grazing.

Main conclusions Our results showed that C4 abundance on the regional scale is mainly driven by environmental conditions and that grazing does not affect this pattern. However, on the small scale (i.e. the farm scale) distinct deviations from the temperature induced P_{C4} pattern exist.

Introduction

The carbon isotope composition (δ^{13}C) of terrestrial ecosystems holds important information on photosynthetic pathways, carbon fluxes and linked biogeochemical cycles (Schimel, 1995; Ehleringer *et al*., 2000). A distinct variation in the ^{13}C signal can especially be anticipated for grasslands with variable proportions of C3 and C4 photosynthetic types (Tieszen *et al*., 1997; Bird & Pousai, 1997; Collatz *et al*., 1998), as the respective carbon isotope discrimination ($^{13}\Delta$) is greatly different between them (Farquhar *et al*., 1989). A shift in the C3/C4 community structure will have major implications on the biogeochemistry of the grasslands, and further will affect land-use, as the magnitude and seasonal distribution of biomass production, soil carbon storage, water use and nutrient cycling will be altered (Tieszen *et al*., 1997; Bird & Pousai, 1997; Epstein *et al*., 1998; Sage & Kubien, 2003; Semmartin *et al*., 2004). As it directly relates to the C3/C4 ratio, δ^{13}C is a useful proxy of the vital functions of grasslands. However, there exist only few regional-scale investigations on δ^{13}C of mixed C3/C4 grasslands (e.g. von Fischer *et al*., 2008; Auerswald *et al*., 2009; Wittmer *et al*., 2010b).

The theory behind the distribution of C4 plants is fairly clear. It appears that the growing season temperature acts as the main driver of current C4 distribution (Ehleringer *et al*., 1997; Collatz *et al*., 1998; Pyankov *et al*., 2000; Still *et al*., 2003b) and that this is related to the higher effective quantum yield of CO_2 fixation (Ehleringer & Bjorkman, 1977) or higher maximum photosynthetic rate of C4 plants (Sage & Kubien, 2003; Still *et al*., 2003b) at higher temperature. Distinct influence of a change in temperature on the C4 abundance can hence be expected for grasslands with temperature close to so-called crossover temperature (i.e. the temperature above which the light use efficiency of C3 plants is inferior compared to that of C4 plants; see Ehleringer *et al.*, 1997). On the regional scale, this was recently corroborated by Auerswald *et al*. (2009). They showed that the pattern of C4 distribution in Inner Mongolia grassland closely follows temperature gradients. However, there are several more factors that can modify the C4 distribution. Land-use changes (Archer *et al.*, 1995), grazing (Wang, 2002a; Derner *et al*., 2006), anthropogenic disturbance (Li *et al*., 2006) and atmospheric CO_2 concentration (Collatz *et al*., 1998) can exert direct effects on the C4 distribution. So far, models predicting the global C4 distribution are based on environmental factors, like temperature, precipitation and the temporal change in ambient CO_2 concentration (Collatz *et al*., 1998), while anthropogenic or socio-economic factors are not taken into account. The same applies for regional studies (e.g. von Fischer *et al*., 2008; Auerswald *et al*.,

2009), as the contrast of anthropogenic or socio-economic factors between herders within a country is manifold, often small or contradictory and thus difficult to quantify. Grazing is often assumed to exert direct effects on the community composition – and hence on the C3/C4 ratio – due to the plants response to defoliation, herbivore selectivity and species physiology (Brown & Stuth, 1993).

However, there exists a large contrast between land-use conditions in the Autonomous region of Inner Mongolia in northern China and the Republic of Mongolia. Both countries share a 4700 km long common border with identical environmental conditions on both sides. However, both countries were strictly separated as belonging to different blocks during the Cold War. The separation and the differences in ruderal development are still largely maintained although both countries partly opened and intensified their economy.

Historically, the Mongolian-Manchurian grassland was inhabited by transhumant pastoralists, who lived in yurts and moved with their livestock to different seasonal pastures, but on the occasion of extended feed shortage also moved over larger distances. Their animals were herded by monastery serfs or banner princes' serfs. In Inner Mongolia theses institutions were replaced in the 1950s, and the pastoralists were collectivized and forced to settle down. The collectives were dissolved again in the early 1980s and individual pastoralists gained ownership of the livestock but remained sedentary (Humphrey & Sneath, 1995; Sneath, 1998). Nowadays, the pastoralists in Inner Mongolia live in permanent settlements (Fig. VII.1 top).

Accompanied with the change in land-use, the economical framework also changed. This manifests in the gross domestic product of Inner Mongolia which increased from approximately 122 Mio Euro (1216 Mio Yuan) in 1952 by about 500% to about 60910 Mio Euro in 2007. During the same time, the number of sheep and goat increased from about 5.7 Mio heads to about 87.7 Mio heads (Statistical Yearbook of Inner Mongolia, 2008). Problems induced by increased grazing pressure were recognized by the Chinese authorities and lead to a prohibition of grazing by sheep and goat from mid April to early June (45 days) since 2001.

In contrast to Inner Mongolia, this mobile system endured even after Mongolia turned socialistic with governmental campaigns to organize the herders in collectives in the early 1950s. In the 1980s, when communism in the former Soviet Union descended, Mongolia moved towards a democratic system, again allowing for private ownership of the livestock. After the complete breakdown of the communistic system in 1990, nearly all livestock was owned by private households, who – in contrast to Inner Mongolia – widely kept their transhumant lifestyle (Sneath, 2002; Fernandez-Gimenez, 2006). In contrast to Inner

Mongolia, the economic condition changed much slower with the gross domestic product increasing by 170% from 1981 to 2007 (from 1540 Mio Euro to 2620 Mio Euro; World Bank, 2009).

The number of sheep and goat increased from about 17 Mio heads in 1950 to about 35.4 Mio heads in 2007 (Suttie, 2000; Statistical Yearbook of Mongolia, 2008). Nowadays, about 30% of the inhabitants of the Republic of Mongolia live in the greater area of Ulaanbaatar (the capital of Republic of Mongolia) and another 30% in other urban areas creating also a large gradient in livestock density within the country. The pastoralists living in rural areas still maintain their mobility and live in yurts (Fig. VII.1 bottom). During winter time, the animals do not receive supplementary fodder but stay at winter grazing pastures (Fernandez-Gimenez, 2006).

Fig. VII.1 Typical farmhouse in Inner Mongolia (top) and typical yurts in Republic of Mongolia (bottom). Pictures were taken by K. Auerswald during sampling trips.

Despite the large contrast in the intensity and management of grazing between both countries, livestock keeping is still the principal source of income for the rural population. Thus, we use

the recently reported method by Auerswald *et al*. (2009) to infer the proportion of C4 plants in aboveground-biomass from $\delta^{13}C$ of woollen samples to answer the following question concerning the C4 abundance in the Mongolian-Manchurian grassland:

(i)   Is there a difference in the pattern and level of relative C4 abundance in aboveground biomass between the grasslands of Inner Mongolia and Republic of Mongolia?

(ii)   Can the observed pattern be explained by differences in the socio-economic conditions (e.g. livestock density and pastoral system), or does this pattern mainly depend on environmental conditions?

Material & Methods

Study area, sampling and transhumance

The study area was situated between 106°28' and 117°49'E (approximately 850 km) and 41°34' and 47°50'N (approximately 800 km) in the Autonomous Region of Inner Mongolia in the Peoples Republic of China and in the Republic of Mongolia (Fig. VII.2). Sampled altitudes ranged from 800-2000 m above sea level. Mean annual precipitation (MAP, mm yr^{-1}) increases from less than 100 mm yr^{-1} in the Gobi Desert to more than 400 mm yr^{-1} in the eastern and northern part of the study area. Most of the precipitation (approximately 75%) falls during the growing period (April-September). Mean annual temperature and mean temperature of the growing period vary between -4 to 6°C and 14 to 19°C, respectively. The spatial variation of temperature and precipitation follows altitudinal and latitudinal trends.

Samples in Inner Mongolia were collected in August/September 2003, August/September 2004, July 2005, July 2006, June/July 2007 and July 2009 (partially from Auerswald *et al*., 2009). Samples in Republic of Mongolia were collected in July/August 2006. The sampling position and altitude were measured with a mobile outdoor global positioning system. Overall, 146 samples dating from 1998-2007 were sampled on 101 sites in Inner Mongolia (partially from Auerswald *et al*., 2009). The constraint of grazing prohibition in the early growing period is partially compensated by the distribution of 0.15 kg d^{-1} animal^{-1} of concentrate pellets, which consist mainly of maize (on average 65%). Overall, this adds about 1% C4 to the annual diet of sheep and goats. Animals do not receive supplements on pasture. Normal pen fodder in the winter and/or spring consists of hay from meadows (information obtained from interviewing the local herders).

In the Republic of Mongolia, 152 samples, dating from 1996-2006 were sampled on 65 sites. The transhumant movement in Republic of Mongolia was elaborated from interviewing the herders (n= 65). The maximum distance between summer and winter places was about 70 km, the arithmetic mean was about 16 km and the geometric mean was about 8 km. Hence, samples could be assigned to the location of sampling given the total extension of the research area. Where long-distance movement has occurred (e.g. induced by droughts), the samples were assigned to the place of origin.
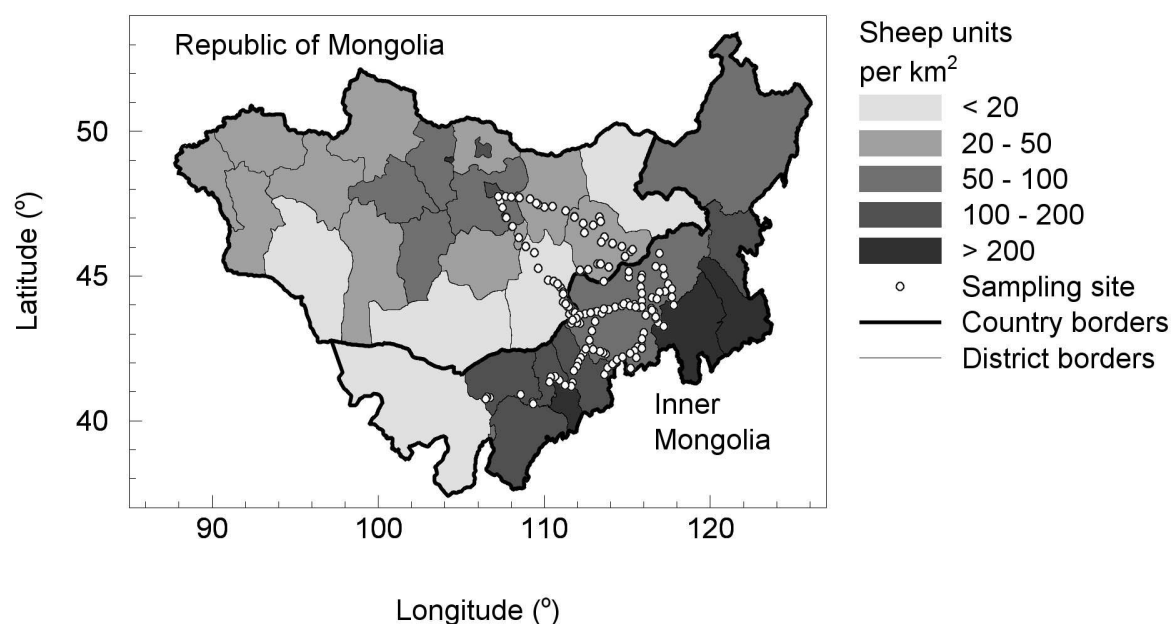
Fig. VII.2 Sampling sites in Inner Mongolia and in the Republic of Mongolia and livestock density averaged for the years 1996 to 2007 expressed as sheep units per km² for the districts (Aimags in Republic of Mongolia and Leagues in Inner Mongolia).

Sample preparation, isotope analysis and estimation of C4 fraction

The samples were cleaned by the procedure of Schwertl *et al.* (2003) and 0.2-0.4 mg wool were packed into tin cups for isotope analysis. The carbon isotope composition of each sample was 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). Carbon isotopic data are presented as $\delta^{13}C$, with $\delta^{13}C = (R_{sample}/R_{standard}) - 1$, where R is the $^{13}C/^{12}C$ ratio and standard is the Vienna Pee Dee Belemnite standard. Each sample was measured against a laboratory working standard CO_2 gas, which was previously calibrated against an IAEA secondary standard (IAEA-CH6, accuracy of calibration 0.06‰ SD). After every tenth sample a solid internal lab standard (SILS) with similar C/N ratio as

the sample material (protein powder) was run as a blind control. The SILS were previously calibrated against an international standard (IAEA-CH6). The precision for sample repeats (SD) was better than 0.1‰.

The relative contribution of C4 plants (P_{C4}) to aboveground biomass at each site was estimated from $\delta^{13}C$ of the woollen samples, following the procedure by Auerswald *et al*. (2009). We accounted for the isotopic fractionation between vegetation and sheep wool (Auerswald *et al*., 2010; Wittmer *et al*., 2010a), the influence of aridity (Wittmer *et al*., 2008) and the influence of altitude (Männel *et al*., 2007) on the C3 end-member. The C4 end-member was taken constant as 6.0‰ (Auerswald *et al*., 2009). A bias due to the selection of sheep or differences in the digestibility of C3 and C4 components could be excluded over the entire range of grazing intensities found in both countries *via* a controlled grazing experiment (Wittmer *et al*., 2010a).

Meteorological, livestock and geographical data

For year-specific data on precipitation and temperature we followed the procedure by Wittmer et al. (2008) and corrected the long-term average high resolution (2 km x 2 km) maps (The Climate Source LLC, 2002) for the deviation of an individual year, which was geostatistically interpolated between meteorological stations. Daily precipitation and temperature data of 63 climate stations, provided by the NOAA Satellite and Information Service (NOAA NCDC Climate Data Online, 2009) were used. For statistical analsysis, we used either the mean July temperature (MJulT) of the respective year from which the wool originated or the average for the observation period 1996 to 2007 indicated by the index '96-07'.

Livestock data and land area of the Leagues (Inner Mongolia) and the Aimags (Republic of Mongolia) were taken from the official statistical yearbooks (National Statistical Office of Mongolia, 2003; 2004; 2007; Statistical Yearbook of Inner Mongolia, 2008). Livestock data were converted to sheep units (SU) according to Table VII.1. Borders between countries and districts (Leagues, Aimags) were taken from GDAM (www.gdam.org, 2009).

Table VII.1 Sheep units for the different types of livestock in the Mongolian-Manchurian grassland

Animal	Sheep unit (SU)
Sheep	1
Goat	0.9
Cattle, horse, donkey or mule	6
Camel	5.4

Statistical and geostatistical analysis

Linear regressions and multiple linear regressions were used to evaluate the datasets. The coefficient of determination was tested with a two-sided test for significance of the regression. Significance thresholds of $P<0.05$, $P<0.01$, and $P<0.001$ were used. Statistical spread is denoted as standard deviation (SD), or 95% confidence interval (CI) of the mean. To test whether the level of P_{C4} differed between Inner Mongolia and Republic of Mongolia we used Student's t-test on parity of the means of two populations (two-sided). This was performed against a 95% confidence interval, preceded by an F-Test on equality of variances. Populations were selected from the data of all sampling points within a 100 km belt to each side along the common border. We only used data which originated from the overlapping period of 1998 to 2006 to exclude of inter-annual fluctuations. All statistical procedures followed standard protocols (Sachs & Hedderich, 2006), and were carried out using R 2.9.0 (R Development Core Team, 2009).

Geostatistics quantify the nature of spatial dependence of a property. This allows separating the data uncertainty from the spatial pattern, to interpret the pattern, and to estimate the property at unrecorded positions (see Rossi *et al.*, 1992, and citations therein). The semivariance (γ) equals the variance for values at points, which are separated by a certain distance called lag (h). The semivariances for classes of different lags yielded the empirical semivariogram (x axis: mean h, y axis: mean γ). A theoretical semivariogram was fitted to minimize weighted least squares, with weights calculated from the ratio of pairs within a class to mean h (Wittmer *et al.*, 2008). The theoretical semivariogram delivers three parameters: the nugget effect, the sill, and the range. The nugget effect quantifies the small-scale variation including data uncertainty. The sill quantifies the total variation caused by the nugget effect and the variation due to a spatial pattern. The nugget/sill ratio hence reflects the ratio of random-(unexplained by a pattern)-to-total variation. The range quantifies the distance of autocorrelation caused by the distance between pattern elements. The quality of the fit between the theoretical and the empirical semivariogram was expressed as weighted root mean squared error with weights as above. Maps were then constructed for a uniform rectangular grid by ordinary block kriging (for 5×5 km² grid cells) using the theoretical semivariogram and all measured data. The quality of the predictions from the resulting maps was given as the (block) krige standard deviation (SD_k), which is a measure of the prediction error of an individual block. All geostatistical and spatial analysis and data handling were carried out, using R 2.9.0 (R Development Core Team, 2009), with the auxiliary packages gstat (Pebesma, 2004) and maptools (Lewin-Koh & Bivand, 2008).

Results

Distinct difference in livestock density between Inner Mongolia and Mongolia

The livestock density averaged for the years 1996 to 2007 of 94 SU km^{-2} in Inner Mongolia differed by a factor of three from that of 36 SU km^{-2} in Republic of Mongolia. The overall grazing gradient ranged from about 10 SU km^{-2} to more than 450 SU km^{-2} with overlapping livestock densities between Inner Mongolia and the Republic of Mongolia (Fig. VII.3). Samples from Inner Mongolia origination prior to the prohibition of spring grazing in 2001 apparently exhibited no distinctly higher P_{C4} (Fig. VII.3, P>0.5). Small ruminants (sheep and goat) contributed 55% in Inner Mongolia and 43% in the Republic of Mongolia to total SU. This indicates that if grazing – especially by small ruminants – influences P_{C4} an effect must be visible, as the two countries differ markedly in their livestock density. Within a 100-km belt along the common border, livestock densities also differed by a factor of three between both countries although densities are smaller then the respective country averages (Table VII.2).

Table VII.2 Number of data points within a 100 km belt along the country border, the mean stocking density of the respective years (expressed as sheep units; SU km^{-2}), the mean P_{C4}, the respective 95% confidence interval of the mean (CI$_{95\%}$), and the level of significance (P<0.05) for Inner Mongolia and Republic of Mongolia.

	Inner Mongolia	Republic of Mongolia
n	38	67
mean SU (SU km^{-2})	67.7	24.1
SU CI$_{95\%}$ (SU km^{-2})	2.6	3.0
Significance (two-sided)	A	B
mean P_{C4} (%)	17.5	21.9
P_{C4} CI$_{95\%}$ (%)	4.2	3.1
Significance (two-sided)	C	C

P_{C4} is not different between Inner Mongolia and Mongolia

For Inner Mongolia, 38 data points fell into the 100 km belt along the border, for Republic of Mongolia 67 data points fell into the 100 km belt. The means of the respective P_{C4} populations were not significantly different (P>0.05, Table VII.2) despite a highly significant difference of approximately 44 SU km^{-2} between the sampling points in Inner Mongolia and those in the Republic of Mongolia. Even more, P_{C4} was (insignificantly) higher in Republic of Mongolia despite a much lower stocking density.

Geographical variation of P_{C4} and the relation to temperature and livestock density

C4 plants accounted for $18 \pm 2\%$ (CI) to aboveground biomass when averaged but made up to 85% at the site-level. No influence of livestock density could be identified despite its large variation (42-fold) between years and districts (Fig. VII.3). Also in combination with MJulT, no significant influence of livestock density either in multiple regression nor in interaction terms (SU $\times$ MJulT, SU / MJulT) could be identified (Table VII.3). In contrast MJulT was highly significantly related to P_{C4}.
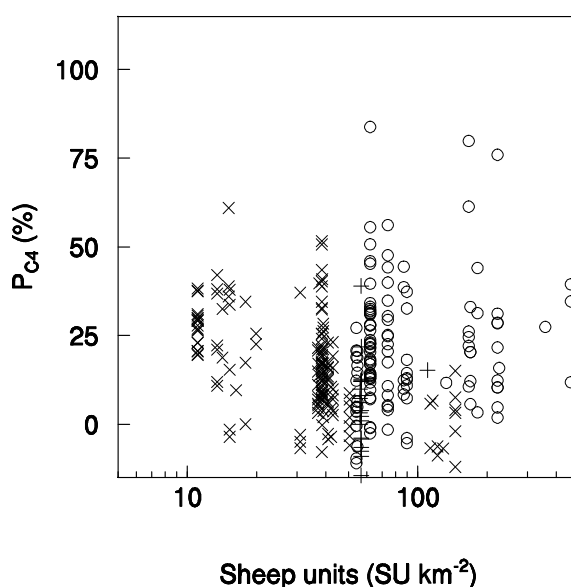
Fig. VII.3 Relative contribution of C4 plants to aboveground biomass (P_{C4}) in relation to year-specific sheep units (log-scaled to increase readability) in the respective district and mean for samples from the Republic of Mongolia ($\times$) and for samples from Inner Mongolia prior to 2001 (+) and since 2001 ($\circ$).

Table VII.3 Linear (1,2,4,5) and multiple (3) regression parameters for regressions of the form $y = \beta_0 + x_1 \times \beta_1 + x_2 \times \beta_2$ with y denoting P_{C4} and x_1 and x_2 denoting either mean July temperature (MJulT) or sheep units (SU) or a combination of both for the respective year from which the sample originated (n=298).

Model	β_0	x_1			x_2			r^2
		Parameter	β_1	P	Parameter	β_2	P	
1	-26.40	MJulT	2.02	<<0.001				0.060
2	16.77	SU	0.01	0.358				0.003
3	-28.95	MJulT	2.08	<<0.001	SU	0.02	0.18	0.060
4	16.46	SU $\times$ MJulT	0.001	0.203				0.005
5	17.16	SU / MJulT	0.16	0.613				0.001

The theoretical semivariogram followed a spherical model (nugget = 140%², sill = 290%², range = 680 km, RMSE = 2%²) and the nugget effect corresponded to an uncertainty of approximately 17%. The nugget/sill ratio was 0.48 indicating a spatial pattern, which explained 52% of the variation in P_{C4}.

Kriged P_{C4} ranged from 1% to 34% (mean SD_k for individual 5 × 5 km² blocks were samples were available: 4%; mean SD_k for all blocks of the map shown in Fig. VII.4: 8.9%). with a mean of 18%. The kriged block means highly significantly increased by 4.5% per 1°C increase in $MJulT_{96-07}$ (Fig. VII.4). The linear regression with $MJulT_{96-07}$ explained 71% of the observed (regional scale) variation.
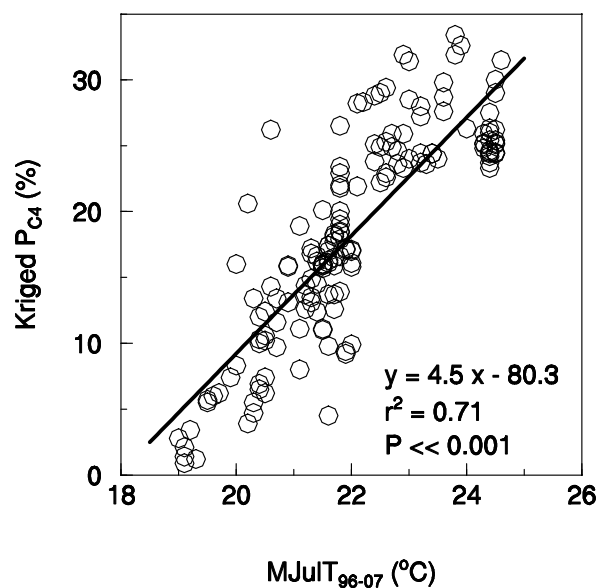
Fig. VII.4 Kriged relative contribution of C4 plants to aboveground biomass (Kriged P_{C4}) at the sampling sites (n = 166) in relation to the mean July temperature, averaged for the years 1996 – 2007 ($MJulT_{96-07}$). The line denotes the linear regression.

The map (Fig. VII.5) indicated an increase of P_{C4} from <10% in the north and east of the study area to >30% in the Gobi desert (west part of the study area). The spatial pattern of P_{C4} greatly agreed with isotherms of $MJulT_{96-07}$, which successfully deliminated areas of 'high' P_{C4} from areas of 'low' P_{C4}. Both parameters agreed well even in areas where sampling density was low, which additionally underlines the pronounced and closely correlated nature of their patterns.

Within the research area, growing period precipitation and MJulT are closely correlated (sample year specific r² = 0.37, last normal period r² = 0.75) with lower precipitation within the warmer area of the Gobi desert. Both influences can hence not be separated. In the drier and warmer part of the research area sufficient rain mainly falls during the warmest months

(June, July and August) while in the cooler areas also spring and autumn months receive rain to sustain some growth. Hence, differences in temperature averaged over the growing period – which is determined by rain availability – are even somewhat larger than differences in MJulT.
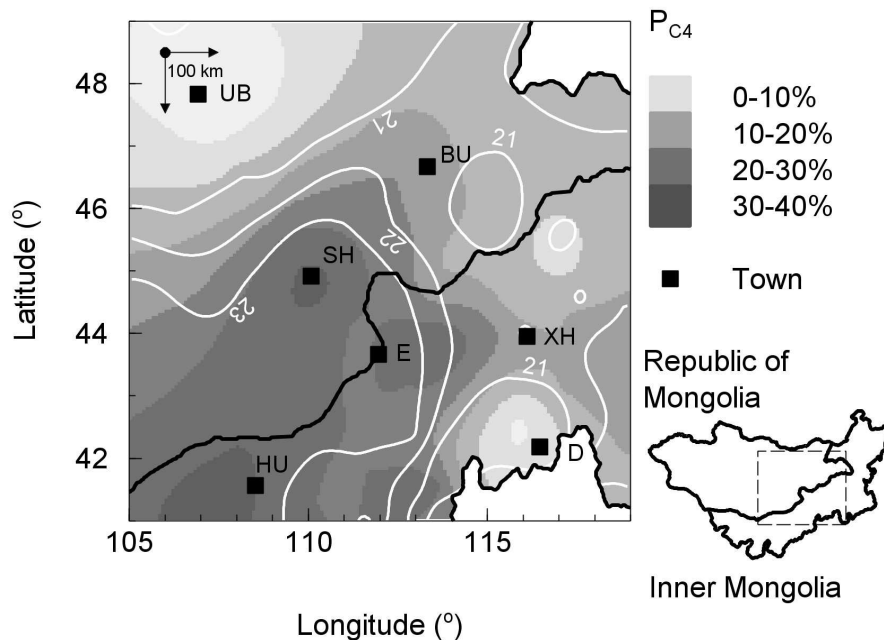
Fig. VII.5 Regional P_{C4} estimated by ordinary block (5× 5 km²) kriging, derived from wool originating from 1996 – 2007. The white lines denote the 21, 22 and 23°C isotherms of the mean July temperature, averaged for the years 1996 – 2007. Towns are UB = Ulaanbaatar, SH = Sainshand, E = Erenhot + Zamny-Uud, XH = Xilinhot, D = Duolun and HU = Haliut.

Discussion

This study clearly showed that livestock density and grazing regime had no influence on P_{C4}, as both variables covered almost their entire cogitable range. Even under ungrazed conditions, which could not be captured by our method based on woollen samples Wang *et al.* (2003b) and Chen *et al.* (2005b) report a contribution of *Cleistogenes squarrosa* – which is one of the two main species contributing to the C4 community in the research area – of 7 to 24 % during 1996 to 2002 in an exclosure experiment. From our map, a mean P_{C4} of 16% during 1996 to 2007 follows for the area (near Xilinhot) in which the exclosure experiment is located. There are two likely explanations how grazing may exert an influence on P_{C4} which need to be discussed in terms of their effective scale.

(i) With increasing grazing intensity and thus biomass removal (micro-)climate may change by reduced transpiration leading to more sensible heat, which then favours C4 species (Terri & Stowe, 1976; Li *et al.*, 2000).

(ii) With increasing grazing intensity the early spring growing C3 species (Wang *et al.*, 2003b) are damaged more that the later developing C4 species because feed shortage is always most pronounced after winter. This may additional reduce early water consumption and leave more soil moisture for the C4 species. Transhumant grazing damages vegetation less the sedentary grazing because seasonal or inter-annual feed shortage is compensated by movement, which should even increase the suppressive effect on C4 if combined with low livestock densities.

Thus the questions arise, why grazing obviously does not influence the pattern of P_{C4} on the regional scale and whether it may have an influence on the local scale. Livestock densities differ on such large areas that an influence on temperature should even be visible on the regional scale. The isotherms, however, show no discontinuity along the Mongolian border. It may still be speculated that temperature may be changed on the local scale but it is difficult to argue why such a change should not scale to the region if not compensated by an opposing influence. Such an opposing influence – working only on the regional scale – is not known. More likely, it already acts on the local scale. And indeed, measurements show that the albedo of bare soil under dry conditions is higher than of vegetation covered surfaces (Li *et al.*, 2000; Lei *et al.*, 2010). This leaves less energy for heating, even if evapotranspiration is lowered by biomass removal due to grazing (see discussion by Li *et al.*, 2000). The reasons why the second explanation is unlikely are less clear. First, all species are adapted to (normal) grazing and sever drought and can survive with little growth over several years (Christensen *et al.*, 2004). Second, C3 can partly escape from mild grazing in spring by adapting their growth habit (Oesterheld & McNaughton, 1991), while a sharp grazing, which takes off nearly all aboveground biomass, would then also cause feed shortage and damage the C4 later in the season, when C4 starts to turn green. Finally, the prohibition of spring grazing, which was set into action in Inner Mongolia in 2001 following years of severe biomass removal and subsequent wind erosion problems, may have beneficial effects for C3. However, it is unlikely that this measure could elevate the effect of grazing on C4 if this effect would be strong. Furthermore, no difference between the years before and after 2001 was obvious from our data (see Fig. VII.3), which would indicate an influence of prohibition of spring grazing on P_{C4}. Hence neither the regional differences nor the temporal difference provide any hint of an effect of grazing pressure in early spring on P_{C4}. On the local scale (i.e. the farm scale) we

observed P_{C4} of up to 85% which might be caused by grazing, but could not be captured by our method. Anyhow, only 10 samples (7 from Inner Mongolia and 3 from Republic of Mongolia) from a total of 298 samples were associated with a P_{C4} larger than 50%. Hence it is not clear and furthermore arguable, if grazing or livestock density really can be considered as a factor altering the C3/C4 ratio even on the local scale.

In contrast to livestock density, MJulT had an overwhelming influence on P_{C4} (Table VII.1; Fig. VII.4) despite the relatively flat gradient. An increase in MJulT of only 4°C caused an increase in P_{C4} of about 20%. Several reasons may contribute to this large effect. First, the area is close to the estimated crossover temperature where a change in temperature should have the largest impact. The crossover temperature is given, where the lines of net photosynthesis *versus* temperature of C3 and C4 species intersect (Ehleringer *et al.*, 1997; Collatz *et al.,* 1998; Still *et al.*, 2003b). For the CO_2 during the growing period of the research period (1996 – 2007) an average crossover temperature of approximately 22°C can be computed for a light saturated stand following Collatz *et al.* (1998). For the observed range in temperature in July (17.7°C – 27.0°C) an increase in net photosynthesis of approximately 120% can be expected for C4 species while net photosynthesis only increases by 15% for C3 species following Still *et al.* 2003b (in both cases assuming a mean atmospheric CO_2 concentration of 350 ppm_v and that leaf temperature equals air temperature). Further temperature related processes than net photosynthesis may, however, also contribute to the success of the C4.

Germination temperature may contribute to this as all C4 in the research area can be classified as ruderals and one of the two dominating C4 species, *Salsola collina*, is an annual species. As germination temperature follows the temperature preference of the plant, C4 have a higher germination temperature than C3 (Baskin & Baskin, 1998). Furthermore, with increasing MJulT also the period favourable for C4 growth increases in length relative to the period of growth for C3 species (data not shown; NOAA NCDC Climate Data Online, 2009). Aridity for that region also increased, as precipitation exhibited no significant trend, but temperature did (data not shown; NOAA NCDC Climate Data Online, 2009). As aridity promotes C4 species (Pyankov *et al.*, 2000; Sage, 2004) it can be expected that ongoing aridification will exhibit further benefits for the C4 species. Furthermore, high growth temperatures tend to decrease the leakiness (i.e the fraction of CO_2 generated by C4 acid decarboxylation that subsequently leaks from bundle-sheath cells; Hatch *et* al., 1995) in C4 grasses (Kubásek *et al.*, 2007). The most abundant C4 species *C. squarrosa* (Yang *et al*., 2010) is characterized by a high leakiness, and may thus additionally profit from high temperatures.

Even though C3 species are favoured by increasing atmospheric CO_2 concentration (Ehleringer *et al*., 1997) this will most likely only attenuate the spread of C4 species in the Mongolian-Manchurian grassland, as the percentage of C4 species in aboveground biomass already increased by about 10% (Wittmer *et al*., 2010b) despite an increase in atmospheric CO_2 concentration. Recent evidence from the Mongolian-Manchurian grassland further suggests that increasing CO_2 concentration promotes C3 species, but also *C. squarrosa* may benefit due to its relatively high leakiness as this might result in increased assimilation under elevated atmospheric CO_2 concentration (Ziska *et al*., 1999).

Conclusions

The conditions in Inner Mongolia and Republic of Mongolia differ greatly in their grazing pressure. Livestock density expressed in sheep units is about threefold higher in Inner Mongolia and in addition, sedentary grazing does not allow adjusting grazing to the annually and regionally changing precipitation sustaining growth. Despite this large contrast no influence of grazing conditions on P_{C4} could be identified. The regional pattern of P_{C4} greatly agreed with MJulT, the temperature during the warmest month.

CHAPTER VIII – What drives the C4 increase - grazing pressure or warming? Isotopic evidence from a multi-year grazing experiment in semi-arid Inner Mongolia

Abstract

Warming and disturbances like grazing are assumed to promote the spread of C4 plants. Here we present data on the abundance of C4 plants from a five-year (2005 – 2009) grazing experiment in the semi-arid grassland of Inner Mongolia. Sheep grazed the grassland with six stocking rates (0.375, 0.75, 1.125, 1.5, 1.875 and 2.25 sheep ha^{-1} $year^{-1}$) from June until September. Wool that had been growing during the grazing period of each year was analysed for its carbon isotopic composition ($\delta^{13}C$). The C4 abundance was then calculated from a two-member mixing model, as $\delta^{13}C$ distinctly differs between C3 and C4 grasses. We accounted for effects of aridity, altitude and isotopic fractionation. Average P_{C4} was 5.8% in 2005 and increased to 12.1% in 2009. In no year, P_{C4} exhibited a significant relationship towards stocking rate. This increase in P_{C4}, however, was highly significantly related to an increase of about 2°C in mean July temperature. Similar findings of an increase in C4 abundance were reported from floristic evidence under grazed and ungrazed conditions. All this suggests that warming is the main driver of the increase in the C4 abundance in semi-arid Inner Mongolia grassland while an influence of grazing pressure could not be verified.

Introduction

Global warming is expected to be a promoter for changes in the distribution and patterns of the vegetative ecotype spectrum (Alward *et al*., 1999; Collatz *et al*., 1998; Epstein *et al*., 1997; Sage & Kubien, 2007), with special relevance for the distribution of the C3 and C4 photosynthetic mechanism (Ehleringer *et al*., 1997). Thus, a notable impact on global (semi-)arid C3/C4 grasslands can be anticipated. The arising shift in the C3/C4 community structure will have major implications on the biogeochemistry of the grasslands, and further will affect land-use, by altering the magnitude and seasonal distribution of biomass production, soil carbon storage, water use and nutrient cycling (Bird & Pousai, 1997; Epstein *et al*., 1998; Sage & Kubien, 2003; Semmartin *et al*., 2004; Tieszen *et al*., 1997). There are clear indications that temperature exerts direct effects on the distribution of C3 and C4 vegetation in (semi-)arid grasslands (e.g. Auerswald *et al*., 2009; von Fischer *et al*., 2008; Wittmer *et al*., 2010b). Disturbances caused by land-use changes (Archer *et al*. 1995), anthropogenic disturbances like dirt roads (Li *et al*., 2006), increasing ambient CO_2 concentration (Collatz *et al*., 1998) and grazing (Derner *et al*., 2006; Hickman *et al*., 2004; Hoshino *et al*., 2009; Wang, 2002a) can also strongly affect the C3/C4 community structure. Especially the impact of grazing on the C3/C4 community structure is conversely presented in literature, as there are reports of a positive effect of grazing on C4 abundance (e.g. Hickman *et al*., 2004; Tong *et al*., 2004), ambiguous effects of grazing on C4 abundance (e.g. Derner *et al*., 2006) and under certain conditions even hampering effects on C4 abundance (e.g. Howe, 1994). Such controversial results may be partly attributed to the fact that it is often difficult to separate grazing effects from other ongoing changes. This is especially true where time series are analysed because all parameters which exhibit a trend will be correlated irrespective whether they are physically connected or not.

One likely explanation why grazing might be the main driver of C4 abundance is the earlier development of C3 species (Liang *et al*., 2002), which then might get more damaged by early grazing (Wang *et al*., 2003b). This additionally promotes the C4 species, as early water-consumption might be reduced, leaving more soil water for later developing species. However, in our experiment grazing did not start before June, thus this scenario is unlikely to explain the observed increase in P_{C4}.

Another possible explanation would be a promotion of C4 species due to a change in micro-climatic conditions (Li *et al*., 2000; Teeri & Stowe, 1976) caused by differential biomass removal among different stocking rates (between 5% and >80%, see Wittmer *et al*., 2010a).

However, this is also unlikely as this should have manifested in a differential P_{C4} among different stocking rates.

To disentangle the possible effects of environmental (i.e. temperature and ambient CO_2 concentration) and other (i.e. grazing) factors on the C3/C4 community structure, a controlled quasi-natural experiment in a suitable habitat is required.

Monitoring changes in the C3/C4 ratio is difficult due to the spatial and temporal variation. Presumably, the most elegant way is to take advantage of the 'sampling activity' of the grazing livestock itself. The carbon isotope composition ($\delta^{13}C$) is distinctly different between C3 and C4 vegetation, due to the pronounced differences in the respective carbon isotope discrimination ($^{13}\Delta$) (Farquhar *et al.*, 1989). Hence, $\delta^{13}C$ of the standing biomass is a mixture of the respective $\delta^{13}C$ of the C3 and C4 component and this signal is propagated in the food web. Since 'you are what you eat, isotopically' (De Niro & Epstein, 1978) this fact has been harnessed in many studies.

Here we report on C3/C4 ratio changes from a five-year (2005 – 2009) grazing experiment with varying stocking rates located in the semi-arid grassland of Inner Mongolia. During the experimental period, the mean July temperature increased by about 2°C. Specifically we want to address to the question, to which degree changes in P_{C4} can be attributed to grazing pressure and/or to July temperature.

Experimental design, isotope analysis, estimation of C4 abundance and statistical analysis

The grazing experiment is located in a typical steppe area of the Xilin river catchment (116°40'E and 43°33'N) in the Inner Mongolia Autonomous Region, P.R. China and is operated by the Inner Mongolia Ecosystem Research Station (IMGERS) and the DFG research group 536 MAGIM (www.magim.net). The vegetation is dominated by perennial C3 grasses (mainly *Stipa grandis* and *Leymus chinensis*) but perennial C4 grasses (mainly *Cleistogenes squarrosa*) and annual C4 herbs (mainly *Salsola collina*) are also present. Altitude is about 1200 m above sea level, mean annual precipitation (last normal period, 1961 – 1990) is about 320 mm yr^{-1} and mean July temperature is about 19.5°C. Yearly meteorological data were obtained from a monitoring station operated within the MAGIM project. 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 two replicates. This yielded a total of 12 plots, each of about 2 ha in size to allow a minimum of three sheep per

plot at the lowest stocking rate. The experiment covered a total area of approximately 2 km². The sheep (*Ovis aries*) were about 2 years old, non-pregnant, and non-lactating ewes from the local Mongolian fat-tail breed. The sheep grazed the pasture from beginning of June, when biomass was sufficient after the onset of growth until September when the growing period ends. Previous to the experiment the whole area was moderately grazed by local semi-nomadic herders until 2003. Then the grass sward was left to recover for two years prior to the beginning of the experiment in 2005. The sheep were shorn before and after the grazing period. Wool samples from the end of the grazing period were used in this study, as this integrated the whole vegetation grazed between June and September. We obtained 12 samples from the years 2005, 2007, 2008 and 2009. No samples were taken in 2006. We excluded one outlier from the 2005 data, thus we yielded an overall of 47 samples.

The distal first cm of the wool samples was discarded. This accounted for the stubbles left after shearing, which were marked by dye, to account for the proportion of hair within the hair channel and turnover of body pools. The remaining hair (approximately 4 to 6 cm) was cleaned following Schwertl *et al*. (2003), ground to homogeneity with a ball mill, and 0.2-0.4 mg hair material was packed into tin cups for isotope analysis. The $\delta^{13}C$ of each sample was 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). Carbon isotopic data are presented as $\delta^{13}C$, with $\delta^{13}C = (R_{sample}/R_{standard}) - 1$, where R is the $^{13}C/^{12}C$ ratio and standard is the Vienna Pee Dee Belemnite standard. Each sample was measured against a laboratory working standard CO_2 gas, which was previously calibrated against an IAEA secondary standard (IAEA-CH6, accuracy of calibration 0.06‰ SD). After every tenth sample a solid internal lab standard (SILS) with similar C/N ratio as the sample material (protein powder) was run as a blind control. The SILS were previously calibrated against an international standard (IAEA-CH6). The precision (standard deviation) or repeats was better then 0.1‰ for SILS, better than 0.2‰ for samples, better than 0.4‰ for sheep and better than 0.7‰ for plots.

P_{C4} from each sample was estimated following Auerswald *et al*. (2009). We accounted for the isotopic fractionation between vegetation and sheep wool (3.2‰; Auerswald *et al*., 2010; Wittmer *et al*., 2010a) on the C3 end-member. The C4 end-member was taken constant as 6.0‰ (Auerswald *et al*., 2009). A bias due to the selection of sheep or differences in the digestibility of C3 and C4 components could be excluded over the entire range of grazing intensities found from another study from the same gazing experiment (Wittmer *et al*., 2010a).

Linear regressions were used to evaluate the datasets. The coefficient of determination was tested with a two-sided test for significance of the regression. Significance thresholds of $P<0.05$, $P<0.01$, and $P<0.001$ were used. Statistical spread is denoted as 95% confidence interval ($CI_{95\%}$) of the mean. To test whether levels of P_{C4} differed between years we used Student's t-test on parity of the means of two populations (two-sided), performed against a 95% confidence interval, preceded by an F-Test on equality of variances. In principle many regressions are possible (e.g. P_{C4} *versus* stocking rate for each individual year or change in P_{C4} of consecutive years *versus* stocking rates). Calculating all possible regressions would call for a Bonferroni correction (Hochberg, 1988), which would pronouncedly decrease the degrees of freedom. The strongest effect of grazing can be expected after the fifth year of the experiment. We will base our conclusions on the results of this year. A Bonferroni correction is then not necessary any more, although we also report regressions of the other years to illustrate that the result is consistent and not caused by this specific year. All statistical procedures followed standard protocols (Sachs & Hedderich, 2006), and were carried out using R 2.9.1 (R Development Core Team 2009).

Results

At the beginning of the experiment in 2005 the mean initial P_{C4} was $5.8 \pm 2.2\%$ (Table VIII.1). After 5 years of grazing, the mean P_{C4} of $12.1 \pm 2.0\%$ (Table VIII.1) was highly significantly higher ($P \ll 0.001$) however no pattern towards stocking rate has established (Fig. VIII.1). The same was true for the years 2005, 2007 and 2008, as there was no significant relation between P_{C4} and stocking rate (Table VIII.1). Furthermore, the pattern was not unique, as the direction of the slopes also changed as indicated by the coefficient of determination (Table VIII.1).

Table VIII.1 Sampling year, duration of the experiment, sampling size, mean contribution of C4 plants to aboveground biomass (P_{C4}), the 95% confidence interval of the mean ($CI_{95\%}$), the respective mean July temperature (MJulT) and the correlation coefficient (r) and the P-value for the linear regression between P_{C4} and stocking rate.

Year	Duration of experiment	n	mean $P_{C4} \pm CI_{95\%}$ (%)	MJulT (°C)	r	P
2005	1	11	5.8 ± 2.2	20.0	-0.55	0.08
2007	3	12	5.8 ± 2.2	20.7	-0.21	0.49
2008	4	12	11.6 ± 1.9	21.5	0.36	0.24
2009	5	12	12.1 ± 2.0	21.9	-0.13	0.70

The yearly differences for the individual plots were also not conclusive. For instance, from 2007 to 2008, P_{C4} increased significantly with increasing stocking rate (r = 0.72; P<0.01), but between 2009 and 2008, P_{C4} decreased significantly with increasing stocking rate (r = -0.63; P<0.05) indicating a considerable interannual fluctuation in P_{C4} within the 2-ha plots. More importantly, the change in P_{C4} between 2009 and 2005 was not significantly related to stocking rate (r = 0.38; P>0.2), even though mean P_{C4} highly significantly increased by about 6.3% during the experiment. This increase in P_{C4} was highly significantly correlated to mean July temperature (MJulT). P_{C4} increased by about 4% per increase of 1°C (Fig. VIII.2). Other meteorological variables will show a similar response, as they are highly auto-correlated.
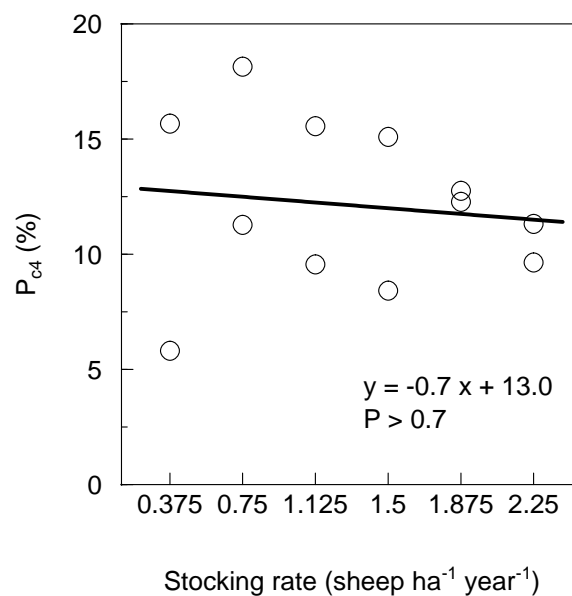
Fig. VIII.1 Relationship between stocking rate and contribution of C4 plants to aboveground biomass (P_{C4}) in 2009 after five years of grazing. The line denotes the linear regression.
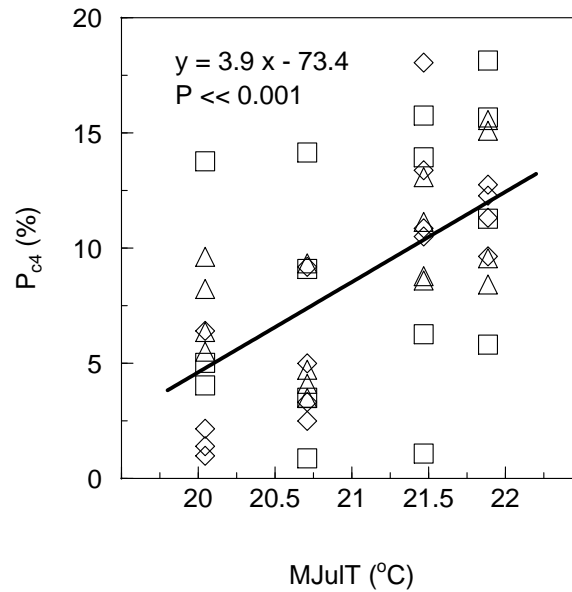
Fig. VIII.2 Relationship between mean July temperature (MJulT) and contribution of C4 plants to aboveground biomass (P_{C4}). The markers denote low ($\square$, 0.375 and 0.75 sheep ha^{-1} year^{-1}), medium (Δ, 1.125 and 1.5 sheep ha^{-1} year^{-1}1) and high ($\Diamond$, 1.875 and 2.25 sheep ha^{-1} year^{-1}) stocking rates and the line denotes the linear regression.

Discussion

Different grazing pressure – applied for five years – apparently did not alter the C4 abundance (Fig. VIII.1), but increasing temperature did (Fig. VIII.2). Schönbach *et al.* (2009) reported an increase in the relative abundance of *C. squarrosa* from 9.7% to 13.2% under grazed conditions from 2005 to 2006 based on the floristic composition of the same experiment as from our study. This is not only supporting our results, but also is giving proof of the high reliability of the isotopic approach used to monitor P_{C4} as done here. Furthermore, this is evidenced by the floristic composition reported by Wang *et al.* (2003b) and Chen *et al.* (2005b) showing that the percentage of *C. squarrosa* increased from 7% to 24% within six years (1996 – 2002) under ungrazed conditions. However, the perennial C4 grass *C. squarrosa* is expected to profit from proceeding degradation caused by enduring grazing in Inner Mongolia grassland (Tong *et al.*, 2004), as it is usually regarded to be vastly grazing tolerant (Gao *et al.*, 2007; Liang *et al.*, 2002; Wang *et al.*, 2008). Hence, most studies suggest management adaptation to prevent the grasslands from further degradation (e.g. Liang *et al.*, 2002; Tong *et al.*, 2004) apparently indicated by an increasing C4 abundance. Based on our findings however, this might be at least partially questioned.

From our data, it became obvious that temperature appears to be the main driver behind the increase of P_{C4}. This is in line with mean July temperature isotherms delineating the spatial extent of C4 abundance in semi-arid Inner Mongolia grassland (Auerswald *et al*., 2009; Wittmer *et al*., 2010b). This temperature related increase in P_{C4} can be attributed to physiological parameters of C3 and C4 species, e.g. variation in the quantum yield (Ehleringer *et al*., 1997) or a decrease in the leakiness (i.e the fraction of CO_2 generated by C4 acid decarboxylation that subsequently leaks from bundle-sheath cells; Hatch *et al*., 1995) of C4 grasses (Kubásek *et al*., 2007).

We used MJult as a proxy for the interannual variation in metrological conditions. However, there is no indication that P_{C4} is actually related to the MJulT of the respective year, as (i) a high degree of autocorrelation between different temperature variables (e.g. MJulT and growing period temperature) exist and (ii) carry over-effects of previous years cannot be excluded e.g. as the area experienced a July temperature increase of 1°C per decade (data from 1987 – 2009 from the long-term monitoring station in Xilinhot about 70 km north-west of the grazing experiment; NOAA NNDC Climate Data Online, 2010). In fact, when using moving averages of mean July temperature from the Xilinhot monitoring station, the presented relationship in Fig. VIII.2 did not change markedly in strength (not shown).

During the past decades temperature has increased in Inner Mongolia and simultaneously stocking rates have increased. An increase in P_{C4} caused by the increase in temperature can thus easily be misinterpreted as an effect of obviously increasing grazing pressure. This and the grazing tolerance of *C. squarrosa*, may explain the frequently found assumption that a high grazing pressure promotes C4 species. The controlled grazing experiment gave no indication of such a relation.

However, all this does not mean that grazing has no influence on the community composition and productivity de-stocking is indeed urgently needed, as the increasing temperature will decrease water use efficiency and thus lower productivity.

CHAPTER IX – General and summarizing discussion

The work presents a comprehensive view on the carbon and partly on the nitrogen isotope composition of the semi-arid grasslands of Inner Mongolia. Particularly, the focus was set on the relationship between the ^{13}C discrimination of C3 vegetation and precipitated water (Chapter II), the influence of different stocking rate regimes on the ^{13}C and ^{15}N fractionation between vegetation, soil and sheep wool and faeces (Chapter III & IV), the estimation and regionalization of the C3/C4 ratio from δ^{13}C of wool (Chapter V), the comparison of the recent, woollen-derived C3/C4 ratio with that of some former times derived from δ^{13}C of soil organic carbon (Chapter VI) and finally a comparison of sedentary *versus* transhumant grazing (Chapter VII) including the influence of stocking rate, which was further evaluated in a controlled grazing experiment (Chapter VIII). Additionally to the analysis of isotopic data, a geostatistical approach to yield actual precipitation at ungauged sites despite a thin network of meteorological stations was established (Chapter II) as well as a review of statistical problems arising from retrieving the isotopic composition of vegetation from consumers' tissues (Annex), a problem which arises for all steps of carbon flow within the carbon cycle (and analogously for nitrogen).

Interpolation of climatic parameters and the relation to δ^{13}C of C3 and C4 vegetation

Regional scale investigations that include sampling trips to remote places usually lack detailed information on site conditions, including meteorological parameters, as sampling sites are mostly far apart from meteorological observatories. Long term data (i.e. from the last normal period 1961-1990) are available in sufficient resolution (approximately 1.5×1.5 km²), but are not suited for multi-year investigations, due to pronounced intra- and inter-annual variations in e.g. precipitation (Ketzer *et al*., 2008). One possibility to overcome theses limitations would be to follow the so called PRISM algorithm (parameter-elevations regressions on independent slopes model) as for the long-term data (see Daly *et al*., 2002), but this would be extensively time-consuming and would require various additional data (e.g. digital elevation model and lake induced effects).

The approach presented here (Chapter II) is predicated on the assumption that the causes of small scale variation as quantified by the PRISM data are generally valid but modified every

year by the respective meteorological conditions. The workflow of the geostatistical approach was then to get data from the meteorological stations (e.g. Feng *et al.*, 2004 or *via* the NOAA network), calculate the deviation from the long term mean and interpolate this deviation for all sampling sites *via* kriging. Thus the deviation from the long term is then known for the sampling sites, as well as the long term mean from the high resolution map. This allows to calculate precipitation at an arbitrary sampling site for an arbitrary period of time defined by the research question (temperature would work in the same way). This approach greatly cuts down the data requirement as no additional data than the parameter under focus is necessary. Further this approach can be applied for all regions were (i) maps of long-term averages and (ii) sufficient meteorological stations are available to apply kriging. In case that also the number of meteorological stations is insufficient for a proper calculation of the semivariogram within the geostatistical analysis, which requires at least 30 – 50 stations, the number of stations can be further cut down by substituting station with measuring periods of the desired length but from other than the desired period of time. This is possible because the semivariogram is largely time-invariant (Schuurmans *et al.*, 2007). Fiener & Auerswald (2009) thus were able to produce high-resolution rain maps based on twelve stations only.

Therewith obtained year specific precipitation during the growing period greatly explained the observed patterns in ^{13}C discrimination of *Stipa* grasses (Figure II.4) as well as of other individual C3 samples (Figure II.5) and of C3 communities as a whole (Figure II.6). A similar relationship between δ^{13}C and a measure for water availability was reported by Murphy & Bowman (2009) for C3 grasses sampled in Australia. Interestingly, in the same study by Murphy & Bowman (2009) a relationship between δ^{13}C of C4 grasses and water availability was also reported, which however run opposite (decreasing ^{13}C discrimination with increasing water availability) to that observed in C3 grasses. This effect was visible within the different photosynthetic groups of C4 vegetation (NADP-ME, NAD-ME and PEP-CK) and hence was not caused by species replacement. Further, this is one of the rare studies reporting effects of water availability on δ^{13}C of C4 grasses on a broad regional scale.

In the present study however, no effect of water availability or any other environmental parameter (e.g. temperature or plant available soil water) on the regional variation of ^{13}C discrimination by C4 vegetation was observed (Chapter V). But we found that *Cleistogenes squarrosa*, the dominant C4 grass of the NAD-ME photosynthetic type, exhibited a significantly higher ^{13}C discrimination of about 1.1‰ compared to NADP-ME species (Chapter V), which was also apparent from the study of Murphy & Bowman (2009). It might be questioned, whether the lack of any relationship was either caused by the relatively small

sample size (n=19 for *C. squarrosa* and n=27 for other C4 species) or a flat gradient in mean annual precipitation of about 250 mm year^{-1} compared to that of about 1400 mm year^{-1} reported by Murphy & Bowman (2009).

Indeed in another study within the MAGIM framework, an effect of precipitation on the ^{13}C discrimination of *C. squarrosa* became apparent (n=47; Yang *et al*., in preparation), but the response was opposite to that reported by Murpyh & Bowman (2009) and seems to be specific for this species, which is characterized by an extraordinary high leakiness. For the C4 community, including other species with different/opposite behaviour, the effect will be dampened or cancelled out. The effect only amounted to about 1.0‰ and would not change the findings of the study. Furthermore, it also disappeared, when influences varying on other scales (including samples on the local and on the tiller scale) than precipitation were included (n=306, r²= 0.001). As discussed by Auerswald *et al*. (2009), a change of 1.0‰ within the C4 end-member would only result in a change of about 1.6% in the estimation of the C4 abundance. This is negligible compared to the overall reported span of 0 to 100% in C4 abundance of the Mongolian-Manchurian grassland.

A main reason for the lack or small extent of an effect within the Mongolian-Manchurian grassland may be that the reaction of C3 and C4 plants towards precipitation anomalies differs. C3 plants start growth in spring and thus have to cope with water shortage during summer. In contrast, C4 plant, which start growing in early summer, when winter moisture is depleted already, adapt to water shortage by delaying their start of growth (Yang *et al*., 2010).

^{13}C and ^{15}N fractionation between diet and the animals' tissue

Analysing the isotopic composition of animals' tissues represents an elegant way to track the isotopic composition of diet of the respective animal. This offers great opportunities for ecological and paleoecological research, as some tissues may even persist for millennia (e.g. bones, teeth and hair). While this was utilized in several studies to investigate the relationship between tissue and diet (cf. Chapter III & IV), the requirements to investigate the relationship between tissue and vegetation as done in this study are extended. First of all, there are two requirements to investigate the relationship between diet and tissue. (i) The fractionation between the diet and the tissue must be known, to allow estimation of the dietary isotopic composition. (ii) The animal needs to be isotopically equilibrated, otherwise a bias in the estimation and application of the fractionation might occur (see Annex). Ruminants are particularly suitable due to the 'isotopic scrambling' in the alimentary tract. However, even if

the true fractionation between tissue and diet is know, the apparent fractionation between tissue and standing vegetation can differ due to selective grazing and differential digestibility, e.g. caused by heterogeneous vegetation. In Chapter III, it was highlighted that the ^{13}C discrimination between vegetation and grazer's tissue, was not influenced by differential digestibility or selective grazing of either the C3 or C4 component. Hence, the reported ^{13}C discriminations can be applied for animals grazing a pasture with variable proportions of C3 and C4 derived biomass.

Concerning nitrogen, the situation was a bit more complex as recent evidence suggests that the fractionation between dietary δ^{15}N and tissue δ^{15}N is influenced by the nitrogen content of the diet (e.g. Adams & Sterner, 2000; Robbins *et al*. 2005). Robbins *et al*. (2005) hypothesized that this is related to an increase in nitrogen metabolism and excretion, which causes altered ^{15}N in the animal, due to selective renal retention. Their review showed that enrichment decreases by 1.0‰ per 10.0% increase in dietary nitrogen content (for a range of dietary nitrogen content of 2.5 to 12.0%). In contrast, neither for faeces nor for wool a significant influence of dietary nitrogen content on the ^{15}N enrichment was found (Chapter IV).

The lack of any effect does not necessarily invalidate the results by Robbins *et al*. (2005) and others, but it might just be not crucial, when the rage of dietary nitrogen content is as narrow as in this study (0.9 to 4.4% including Chapter IV, V and VI), from which an effect of only 0.3‰ may be expected. This effect is much smaller than the unexplained scatter (usually several per mill) and thus does not become statistically significant although it seems to exist (Fig. IX.1) and cannot be classified. Anyhow, a range of 10% in dietary vegetation nitrogen content is not to be expected for semi-arid Inner Mongolia grassland as apparent from this study or reported by others (Liu, 1993; Chen *et al*., 2007).
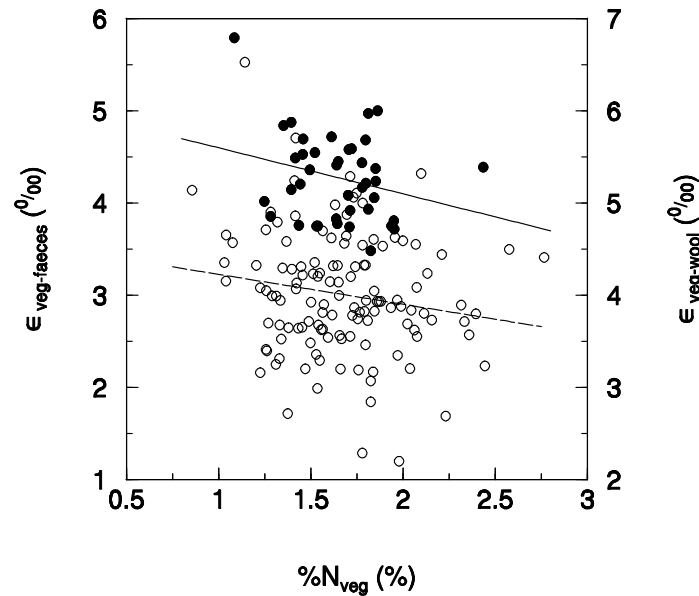
Fig. IX.1 Relationship between nitrogen content of the vegetation (%N$_{veg}$) and ^{15}N enrichment between vegetation and faeces ($\varepsilon_{veg\text{-}faeces}$; ○; dashed line) and between vegetation and wool ($\varepsilon_{veg\text{-}wool}$; ●; solid line). Both relationships are not significant (P>0.05). Note that both y-axes span 5.0‰.

Furthermore, maturation of biomass came along with a distinct difference in δ^{15}N, which amounted up to 8.0‰ (Chapter IV). Hence, if selection on e.g. younger material would have occurred, this would have been revealed. However, there was no evidence of selection towards younger biomass.

Depending on the type of tissue, the retrieved information is valid on different scales. For instance, faeces reflect the diet of a grazer of the last few days (Raymond, 1948) while hair offers a consecutive integration. This distinct difference in temporal integration also leads to a difference in spatial integration: the grazed area reflected in the faecal isotopic composition is smaller than the grazed area reflected in the isotopic composition of the hair (Fig. IX.2).
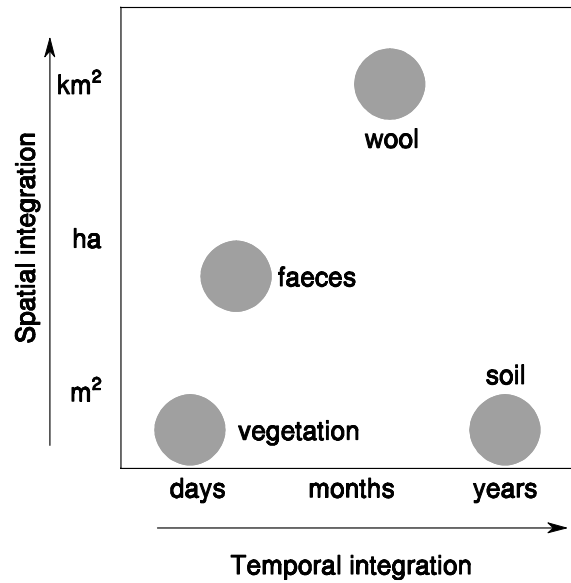
Fig. IX.2 Schematic illustration of the temporal and spatial integration for the different samples used in this thesis.

C4 abundance in Inner Mongolia grassland

Inner Mongolia as a whole has been changing. For instance, since 1960 the crop production area increased by approximately 10000 km² to a total of 67615 km² in 2007 (Inner Mongolia Statistical Yearbook, 2008). Furthermore, not only the share of population of Mongolians is more and more reduced by the ongoing settlement of Han-Chinese – which will accelerate the loss of traditionalistic habits – but also stocking densities and temperature increased (Fig. IX.3). Furthermore, changing enivronmental conditions are know to affect the productivity of grasslands (Bai *et al.*, 2004) and especially that of grasslands with low annual precipitation as they are expected to be even more vulnerable to summer droughts (Gilgen & Buchmann, 2009).
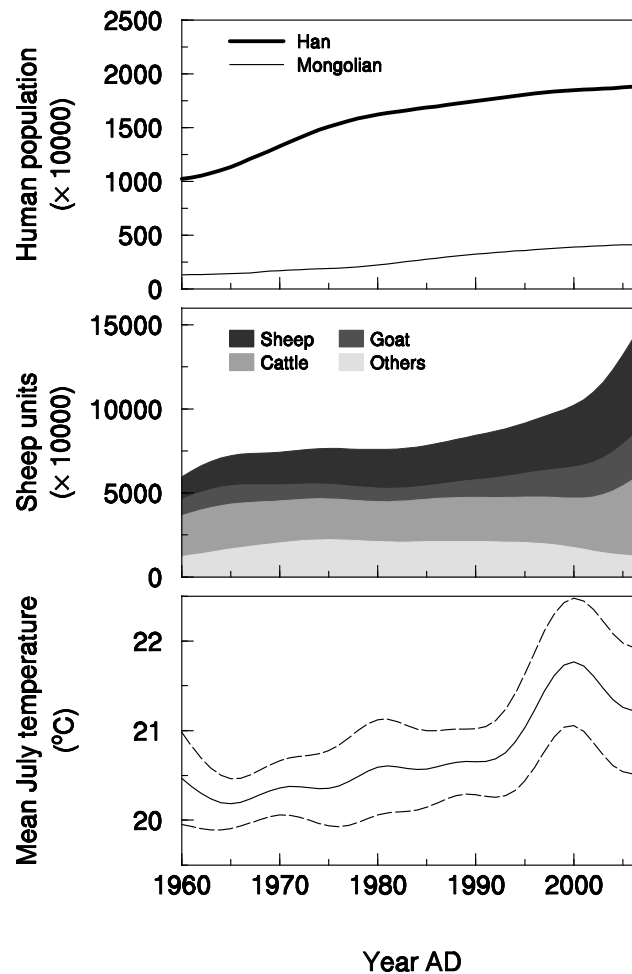
Fig. IX.3 Changes in population with different ethnic background (top), stocking density (expressed as sheep units; middle) and mean July temperature (bottom; solid line; dashed lines denote the 95% confidence interval) in Inner Mongolia since 1960. Data were smoothed by kernel regressions (Hayfield & Racine, 2008) and were taken from Inner Mongolia Statistical Yearbook (2008), Feng *et al.* (2004) and NOAA NCDC Climate Data Online (2009).

The performance of the plants is particularly controlled by the prevailing climatic conditions. By now, it is well know, that temperature is the dominant control of C4 abundance in grasslands (e.g. Teeri & Stowe, 1976; Ehleringer *et al.*, 1997; Still *et al.*, 2003). Due to their CO_2-concentrating mechanism that greatly excludes photorespiration, C4 plants cope well with high temperatures and low ambient CO_2 concentrations (Ehleringer *et al.*, 1997). From a comparison of the quantum yield efficiencies of C3 and C4 plants under certain levels of ambient CO_2 concentration and temperature, Ehleringer *et al.* (1997) developed the concept of the crossover temperature (Fig. IX.4). The crossover temperature denotes the temperature from above which C3 plants have an inferior quantum yield efficiency compared to C4 plants.
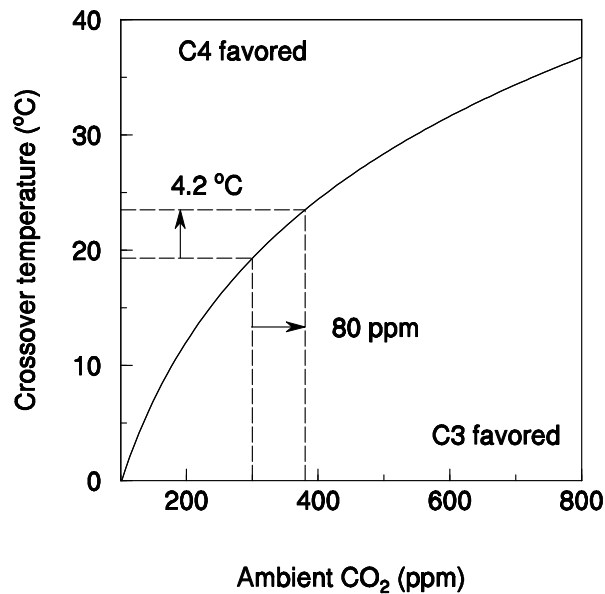
Fig. IX.4 Modelled crossover temperature of the quantum yield for CO_2 uptake as a function of atmospheric CO_2 concentrations (after Ehleringer *et al.*, 1997). Dashed lines denote a shift of 80 ppm (from 300 to 380 ppm) in ambient CO_2 concentration and the resultant increase of 4.2°C in the crossover-temperature.

However, this is suggestive for the dilemma of mixed C3/C4 grasslands, which are facing both: an increase in ambient CO_2 concentrations as well as an increase in temperature. Collatz *et al.* (1998) modelled the global distribution of C3 and C4 plants plainly based on meteorological parameters (like annual precipitation and the temperature of the warmest month). Thereby, in varying the input parameters, they were able to produce global maps of former (i.e. at lower CO_2 concentration) and present C3/C4 distributions. In this context, the Inner Mongolia grassland is especially interesting as it exhibits a mean July temperature (which is the temperature of the warmest month), which is close to the modelled crossover temperature (about 22°C). In fact, one major finding of this thesis supports that model, as the predicted crossover temperature for the period 1998 to 2007 greatly delineated the spatial pattern of the C4 distribution estimated from $\delta^{13}C$ of wool originating from the respective period (Chapter V & VI). The 22°C crossover temperature isotherm highly significantly separated areas of 'high' C4 abundance (approximately 23%) from those with 'low' C4 abundance (approximately 10%) (Fig. VI.4b). However, this was not the case when the long-term mean (last normal period 1961-1990) of the July temperature was used. This indicates that the regional differences of the ongoing climate change is already reflected in the C3/C4 ratio of Inner Mongolia grassland, although it is assumed that it may take at least 50 years or more until a severe impact on the C3/C4 ratio (Sage & Kubien, 2003).

This finding is further promoted by the spatial pattern of C3/C4 derived from the analysis of $\delta^{13}C$ of soil organic carbon (Chapter VI). Top soil integrates the biomass carbon over a certain

(but unknown) time and hence reflects the pattern of some former times. Despite the uncertainty concerning the true age of the soil organic carbon, the long term crossover temperature isotherm (of approximately 19°C for a atmospheric CO_2 concentration of 300 ppm prevailing about 100 years b.p.), greatly separated areas of 'high' C4 abundance (approximately 16%) from areas with 'low' C4 abundance (less than 5%) (Fig. VI.4a). Direct comparison of the recent C4 abundance and the C4 abundance derived from soil organic carbon revealed an increase of the C4 abundance in the mountainous eastern region of the study area and along an east-west belt at a latitude of about 44°North (Fig. VI.5). This pattern gives proof of C4 spreading to formerly cool regions, in moving northwards and to higher altitudes. Anyhow, it could not definitely be clarified how the chronological sequence of this change looked like. This can be attributed to the unknown age of the soil organic carbon as well as to the lack of any temporal resolution.

Despite this resilient indication of a strong relationship between global warming and increasing C4 abundance, other factors might also intensify the spreading of C4 plants. For instance disturbances, like overgrazing, land-use changes and other human activities can also lead to an increase in the abundance of C4 plants (Wang, 2002a; Li *et al.*, 2006). Especially overgrazing possibly could increase the abundance of C4 plants, based on two mechanisms. First, the fed gap is largest after winter, when C3 plants start growing while C4 plants will appear up to one month later (Liang *et al.*, 2002; Chapter III). This might cause a selective damage of C3 plants and may thus promote C4 plants. Second, overgrazing by increasing percentage of bare soil will change the microclimate. Soil temperature will increase, water will accumulate (Garner & Steinberger, 1989; Bhark & Small, 2003), and this may promote C4 plants. Wang *et al.* (2010), however, have shown that despite this increase in soil temperature, canopy temperatures change only marginally, because the canopy – in contrast to soil – is exposed to full sun light in any case.

Despite these two possible mechanisms of grazing impact, no indication – neither on the regional scale (Chapter VII) nor in a controlled grazing experiment (Chapter VIII) – could be found that grazing pressure influences the C3/C4 ratio.

Even though the multitude of influences on species composition on the regional scale is difficult to disentangle and even though the temporal course of change in the C3/C4 ratio was not fully resolved and connected to environmental and land-use changes, there is overwhelming evidence that the pronounced temperature increase in the Mongolian-Manchurian grassland has caused the C3/C4 ratio to increase. This in turn has a significant impact on global biogeochemical cycles and the sustainability of land-use.

Outlook

The present work clearly demonstrated an increase in the abundance of C4 grassland species in Inner Mongolia grassland (Chapter VI) but the timing of that increase is still unclear. That points to the major drawback in using soil carbon as an isotopic archive: the lack of any temporal resolution and the uncertainties in the estimation of the age of the soil organic carbon. Even though, the latter was recently assessed by Steffens et al. (2009), the age of individual soil matter fractions varied from modern to >300 years. Hence, the question whether the increase in P_{C4} occurred gradually over several decades to centuries or whether the increase in P_{C4} occurred rapidly over years to decades could not be answered with contemporary samples.

This study however opened the opportunity to get resilient estimates of P_{C4} from woollen samples of known age. During the sampling campaigns from this study (2003, 2004, 2005 and 2007) and as well from a further sampling campaign (2009), sheep wool of known age was sampled, ranging back to the 18th century. Thus, one might establish a time course of P_{C4}, with a high temporal resolution and a high precision in the estimation of P_{C4} as environmental influences could directly be captured by the P_{C4} mixing model (Chapter II, III & V). This can especially shed light on the above evoked question on the timing of the C4 increase and the potential drivers behind that, as detailed data on stocking rates (Statistical Yearbook of Inner Mongolia, 2008; Fig. IX.3) and resilient meteorological information (e.g. Feng *et al.*, 2004; Fig. IX.3) are available back to the early 1960´s.

References

Adams TS, Sterner RW (2000) The effect of dietary nitrogen content on trophic level ^{15}N enrichment. *Limnology and Oceanography*, **45**, 601-607.

Allen GA, Pereira LS, Raes D, Smith M (1998) Crop Evapotranspiration – Guidelines for Computing Crop Water Requirements. *FAO Irrigation and Drainage Paper*, **56**, 78-86.

Allison CE, Francey RJ, Krummel PB (2003) δ^{13}C in CO_2 from sites in the CSIRO Atmospheric Research GASLAB air sampling network, *Trends: A Compendium of Data on Global Change*. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, TN, USA.

Alward RD, Detling JK, Milchunas DG (1999) Grassland vegetation changes and nocturnal global warming. *Science*, **283**, 229-231.

Amundson R, Austin AT, Schuur EAG, Yoo K, Matzek V, Kendall C, Uebersax A, Brenner DL, Baisden WT (2003) Global patterns of the isotopic composition of soil and plant nitrogen. *Global Biogeochemical Cycles*, **17**, 1013.

Amundson R, Austin AT, Schuur EAG, Yoo K, Matzek V, Kendall C, Uebersax A, Brenner DL, Baisden WT (2003) Global patterns of the isotopic composition of soil and plant nitrogen. *Global Biogeochemical Cycles*, **17**, 1013.

Archer S, Schimel DS, Holland EA (1995) Mechansims of shrubland expansion – land-use, climate or CO_2? *Climatic Change*, **29**, 91-99.

Arnold GW (1960) Selective grazing by sheep of two forage species at different stages of growth. *Australian Journal of Agricultural Research*, **11**, 1026-1033.

Auerswald K, Wittmer MHOM, Männel TT, Bai YF, Schäufele R, Schnyder H (2009) Large regional-scale variation in C3/C4 distribution pattern in Inner Mongolia steppe is revealed by grazer wool carbon isotope composition. *Biogeosciences*, **6**, 795-805.

Auerswald K, Wittmer MHOM, Zazzo A, Schäufele R, Schnyder H (2010) Biases in the analysis of stable isotope discrimination in food webs. *Journal of Applied Ecology*, doi: 10.1111/j.1365-2664.2009.01764.x

Ayliffe LK, Cerling TE, Robinson T *et al.* (2004) Turnover of carbon isotopes in tail hair and breath CO_2 of horses fed an isotopically varied diet. *Oecologia*, **193**, 11-22.

Ayyub BM, McCuen RH (1990) Optimum sampling for structural strength evaluation. *Journal of Structural Engineering*, **116**, 518-535.

Bachmaier M, Backes M (2008) Variogram or semivariogram? Understanding the variances in a variogram. *Precision Agriculture*, **9**, 173-175.

Baertschi P (1952) Die Fraktionierung der Kohlenstoffisotopen Bei der Absorption von Kohlendioxyd. *Helvetica Chimica Acta*, **35**, 1030-1036.

Baertschi P (1953) Die Fraktionierung der Natürlichen Kohlenstoffisotopen im Kohlendioxydstoffwechsel grüner Pflanzen. *Helvetica Chimica Acta*, **36**, 773-781.

Bai YF, Han XG, Wu JG, Chen ZZ, Li LH (2004) Ecosystem stability and compensatory effects in the Inner Mongolian grassland. *Nature*, **431**, 181-184.

Bai YF, Zhang LX, Zhang Y, Chen ZZ (2002) Changes in plant functional compositions along gradients of precipitation and temperature in the Xilin river basin, Inner Mongolia. *Acta Phytoecologica Sinica*, **26**, 308-316.

Barbehenn RV, Chen Z, Karowe DN, Spickard A (2004) C_3 grasses have higher nutritional quality than C_4 grasses under ambient and elevated atmospheric CO_2. *Global Change Biology*, **10**, 1565-1575.

Barbosa ICR, Kley M, Schäufele R, Auerswald K, Schröder W, Filli F, Schnyder H (2009a) Analysing the isotopic life history of the alpine ungulates *Capra ibex* and *Rupicapra rupicapra rupicapra* through their horns. *Rapid Communications in Mass Spectrometry*, **23**, 2347-2356.

Barbosa ICR, Köhler IH, Auerswald K, Lüps P, Schnyder H (2009b) Last-century changes of alpine grassland water-use efficiency – a reconstruction through carbon isotope analysis of a time-series of *Capra ibex* horns. *Global Change Biology*, DOI: 10.1111/j.1365-2486.2009.02018.x.

Baron VS, Mapfumo E, Dick AC, Narth MA, Okine EK, Chanasyk DS (2002) Grazing intensity impacts on pasture carbon and nitrogen flow. *Journal of Rangeland Management*, **55**, 535-541.

Baskin CC, Baskin JM (1998) Seeds. Ecology, biogeography and evolution of dormancy and germination. *Academic Press*. San Diego.

Bhark EW, Small EE (2003) Association between plant canopies and the spatial patterns of infiltration in shrubland and grassland of the Chihuahuan Desert, New Mexico. *Ecosystems*, **6**, 185-196.

Bird MI, Pousai P (1997) Variations in $\delta^{13}C$ in the surface soil organic pool. *Global Biogeochmical Cycles*, **11**, 313-322.

Bird MI, Santruckova H, Arneth H, Grigoriev S, Gleixner G, Kalaschnikov YN, Lloyd J, Schulze ED (2002) Soil carbon inventories and carbon-13 on a latitudinal transect in Siberia. *Tellus*, **54B**, 631-641.

Boutton TW, Archer SR, Midwood AJ, Zitzer SF, Bol R (1998) $\delta^{13}C$ values of soil organic carbon and their use in documenting vegetation change in a subtropical savanna ecosystem. *Geoderma,* **82**, 5-41.

Boval M, Archimede H, Fleury J, Xande A (2003) The ability of faecal nitrogen content to predict digestibility for goats and sheep with tropical herbage. *Journal of Agricultural Sciences*, **140**, 443-450.

Brown JR, Stuth JW (1993) How herbivory affects grazing tolerant and sensitive grasses in a central Texas grassland - integrating plant-response across hierarchical levels. *Oikos*, **67**, 291-298.

Buchmann N, Brooks JR, Rapp KD, Ehleringer JR (1996) Carbon isotope composition of C4 grasses is influenced by light and water supply. *Plant Cell and Environment*, **19,** 392-402.

Buxton DR (1996) Quality-related characteristics of forages as influenced by plant environment and agronomic factors. *Animal Feed Science and Technology*, **59**, 37-49.

Cabido M, Pons E, Cantero JJ, Lewis JP, Anton A (2008) Photosynthetic pathway variation among C_4 grasses along a precipitation gradient in Argentina. *Journal of Biogeography*, **35**, 131-140.

Caswell H, Reed F, Stephens SN, Werner PA (1973) Photosynthetic pathways and selective herbivory: A hypothesis. *American Naturalist*, **107**, 465-480.

Cerling TE, Harris JM (1999) Carbon isotope fractionation between diet and bioapatite in ungulate mammals and implications for ecological and paeloecological studies. *Oecologia*, **120**, 347-363.

Cerling TE, Quade J, Wang Y, Bowman JR (1989) Carbon isotopes in soils and paleosoils as ecology and paleoecology indicators. *Nature*, **341**, 138-139.

Cerling TE, Wittemyer G, Ehleringer JR, Remien CH, Douglas-Hamilton I (2009) History of Animals using Isotope Records (HAIR): A 6-year dietary history of one family of African elephants. *Proceedings of the National Academy of Sciences of the United States of America*, **106,** 8093-8100.

Chase TN, Pielke RA, Knaff JA, Kittel TGF, Eastman JL (2000) A comparison of regional trends in 1979-1997 depth-averaged tropospheric temperatures. *International Journal of Climatology*, **20**, 503-518.

Chen S, Bai Y, Lin G, Huang J, Han X (2007) Isotopic carbon composition and related characters of dominant species along an environmental gradient in Inner Mongolia, China. *Journal of Arid Environments*, **71**, 12-28.

Chen S, Zhang H, Wang L, Hai B, Zhao M (2001) Roots of grassland plants in northern China. Jilin University Press, Changchun, 531 pp.

Chen SP, Bai YF, Guanghui L, Han XG (2005a) Variations in life-form composition and foliar carbon isotope discrimination among eight plant communities under different soil moisture conditions in the Xilin River Basin, Inner Mongolia, China. *Ecological Research*, **20**, 167- 176.

Chen SP, Bai YF, Han XG (2002) Variation of Water-Use Efficiency of *Leymus chinensis* and *Cleistogenes squarrosa* in different plant communities in Xilin River Basin, Nei Mongol. *Acta Botanica Sinica*, **44,** 1484-1490.

Chen SP, Bai YF, Lin GH, Liang Y, Han XG (2005b) Effects of grazing on photosynthetic characteristics of major steppe species in the Xilin River Basin, Inner Mongolia China. *Photosynthetica*, **43**, 559-565.

Cheng WX, Chen QS, Xu YQ, Han XG, Li LH (2009) Climate and ecosystem ^{15}N natural abundance along a transect of Inner Mongolia grasslands: Contrasting regional patterns and global patterns. *Global Biogeochemical Cycles*, **23**, GB2005.

Christensen L, Coughenour MB, Ellis JE, Chen ZZ (2004) Vulnerability of the Asian typical steppe to grazing and climatic change. *Climatic Change*, **63**, 351-368.

Coates DB, Van der Weide APA, Keer JD (1991) Changes in faecal $\delta^{13}C$ in response to changing proportions of legume (C_3) and grass (C_4) in the diet of sheep and cattle. *Journal of Agricultural Science*, **161**, 287-295.

Codron D, Codron J, Lee-Thorp JA, Sponheimer M, de Ruiter D, Sealy J, Grant R, Fourie N (2007) Diets of savanna ungulates from stable carbon isotope composition of faeces. *Journal of Zoology*, **273**, 21-29.

Codron D, Lee-Thorp JA, Sponheimer M, de Ruiter D, Codron J (2006) Inter- and intrahabitat dietary variability of chacma baboons (*Papio ursinus*) in South African savannas based on fecal $\delta^{13}C$ $\delta^{15}N$, and %N. *American Journal of Physical Anthropology*, **129**, 204-214.

Collatz GJ, Berry JA, Clark JS (1998) Effects of climate and atmospheric CO_2 partial pressure on the global distribution of C4 grasses: present, past, and future. *Oecologia,* **114**, 441-454.

Condon AG, Farquhar GD, Richards RA (1990) Genotypic variation in carbon isotope discrimination and transpiration efficiency in wheat - leaf gas-exchange and whole plant studies. *Australian Journal of Plant Physiology*, **17**, 9-22.

Conway TJ, Tans PP, Waterman LS (1994) Atmospheric CO_2 records from sites in the NOAA/CMDL air sampling network. *Trends ´93: A Compendium of Data on Global Change*. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, TN, USA.

Craig H (1954) Carbon 13 in plants and the relationships between carbon 13 and carbon 14 variations in nature. *The Journal of Geology*, **62**, 115-149.

Daly C, Gibson WP, Taylor GH, Johnson GL, Pasteris P (2002) A knowledge-based approach to the statistical mapping of climate. *Climate Research*, **22**, 99-113.

Davies RO, Milton EJ, Lloyd JR (1950) Pasture productivity in Mid-Wales. Part I. Experimental methods of measurement. *Empire Journal of Experimental Agriculture*, **18**, 203-217.

De Niro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta*, **45**, 341-351.

De Niro MJ, Epstein S (1978) Influence of diet on distribution of carbon isotopes in animals. *Geochimica and Cosmochimica Acta*, **42**, 495-506.

Derner JD, Boutton TW, Briske DD (2006) Grazing and ecoystem carbon storage in the North American Great Plains. *Plant and Soil*, **280**, 77-90.

Dümig A, Schad P, Rumpel C, Dignac MF, Kögel-Knabner I (2008) Araucaria forest expansion on grassland in the southern Brazilian highlands as revealed by 14C and δ^{13}C studies. *Geoderma*, **145**, 143-157.

Edwards GR, Newmann JA, Parsons AJ, Krebs JR (1994) Effects on the scale and spatial distribution of the food resource and animal state on diet selection: an example with sheep. *Journal of Animal Ecology*, **63**, 816-826.

Ehleringer JR, Bjorkman O (1977) Quantum yields for CO_2 uptake in C3 and C4 plants – dependence on temperature, CO_2, and O_2 concentration. *Plant Physiology*, **59**, 86-90.

Ehleringer JR, Buchmann N, Flanagan LB (2000) Carbon isotope ratios in belowground carbon cycle processes. *Ecological Applications*, **10**, 412-422.

Ehleringer JR, Cerling TE, Helliker BR (1997) C4 photosynthesis, CO_2 and climate. *Oecologia*, **112**, 285-299.

Ehleringer JR, Phillips SL, Comstock JP (1992) Seasonal-variation in the carbon isotopic composition of desert plants. *Functional Ecology*, **6**, 396-404.

Epstein HE, Burke IC, Mosier AR (1998) Plant effects on spatial and temporal patterns of nitrogen cycling in shortgrass steppe. *Ecosystems*, **1**, 374-385.

Epstein HE, Lauenroth W., Burke IC, Coffin DP (1997) Productivity patterns of C-3 and C-4 functional types in the US Great Plains. *Ecology*, **78**, 722-731.

FAO (2005) Global Forest Resources Assessment.

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, **40**, 503-537.

Feng S, Hu Q, Qian WH (2004) Quality control of daily meteorological data in China, 1951-2000: A new dataset. *International Journal of Climatology*, **24,** 853-870.

Fernandez-Gimenez ME (2006) Land use and land tenure in Mongolia: a brief history and current issues. In: Bedunah DJ, McArthur ED, Fernandez-Gimenez ME (eds) *Rangelands of Central Asia: Proceedings of the Conference on Transformations, Issues, and Future Challenges*. **RMRS-P-39**, 30-36.

Fick GW, Wilkens PW, Cherney JH (1994) Modeling forage quality changes in the growing crop. *Forage Quality, Evaluation, and Utilization, Fahey*, American Society of Agronomy, Madison, WI, USA, 757-795.

Field C (1983) Allocating leaf nitrogen for the maximization of carbon gain: Leaf age as control of the allocation program. *Oecologia*, **56,** 341-347.

Fiener P, Auerswald K (2009) Spatial variability of rainfall on a sub-kilometre scale. *Earth Surface Processes and Landforms*, **34,** 848-859.

Francey RJ, Allison CE, Etheridge DM *et al.* (1999) A 1000-year high precision record of $\delta^{13}C$ in atmospheric CO_2. *Tellus*, **51B**, 170-193.

Friedli H, Lötscher H, Oeschger H, Siegenthaler U, Stauffer B (1986) Ice core record of the $^{13}C/^{12}C$ ratio of atmospheric CO_2 in the past two centuries. *Nature*, **324**, 237-238.

Galloway JN (1998) The global nitrogen cycle: changes and consequences. *Environmental Pollution*, **102**, 15-24.

Gao Q, Yu M, Yang XS (2000) An analysis of sensitivity of terrestrial ecosystems in China to climatic change using spatial simulation. *Climatic Change*, **47**, 373-400.

Gao YZ, Wang SP, Han XG, Chen QS, Zhou ZY, Patton BD (2007) Defoliation, nitrogen, and competition: effects on plant growth and resource allocation of *Cleistogenes squarrosa* and *Artemisia frigida*. *Journal of Plant Nutrition and Soil Science*, **170**, 115-122.

Garcia F, Carrere P, Soussana JF, Baumont R (2003) The ability of sheep at different stocking rates to maintain the quality and quantity of their diet during the grazing season. *Journal of Agricultural Science*, **140**, 113-124.

Garner W, Steinberger Y (1989) A proposed mechanism for the formation of fertile islands in the desert ecosystem. *Journal of Arid Environments*, **16**, 257-262.

Garten CT, Taylor GE (1992) Foliar $\delta^{13}C$ within a temperate deciduous forest: spatial, temporal and species sources of variation. *Oecologia*, **90**, 1-7.

Gat JR, Mook WG, Meijer HA.J (2001) Environmental isotopes in the hydrological cycle, principles and applications. *Vol. II, Atmospheric water*, http://www-naweb.iaea.org/napc/ih/IHS_resources3_publication_en.html.

Geist J, Auerswald K, Boom A (2005) Stable carbon isotopes in freshwater mussel shells: Environmental record or marker for metabolic activity? *Geochimica et Cosmochimica Acta*, **69**, 3545-3554.

Ghannoum O, von Caemmerer S, Conroy JP (2002) The effect of drought on plant water use efficiency of nine NAD-ME and nine NADP-ME Australian C4 grasses. *Functional Plant Biology*, **29**, 1337-1348.

Gifford RM (1994) The global carbon cycle: A viewpoint of the missing sink. *Australian Journal of Plant Physiology*, **21**, 1-15.

Gilgen AK, Buchmann N (2009) Response of temperate grasslands at different altitudes to simulated summer drought differed but scaled with annual precipitation. *Biogeosciences*, **6**, 2525-2539.

Golluscio RA, Oesterheld M (2007) Water use efficiency of twenty-five co-existing Patagonian species growing under different soil water availability. *Oecologia*, **54**, 207-217.

Gong DY, Shi PJ, Wang JA (2004) Daily precipitation changes in the semi-arid region over northern China. *Journal of Arid Environments*, **59**, 771-778.

Gong X, Brück H, Giese M, Limeng Z, Sattelmacher B, Shan L (2008) Slope direction has effects on productivity and species composition of hilly grassland in the Xilin River Basin, Inner Mongolia. *Journal of Arid Environments*, **72**, 483-493.

Google Earth (2007) Google Inc. CA 94043, USA, Version 4.2.0205.5730.

Griffiths WM, Hodgson J, Arnold GC (2003) The influence of sward canopy structure on foraging decision by grazing cattle. II. Regulation of bite depth. *Grass and Forage Science*, **58**, 125-137.

Handley LL, Odee D, Scrimgeour CM (1994) $\delta^{15}N$ and $\delta^{13}C$ patterns in savannah vegetation: dependence on water availability and disturbance. *Functional Ecology*, **8**, 306-314.

Harris D, Horwath WR, van Kessel C (2001) Acid fumigation of soils to remove carbonates prior to total carbon or carbon-13 isotopic analysis. *Soil Science Society of America Journal*, **65**, 1853-1856.

Hatch MD, Agostino A, Jenkins CLD (1995) Measurement of the leakage of CO_2 from bundle-sheath cells of leaves during C4 photosynthesis. *Plant Physiology*, **108**, 173-181.

Hattersley PW (1983) The distribution of C3 and C4 grasses in Australia in relation to climate. *Oecologia*, **57**, 113-128.

Hayfield T, Racine JS (2008) Nonparametric econometrics: The np package. *Journal of Statistical Software*, **27,** 1-32.

Hickman KR, Hartnett DC, Cochran RC, Owensby CE (2004) Grazing management effects on plant species diversity in tallgrass prairie. *Journal of Range Management*, **57**, 58-65.

Hobson KA (1999) Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia*, **120,** 314-326.

Hochberg Y (1988) A sharper Bonferroni procedure for multiple tests of significance. *Biometrika*, **75**, 800-802.

Hodgkinson KC, Terpstra JW, Müller WJ (1995) Spatial and temporal pattern in the grazing of grasses by sheep within a semi-arid wooded landscape. *Rangeland Journal*, **17**, 154-165.

Högberg P (1997) ^{15}N natural abundance in soil-plant systems. *New Phytologist*, **137**, 179-203.

Holst J, Liu CY, Brüggemann N *et al*. (2007) Microbial N turnover and N-oxide (N_2O / NO / NO_2) fluxes in semi-arid grassland of Inner Mongolia. *Ecosystems*, **10**, 623-634.

Hoshino A, Yoshihara Y, Sasaki T, Okayasu T, Jamsran U, Okuro T, Takeuchi K (2009) Comparison of vegetation changes along grazing gradients with different numbers of livestock. *Journal of Arid Environments,* **73**, 687-690.

Houghton RA (1999) The annual net flux of carbon to the atmosphere from changes in land use 1850-1990. *Tellus Series B-Chemical and Physical Meteorology*, **51,** 298-313.

Howe HF (1994) Managing Species-Diversity in Tallgrass Prairie - Assumptions and Implications. *Conservation Biology*, **8,** 691-704.

Hülber K, Ertl S, Gottfried M, Reiter K, Grabherr G (2005) Gourmets or gourmands? – Diet selection by large ungulates in high-alpine plant communities and possible impacts on plant propagation. *Basic and Applied Ecology*, **6**, 1-10.

Humphrey C, Sneath D (1995) Pastoralism and institutional change in Inner Asia: Comparative perspectives from the findings of the M.E.C.C.I.A. research project. *Pastoral Development Network Series*, **39**, 2-23.

Inner Mongolia Statistical Yearbook (2008) China Statistic Press, ISBN 978-7-89468-020-4.

Ivanov LA, Ivanova LA, Ronzhina DA, Ziegler H, Deigele K, Gunin PD, Pyankov VI (2007) Effects of interspecific competition on functional properties of plants in mountain-steppe communities of the Gobi. *Russian Journal of Ecology*, **38**, 155-160.

Jenkinson DS (1990) The turnover of organic carbon and nitrogen in soils. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, **329**, 361-368.

Jones RJ, Hu GD (2006) Diet selection of steers grazing *Stylsanthes hamata* cv. Verano-grass pastures in north Queensland and its potential influence on botanical composition. *Tropical Grasslands*, **40**, 65-69.

Jones RJ, Ludlow MM, Troughton JH, Blunt CG (1979) Estimation of the proportion of C_3 and C_4 plant species in the diet of animals from the ratio of natural ^{12}C and ^{13}C isotopes in the faeces. *Journal of Agricultural Science*, **92**, 91-100.

Jones RJ, Ludlow MM, Troughton JH, Blunt CG (1981) Changes in the natural carbon isotope ratios of the hair from steers fed diets of C4, C3 and C4 species in sequence. *Search,* **12**, 85-87.

Kaplan JO, Prentice IC, Buchmann N (2002) The stable carbon isotope composition of the terrestrial biosphere: modelling at scales from the leaf to the globe. *Global Biogeochemical Cycles*, **16**, 1060.

Kausrud K, Mysterud A, Rekdal Y, Holand O, Austrheim G (2006) Density-dependent foraging behaviour of sheep on alpine pastures: effects of scale. *Journal of Zoology*, **270**, 63-71.

Keeling CD, Mook WG, Tans PP (1979) Recent trends in the C-13-C-12 ratio of atmospheric carbon-dioxide, *Nature*, **277**, 121-123.

Kennedy P, Kennedy H, Papadimitriou S (2005) The effect of acidification on the determination of organic carbon, total nitrogen and their stable isotopic composition in algae and marine sediment. *Rapid Communications in Mass Spectrometry*, **19**, 1063-1068.

Kerven C, Channon J, Behnke R (1996) Planning and policies on extensive livestock development in Central Asia, Overseas Development Institute, *Working Paper 91*, London: Overseas Development Institute.

Ketzer B, Huizhi L, Bernhofer C (2008) Surface characteristics of grasslands in Inner Mongolia as detected by micrometeorological measurements. *International Journal of Biometeorology*, **52**, 563-574.

Kohzu A, Iwata T, Kato M, Nishikawa J, Wada E, Amartuvshin N, Namkhaidorj B, Fujita N (2009) Food webs in Mongolian grasslands: The analysis of C-13 and N-15 natural abundances. *Isotopes in Environmental and Health Studies*, **45**, 208-219.

Körner C, Farquhar GD, Roksandic Z (1988) A global survey of carbon isotope discrimination in plants from high-altitude. *Oecologia*, **74**, 623-632.

Körner C, Farquhar GD, Wong SC (1991) Carbon isotope discrimination by plants follows latitudinal and altitudinal trends. *Oecologia*, **88**, 30-40.

Krull ES, Bray S, Harms B, Baxter N, Bol R, Farquhar G (2007) Development of a stable isotope index to assess decadal-scale vegetation change and application to woodlands of the Burdekin catchment, Australia. *Global Change Biology*, **13**, 1455-1468.

Krull ES, Skjemstad JO, Burrows WH, Bray SG, Wynn JG, Bol R, Spouncer L, Harms B (2005) Recent vegetation change in central Queensland, Australia: Evidence from $\delta^{13}C$ and ^{14}C analyses of soil organic matter. *Geoderma*, **126**, 241-259.

Kubásek J, Šeltík J, Dwyer S, Šantrůček J (2007) Light and growth temperature alter carbon isotope discrimination and estimated bundle sheath leakiness in C4 grasses and dicots. *Photosynthesis Research*, **91**, 47-58.

Le PD, Aarnink AJA, Ogink NWM, Becekr PM, Verstegen WA (2005) Odors from animal production facilities: Its relationship to diet. *Nutrition Research Reviews*, **18**, 3-30.

Lei W, Ketzer B, Liu HZ, Bernhofer C (2010) Effects of grazing on evapotranspiration over a typical steppe in Inner Mongolia, China. *Plant and Soil* (submitted).

Lewin-Koh NJ, Bivand RS (2008) maptools: Tools for reading and handling spatial objects. *R package version 0.7-15*.

Li J, Zhou MZ, Du GH, *et al*. (1978) Soils Map of China (1:4,000,000). In: *Academia Sinica* Science Press, Beijing.

Li SG, Harazono Y, Oikawa T, Zhao HL, He ZY, Chang XL (2000) Grassland desertification by grazing and the resulting micrometeorological changes in Inner Mongolia. *Agricultural and Forest Meteorology*, **102**, 125-137.

Li SG, Tsujimura M, Sugimoto A, Davaa G, Sugita M (2006) Natural recovery of steppe vegetation on vehicle tracks in central Mongolia. *Journal of Biosciences*, **31**, 85-93.

Li YB, Chen T, Zhang YF, An LZ (2007) The relation of seasonal pattern in stable carbon composition to meteorological variables in the leaves of *Sabina przewalskii* Kom. and *Sabina chinensis* (Lin.) Ant. *Environmental Geology*, **51**, 179-1284.

Liang C, Michalk DL, Millar GD (2002) The ecology and growth patterns of *Cleistogenes* species in degraded grassland of eastern Inner Mongolia, China. *Journal of Applied Ecology*, **39**, 584-594.

Liu WG, Feng XH, Ning YF, Zhang QL, Cao YN, An ZS (2005) $\delta^{13}C$ variation of C-3 and C-4 plants across an Asian monsoon rainfall gradient in arid northwestern China. *Global Change Biology*, **11**, 1094-1100.

Liu XQ, Wang RZ, Li YZ (2004) Photosynthetic pathway types in rangeland plant species from Inner Mongolia, North China. *Photosynthetica*, **42**, 339-344.

Liu Y (1993) A study on the dynamic features of nutritive materials in Inner Mongolia steppe. *Grasslands in China*, **4**, 16-20.

Liu Y, An ZS, Linderholm HW, Chen DL, Son HM, Cai QF, Sun JY, Tian H (2009) Annual temperatures during the last 2485 years in the mid-eastern Tibetan Plateau inferred from tree rings. *Science in China D: Earth Sciences*, **52**, 348-359.

Lloyd J, Farquhar GD (1994) ^{13}C discrimination during CO_2 assimilation by the terrestrial biosphere. *Oecologia*, **99**, 201-215.

Lopez-Capel E, Krull ES, Bol R, Manning DAC (2008) Influence of recent vegetation on labile and recalcitrant carbon soil pools in central Queensland, Australia: evidence from thermal analysis-quadrupole mass spectrometry-isotope ratio mass spectrometry. *Rapid Communications in Mass Spectrometry*, **22**, 1751-1758.

Loveland TR, Reed BC, Brown JF, Ohlen DO, Zhu Z, Yang L, Merchant JW (2000) Development of a global land cover characteristics database and IGBP DISCover from 1 km AVHRR data. *International Journal of Remote Sensing*, **21**, 1303-1330.

Ma JY, Chen T, Qiang WY, Wang G (2005) Correlations between foliar stable carbon isotope composition and environmental factors in desert plant *Reaumuria soongorica* (Pall.) Maxim. *Journal of Integrative Plant Biology*, **47,** 1065-1073.

Ma XZ, Wang SP, Jiang GM, Haneklaus S, Schnug E, Nyren P (2007) Short-term effect of targeted placements of sheep excrement on grassland in inner mongolia on soil and plant parameters. *Communications in Soil Science and Plant Analysis*, **38,** 1589-1604.

Männel TT, Auerswald K, Schnyder H (2007) Altitudinal gradients of grassland carbon and nitrogen isotope composition are recorded in the hair of grazers. *Global Ecology and Biogeography*, **16**, 583-592.

Mariotti A, Germon JC, Hubert P, Kaiser P, Letolle R, Tardieux A, Tardieux P (1981) Experimental determination of nitrogen kinetic isotope fractionation: some principle; illustration for the denitrification and nitrification process. *Plant and Soil*, **62**, 413-430.

Meinzer FC, Rundel PW, Goldstein G, Sharifi MR (1992) Carbon isotope composition in relation to leaf gas-exchange and environmental-conditions in Hawaiian metrosideros-polymorpha populations. *Oecologia*, **91**, 305-311.

Milchunas DG, Varnamkhasti AS, Lauenroth WK, Goetz H (1995) Forage quality in relation to long-term grazing history, current-year defoliation, and water-resource. *Oecologia*, **101**, 366-374.

Minson DJ, Ludlow MM, Troughton JH (1975) Differences in Natural Carbon Isotope Ratios of Milk and Hair from Cattle Grazing Tropical and Temperate Pastures. *Nature*, **56**, 602.

Mobæk R, Mysterud A, Loe LE, Holand O, Austrheim G (2009) Density dependent and temporal variability in habitat selection by a large herbivore; an experimental approach. *Oikos*, **118**, 209-218.

Mole S, Joern A, OʹLeary MH, Madhaven S (1994) Spatial and temporal variation in carbon-isotope discrimination in prairie graminoids. *Oecologia*, **97**, 316-321, 1994.

Monger HC, Cole DR, Buck BJ, Gallegos RA (2009) Scale and the isotopic record of C4 plants in pedogenic carbonate: From the biome to the rizosphere. *Ecology*, **90**, 1498-1511.

Murphy BR, Bowman DMJS (2007) Seasonal water availability predicts the relative abundance of C3 and C4 grasses in Australia. *Global Ecology and Biogeography*, **16**, 160-169.

Murphy BP, Bowman DMJS (2009) The carbon and nitrogen isotope composition of Australian grasses in relation to climate. *Functional Ecology*, **23**, 1040-1049.

National Statistical Office of Mongolia (2003) *Statistical Yearbook of Mongolia,* Ulaanbaatar, Mongolia.

National Statistical Office of Mongolia (2004) *Statistical Yearbook of Mongolia,* Ulaanbaatar, Mongolia.

National Statistical Office of Mongolia (2007) *Statistical Yearbook of Mongolia,* Ulaanbaatar, Mongolia.

Ni J (2003) Plant functional types and climate along a precipitation gradient in temperate grasslands, north-east China and south-east Mongolia. *Journal of Arid Environments*, **53**, 501-516.

Nielsen DR, Wendroth O (2003) Spatial and temporal statistics – Sampling field soils and their vegetation, *Catena Verlag, Reiskirchen*, 398 pages.

NOAA ESRL (2008) Earth System Research Laboratory. Global Monitoring Division. http://www.esrl.noaa.gov/gmd.

NOAA NCDC Climate Data Online (2007) http://cdo.ncdc.noaa.gov/CDO/cdo.

NOAA NCDC Climate Data Online (2008) http://cdo.ncdc.noaa.gov/CDO/cdo.

NOAA NCDC Climate Data Online (2009) http://cdo.ncdc.noaa.gov/CDO/cdo.

NOAA NNDC Climate Data Online (2010) http://www7.ncdc.noaa.gov/CDO/cdo.

Norman HC, Wilmot MG, Thomas DT, Masters DG, Revell DK (2009) Stable carbon isotopes accurately predict diet selection by sheep fed mixtures of C_3 annual pastures and saltbush or C_4 perennial grasses. *Livestock Science*, **121**, 162-172.

O`Leary MH (1981) Carbon isotope fractionation in plants. *Phytochemistry*, **20**, 553-567.

Oesterheld M, McNaughton SJ (1991) Effect of stress and time for recovery on the amount of compensatory growth after grazing. *Oecologia*, **85**, 303-313.

Osborne CP (2008) Atmosphere, ecology and evolution: what drove the Miocene expansion of C_4 grasslands? *Journal of Ecology*, **96**, 35-45.

Parton WJ, Scurlock JMO, Ojima DS, Schimel DS, Hall DO, Scopegram Group Members (1995) Impact of climate change on grassland production and soil carbon worldwide. *Global Change Biology*, **1**, 13-22.

Paruelo JM, Lauenroth WK (1996) Relative abundance of plant functional types in grasslands and shrublands of North America. *Ecological Applications*, **6**, 1212-1224.

Pearson SF, Levey DJ, Greenberg CH, Del Rio CM (2003) Effects of elemental composition in the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird. *Oecologia*, **135**, 516-523.

Pebesma EJ (2004) Multivariable geostatistics in S: the gstat package. *Computers & Geosciences*, **30**, 683-691.

Prache S, Roguet C, Petit M (1998) How degree of selectivity modifies foraging behaviour of dry ewes on reproductive compared to vegetative award structure. *Applied Animal Behaviour Science*, **57**, 91-108.

Pyankov VI, Gunin PD, Tsoog S, Black CC (2000) C4 plants in the vegetation of Mongolia: their natural occurrence and geographical distribution in relation to climate. *Oecologia*, **123**, 15-31.

R Development Core Team (2007) R: A language and environment for statistical computing, *R Foundation for Statistical Computing*, Vienna, Austria, ISBN 3-900051-07-0, URL http://www.R-project.org.

R Development Core Team (2008): R: A language and environment for statistical computing, R Foundation for Statistical Computing. ISBN 3-900051-07-0, URL http://www.R-project.org, Vienna, Austria.

R Development Core Team (2009) *R: A language and environment for statistical computing. R Foundation for Statistical Computing*. ISBN 3-900051-07-0, URL http://www.R-project.org, Vienna, Austria.

Randerson JT, Collatz GJ, Fessenden JE, Munoz AD, Still CJ, Berry JA, Fung IY, Suits N, Denning AS (2002) A possible global covariance between terrestrial gross primary production and C-13 discrimination: Consequences for the atmospheric C-13 budget and its response to ENSO. *Global Biogeochemical Cycles*, 1**6**, 1136.

Raymond WF (1948) Evaluation of herbage for grazing. *Nature*, **161**, 937-939.

Reich PB, Peterson DW, Wedin DA, Wrage K (2001) Fire and vegetation effects on productivity and nitrogen cycling across a forest-grassland continuum. *Ecology*, **82**, 1703-1719.

Retzer V (2007) Forage competition between livestock and Mongolian pika. *Basic and Applied Ecology*, **8**, 147-157.

Reuters (2001) Water sources for China Yellow River dry up Xinhua, *Reuters News Service*, Beijing, China, www.planetark.org/dailynewsstory.cfm/newsid/12711/story.htm, 10. Oct. 2001.

Ribeiro PJ, Diggle PJ (2001) geoR: a package for geostatistical analysis. *R-NEWS*, **1**, 15-18.

Richards MP, Pettitt PB, Trinkaus E, Smith FH, Paunovic M, Karavanic I (2000) Neanderthal diet at Vindija and Neanderthal predation: The evidence from stable isotopes. *Proceedings of the National Academy of Sciences of the United States of America*, **97,** 7663-7666.

Robbins CT, Felicetti LA, Sponheimer M (2005) The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds. *Oecologia*, **144**, 534-540.

Rossi RE, Mulla DJ, Journel AG, Franz EH (1992) Geostatistical tools for modeling and interpreting ecological spatial dependence. *Ecological Monographs*, **62**, 277-314.

Rumpel, C, Kögel-Knabner I, Bruhn F (2002) Vertical distribution, age and chemical composition of organic carbon in two forest soils of different pedogenesis. *Organic Geochemistry*, **33**, 1131-1142.

Sachs L, Hedderich J (2006) *Angewandte Statistik – Methodensammlung mit R. Springer,* Berlin.

Sage RF (2004) The evolution of C4 photosynthesis. *New Phytologist*, **161**, 341-370.

Sage RF, Kubien DS (2003) Quo vadis C4? An ecophysiological perspective on global change and the future of C4 plants. *Photosynthesis Research*, **77**, 209-225.

Sage RF, Kubien DS (2007) The temperature response of C-3 and C-4 photosynthesis. *Plant Cell and Environment*, **30,** 1086-1106.

Santruckova H, Bird MI, Lloyd J (2000) Microbial processes and carbon-isotope fractionation in tropical and temperate grassland soils. *Functional Ecology,* **14,** 108-114.

Scheirs J, De Bruyn L, Verhagen R (2001) A test of the C3-C4 hypothesis with two grass miners. *Ecology*, **82**, 410-421.

Schiborra A, Gierus M, Wan HW, Glindemann T, Wang CJ, Susenbeth A, Taube F (2009) Dietary selection of sheep grazing the semi-arid grasslands of Inner Mongolia, China

at different grazing intensities. *Journal of Animal Physiology and Animal Nutrition*, DOI: 10.1111/j.1439-0396.2009.00928.x.

Schimel DS (1995) Terrestrial ecosystems and the carbon-cycle. *Global Change Biology*, **1**, 77-91.

Schimel DS, House JI, Hibbard KA *et al.* (2001) Recent patterns and mechanisms of carbon exchange by terrestrial ecosystems. *Nature*, **414**, 169-172.

Schnute J, Boers N, Haigh R *et al.* (2007) PBSmapping: PBS Mapping 2.54, *R package version 2.54*.

Schnute J, Boers N, Haigh R, *et al.* (2008) PBSmapping: PBS Mapping 2.59, R package version 2.59.

Schnyder H, Schwertl M, Auerswald K, Schäufele R (2006) Hair of grazing cattle provides an integrated measure of the effects of site conditions and inter-annual weather variability on δ^{13} of temperate humid grassland. *Global Change Biology*, **12,** 1315-1329.

Schönbach P, Wan H, Schiborra A *et al.* (2009) Short-term management and stocking rate effects of grazing sheep on herbage quality and productivity of Inner Mongolia steppe. *Crop & Pasture Science*, **60**, 963-974.

Schulze ED, Ellis R, Schulze W, Trimborn P (1996) Diversity, metabolic types and delta C-13 carbon isotope ratios in the grass flora of Namibia in relation to growth form, precipitation and habitat conditions. *Oecologia*, **106**, 352-369.

Schulze ED, Turner NC, Nicolle D, Schumacher J (2006) Species differences in carbon isotope ratios, specific leaf area and nitrogen concentrations in leaves of Eucalyptus growing in a common garden compared with along an aridity gradient. *Physiolgia Plantarum*, **127**, 434-444.

Schulze ED, Williams RJ, Farquhar GD, Schulze W, Langridge J, Miller JM, Walker BH (1998) Carbon and nitrogen isotope discrimination and nitrogen nutrition of trees along a rainfall gradient in northern Australia. *Australian Journal of Plant Physiology*, **25**, 413-425.

Schuurmans JM, Bierkens MFP, Pebesma EJ, Uijlenhoet R (2007) Automatic prediction of high-resolution daily rainfall fields for multiple extents: The potential of operational radar. *Journal of Hydrometeorology*, **8,** 1204-1224.

Schwertl M, Auerswald K, Schäufele R, Schnyder H (2005) Carbon and nitrogen stable isotope composition of cattle hair: ecological fingerprints of production systems? *Agriculture Ecosystems & Environment*, **109,** 153-165.

Schwertl M, Auerswald K, Schnyder H (2003) Reconstructing the isotopic history of animal diets by hair segmental analysis. *Rapid Communications in Mass Spectrometry*, **17**, 1312-1318.

Scurlock JMO, Hall DO (1998) The global carbon sink: a grassland perspective. *Global Change Biology*, **4**, 229-233.

Semmartin M, Aguiar MR, Distel RA, Moretto AS, Ghersa CM (2004) Litter quality and nutrient cycling affected by grazing-induced species replacements along a precipitation gradient. *Oikos*, **107**, 148-160.

Shabtay A, Ravid U, Brosh A, Baybikov R, Eitam H, Laor Y (2009) Dynamics of offensive gas-phase odorants in fresh and aged feces throughout the development of beef cattle. *Journal of Animal Science*, **87**, 1835-1848.

Silvermann BW (1986) Density estimations for statistics and data analysis. In: *Monographs on Statistics and Applied Probability*, **26**, Chapman and Hall/CRC, London, UK:

Smedley MP, Dawson TE, Comstock JP, Donovan LA, Sherrill DE, Cook CS, Ehleringer JR (1991) Seasonal carbon isotope discrimination in a grassland community. *Oecologia*, **85**, 314-320.

Sneath D (1998) Ecology – State policy and pasture degradation in Inner Asia. *Science*, **281**, 1147-1148.

Sneath D (2002) Grassland Ecosystems. Sustaining the steppe: the future of Mongolia's grassland. In: *World resources 2000-2001*, edited by World Resources Institute, Amsterdam:Elsevier Science, p. 212-224.

Sparks JP, Ehleringer JR (1997) Leaf carbon isotope discrimination and nitrogen content for riparian trees along elevational transects. *Oecologia*, **109**, 362-367.

Sponheimer M, Robinson T, Ayliffe L *et al*. (2003c) Nitrogen isotopes in mammalian herbivores: hair δ^{15}N values from a controlled feeding study. *International Journal of Osteoarchaeology*, **13**, 80-87.

Sponheimer M, Robinson T, Ayliffe LK, Passey BH, Roeder B, Shipley L, Lopez E, Cerling TE, Dearing MD, Ehleringer JR (2003a) An experimental study of carbon-isotope

fractionation between diet, hair, and feces of mammalian herbivores. *Canadian Journal of Zoology*, **81**, 871-876.

Sponheimer M, Robinson TF, Cerling TE, Tegland L, Roeder BL, Ayliffe L, Dearing MD, Ehleringer JR (2006) Turnover of stable carbon isotopes in the muscle, liver, and breath CO_2 of alpacas (*Lama pacos*). *Rapid Communications in Mass Spectrometry*, **20**, 1395-1399.

Sponheimer M, Robinson TF, Roeder BL *et al*. (2003b) An experimental study of nitrogen flux in llamas: is ^{14}N preferentially excreted? *Journal of Archaeological Science*, **30**, 1649-1655.

Sponheimer M, Robinson TF, Roeder BL *et al*. (2003d) Digestion and passage rates of grass hays by llamas, alpacas, goats, rabbits, and horses. *Small Ruminant Reearch*, **48**, 149-154.

Steffens M, Kölbl A, Kögel-Knabner I (2009) Alteration of soil organic matter pools and aggregation in semi-arid steppe topsoils as driven by organic matter input. *European Journal of Soil Science*, **60**, 192-212.

Still CJ, Berry JA, Collatz GJ, DeFries RS (2003b) Global distribution of C-3 and C-4 vegetation: Carbon cycle implications. *Global Biogeochemical Cycles*, **17**, 1006.

Still CJ, Berry JA, Ribas-Carbo M, Helliker BR (2003a) The contribution of C_3 and C_4 plants to the carbon cycle of a tallgrass prairie: an isotopic approach. *Oecologia*, **136**, 347-359.

Sutoh M, Koyama T, Yoneyama T (1987) Variations of natural ^{15}N abundance in the tissues and digesta of domestic animals. *Radioisotopes*, **36**, 74-77.

Sutoh M, Obara Y, Yoneyama T (1993) The effects of feeding regime and dietary sucrose supplementation on natural abundance of ^{15}N in some components of ruminal fluid and plasma of sheep. *Journal of Animal Science*, **71**, 226-231.

Suttie JM (2000) Country pasture – forage resources profiles: Mongolia. FAO http://www.fao.org/ag/AGP/AGPC/doc/Counprof/Mongolia/mongol1.htm

Swap RJ, Aranibar JN, Dowty PR, Gilhooly WP, Macko SA (2004) Natural abundance of C-13 and N-15 in C3 and C4 vegetation of southern Africa: patterns and implications. *Global Change Biology*, **10**, 350-358.

Tans PP, Conway TJ (2005) Monthly atmospheric CO_2 mixing ratios from the NOAA CMDL Carbon Cycle Cooperative Global Air Sampling Network, 1968-2002. *A Compendium of Data on Global Change*. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, TN, USA.

Taub DR (2000) Climate and the U.S. distribution of C4 grass subfamilies and decarboxylation variants of C4 photosynthesis. Aerican. *Journal of Botany*, **87**, 1211-1215.

Teeri JA, Stowe LG (1976) Climatic Patterns and Distribution of C4 Grasses in North-America. *Oecologia*, **23**, 1-12.

Teeri, JA (1988) Interaction of temperature and other environmental variables influencing plant distribution. *Symposia of the Society for Experimental Biology*, **42**, 77-89.

The Climate Source LLC (2002) PRISM spatial climate datasets for Mongolia and Inner Mongolia, Corvallis, OR, USA.

Tieszen LL, Boutton TW, Tesdahl KG, Slade NA (1983) Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}C$ analysis of diet. *Oecologia*, **57**, 32-37.

Tieszen LL, Reed BC, Bliss NB, Wylie BK, Dejong DD (1997) NDVI, C3 and C4 production, and distributions in Great Plains grassland land cover classes. *Ecological Applications*, **7**, 59-78.

Tieszen LL, Reed BC, Bliss NB, Wylie BK, Dejong DD (1997) NDVI, C3 and C4 production, and distributions in great plains grassland land cover classes. *Ecological Applications*, **7**, 59-78.

Tong C, Wu J, Yong S, Yang J, Yong W (2004) A landscape-scale assessment of steppe degradation in the Xilin River Basin, Inner Mongolia, China. *Journal of Arid Environments*, **59,** 133-149.

Torn MS, Lapenis AG, Timofeev A, Fischer ML, Babikov BV, Harden JW (2002) Organic carbon and carbon isotopes in modern and 100-year-old-soil archives of the Russian steppe. *Global Change Biology*, **8**, 941-953.

Trumbore S (2000) Age of soil organic matter and soil respiration: radiocarbon constraints on belowground C dynamics. *Ecological Applications*, **10**, 399-411.

Tsialtas JT, Handley LL, Kassioumi MT, Veresoglou DS, Gagianas AA (2001) Interspecific variation in potential water-use efficiency and its relation to plant species abundance in a water-limited grassland. *Functional Ecology*, **15**, 605-614.

Van Soest PJ (1994) Nutritional ecology of the ruminant. *Comstock*, New York.

Vanderklift MA, Ponsard S (2003) Sources of variation in consumer-diet δ^{15}N enrichment: a meta analysis. *Oecologia*, **136**, 169-182.

Vitousek PM, Aber JD, Howarth RD, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman GD (1997) Human alteration of the global nitrogen cycle: Sources and consequences. *Ecological Applications*, **7**, 737-750.

von Fischer JC, Tieszen LL, Schimel DS (2008) Climate controls on C3 vs. C4 productivity in North American grasslands from carbon isotope composition of soil organic matter. *Global Change Biology*, **14**, 1141-1155.

Wallis de Vries MF, Manibazar N, Dügerlham S (1996) The vegetation of the forest-steppe region of Hustain Nuruu, Mongolia. *Plant Ecology*, **122**, 11-127.

Wang CJ, Tas BM, Glindemann T, Rave G, Schmidt L, Weißbach F, Susenbeth A (2009) Fecal crude protein content as an estimate for the digestibility of forage in grazing sheep. *Animal Feed Science and Technology*, **149**, 199-208.

Wang G, Han JM, Liu D (2003a) The carbon isotope composition of C3 herbaceous plants in loess area of Northern China. *Science in China Series D*, **46**, 1069-1076.

Wang GA, Han JM, Zhou LP, Xiong XG, Wu Z (2005) Carbon isotope ratios of plants and occurrences of C_4 species under different soil moisture regimes in arid region of Northwest China. *Physiolgia Plantarum*, **125**, 74-81.

Wang JH, Wang SP, Schnug E, Haneklaus S, Patton B, Nyren P (2008) Competition between *Stipa grandis* and *Cleistogenes squarrosa*. *Journal of Arid Environments*, **72,** 63-72.

Wang L, Ketzer B, Huizhi L, Bernhofer C (2010) Effects of grazing on evapotranspiration over a typical steppe in Inner Mongolia, China. *Plant and Soil* (accepted).

Wang RZ (2002a) Photosynthetic pathway types of forage species along grazing gradient from the Songnen grassland, Northeastern China. *Photosynthetica*, **40**, 57-61.

Wang RZ (2002b) Photosynthetic pathways, life forms, and reproductive types for forage species along the desertification gradient on Hunshandake desert, North China. *Photosynthetica,* **40**, 321-329.

Wang RZ (2003) Photosynthetic pathway and morphological functional types in the steppe vegetation from Inner Mongolia, North China. *Photosynthetica*, **41**, 143-150.

Wang RZ (2004) Photosynthetic and morphological functional types from different steppe communities in Inner Mongolia, North China. *Photosynthetica*, **42**, 493-503.

Wang SP, Wang YF, Chen ZZ (2003b) Effect of climate change and grazing on populations of *Cleistogenes squarrosa* in Inner Mongolia steppe. *Acta Phytoecologica Sinica*, **27**, 337-343.

Warren CR, McGrath JF, Adams MA (2001) Water availability and carbon isotope discrimination in conifers. *Oecologia*, **127**, 476-486.

Watzka M, Buchgraber K, Wanek W (2006) Natural ^{15}N abundance of plants and soils under different management practices in a montane grassland. *Soil Biology & Biochemistry*, **38,** 1564-1576.

Webster R (1991) Local disjunctive kriging of soil properties with change of support. *Journal of Soil Science*, **42**, 301-318.

Webster R, Oliver M (2004) Geostatistics for environmental scientists. *Wiley & Sons*, Chichester, ISBN 0-471-96553-7.

Wedin DS, Tieszen LL, Dewey B, Pastor J (1995) Carbon isotope dynamics during grass decomposition and soil organic matter formation. *Ecology*, **76**, 1383-1392.

Wiesmeier M, Kreyling O, Steffens M, Schönbach P, Wan H, Gierus M, Taube F, Kölbl A, Kögl-Knabner I (2009) Soil and vegetation indicators for degradation of steppes due to short-term grazing in Northern China. *Journal of Arid Environments* (accepted).

Wilson JR, Hacker JB (1987) Comparative digestibility and anatomy of some sympatric C3 and C4 arid zone grasses. *Australian Journal of Agricultural Research*, **38**, 287-295.

Witt GB, Moll EJ, Beeton RJS, Murray PJ (1998) Isotopes, wool, and rangeland monitoring: Let the sheep do the sampling. *Environmental Management*, **22**, 145-152.

Wittmer MHOM, Auerswald K, Bai YF, Schäufele R, Schnyder H (2010b) Changes in the abundance of C3/C4 species of Inner Mongolia grassland: evidence from isotopic composition of soil and vegetation. *Global Change Biology*, **16**, 605-616.

Wittmer MHOM, Auerswald K, Schönbach P *et al*. (2010a) Do grazer hair and faeces reflect the carbon isotope composition of semi-arid C3/C4 grassland? *Basic and Applied Ecology*, **11**, 83-92.

Wittmer MHOM, Auerswald K, Tungalag R, Bai YF, Schäufele R, Schnyder H (2008) Carbon isotope discrimination of C3 vegetation in Central Asian Grassland as related to long-term and short-term precipitation patterns. *Biogeosciences*, **5**, 913-924.

World Bank (2009) Mongolia – Data & Statistics, http://go.worldbank.org/XI5V65BZW0

Wynn JG, Bird MI (2007) C4 derived soil organic carbon decomposes faster than its C3 counterpart in mixed C3/C4 soils. *Global Change Biology*, **13**, 2206-2217.

Wynn JG, Bird MI (2008) Environmental controls on the stable carbon isotopic composition of soil organic carbon: implications for modeling the distribution of C3 and C4 plants, Australia. *Tellus*, **60B**, 604-621.

Xiao X, Ojima DS, Parton WJ, Chen Z, Chen D (1995a) Sensitivity of Inner Mongolian grasslands to Climate Chang. *Journal of Biogeography*, **22**, 643-648.

Xiao X, Wang YF, Jiang S, Ojima DS, Bonham CD (1995b) Interannual variation in the Climate and aboveground biomass of *Leymus chinense* Steppe and *Stipa grandis* steppe in the Xilin River Basin, Inner-Mongolia, China. *Journal of Arid Environments*, **31**, 283-299.

Xu M, Zhang TZ (2007) Material flows and economic growth in developing China. *Journal of Industrial Ecology*, **11**, 121-140.

Yang H, Auerswald K, Bai YF, Han XG (2010) Complementarity in water sources among dominant species in typical steppe ecosystems of Inner Mongolia, China. *Plant and Soil*, accepted.

Yang H, Auerswald K, Bai YF, Wittmer MHOM, Wiesmeier M, Schnyder H (in preparation) Variation in carbon isotope discrimination of *Cleistogens squarrosa*: Patterns, drivers, and mechanisms on plant, local, catchment and regional scales.

Yu FF, Price KP, Ellis J, Shi PJ (2003) Response of seasonal vegetation development to climatic variations in eastern central Asia, *Remote Sensing of Environment*, **87**, 42-54.

Yuan ZY, Liu WX, Niu SL, Wan SQ (2007) Plant nitrogen dynamics and nitrogen-use strategies under altered nitrogen seasonality and competition. *Annals of Botany*, **100**, 821-830.

Zazzo A, Moloney AP, Monahan FJ, Scrimgeour CM, Schmidt O (2008) Effect of age and food intake on dietary carbon turnover recorded in sheep wool. *Rapid Communications in Mass Spectrometry*, **22,** 2937-2945.

Zhao FJ, Spiro B, McGarth SP (2001) Trends in $^{13}C/^{12}C$ ratios and isotope discrimination of wheat since 1845, *Oecologia*, **128**, 336-342.

Zheng SX, Shangguan ZP (2006) Spatial patterns of foliar stable carbon isotope compositions of C3 plant species in the Loess Plateau of China. *Ecological Research*, **22**, 342-353.

Zheng SX, Shangguan ZP (2007) Foliar $\delta^{13}C$ values of nine dominant species in the Loess Plateau of China. *Photosynthetica*, **45**, 110-119.

Ziska LH, Sicher RC, Bunce JA (1999) The impact of elevated carbon dioxide on the growth and gas exchange of three C4 species differing in CO_2 leak rates. *Physiologia Plantarum*, **105**, 74-80.

ANNEX – Biases in the analysis of the stable isotopes in food webs[5]

Abstract

1. Recently Caut, Angulo & Courchamp (2009, Journal of Applied Ecology) published a review on diet-tissue isotopic shifts in animals. They concluded that diet-tissue shifts are influenced by the isotopic composition of the diet for both ^{13}C and ^{15}N in a wide range of animal taxa.

2. We suggest that the conclusion of Caut *et al*. is in error, and provide a discussion of sources of error in the assessment of diet-tissue discrimination.

3. Errors in the derivation of diet-tissue shifts include imprecise definitions, mathematical artefacts and invalid statistical analysis. Likely, the work also suffers from experimental bias. The mathematical artefacts and statistical invalidity result from using the same variable (diet isotopic composition) in the independent and dependent variable for regression analysis and failure to correct for the resulting bias. Experimental bias can result from the incomplete turnover of body pools after diet switches or during natural fluctuations in diet isotope composition. Unfortunately, the main sources of error work in the same direction, strengthening the biased relationship between the diet-tissue shift and diet isotope composition. Therefore, the analysis of Caut et al. does not provide proof of a relationship between diet-tissue shift and diet isotope composition.

4. *Synthesis and application:* The present discussion is relevant to all applications of diet-tissue isotopic shifts in anthropogenic and feral trophic networks, including diet reconstructions.

Introduction

Caut, Angulo & Courchamp have recently reviewed carbon and nitrogen isotope discrimination factors in mammals, birds, fish and invertebrates (Caut *et al.*, 2009). This extended an earlier study on an omnivorous consumer (Caut *et al.*, 2008). These authors concluded that discrimination factors are influenced by the isotopic composition of the diet in all taxonomic groups, with statistically significant effects in most comparisons. If true, this finding is important for all studies of diet-tissue shifts, including nutritional ecology, archaeology and forensics. But, other recent compilations of discrimination (or diet-tissue shifts) have not identified such effects (e.g. McCutchan *et al.*, 2003; Sponheimer *et al.*, 2003a; Hwang *et al.*, 2007; Männel *et al.*, 2007). Also the conclusion of Caut *et al.* conflicts with the rate law of chemical reactions that the rate constant of a reaction is independent of the concentration (IUPAC, 1997; Wright, 2004). This applies to kinetic isotope effects, which are given by the ratio of the reaction constants for the heavier and the lighter isotopes (Baertschi, 1952). In this comment we draw attention to general sources of bias in isotopic food chain experiments and we suggest that the effect reported by Caut *et al.* (2009) is flawed by invalid mathematical/statistical procedures and possibly experimental bias.

Definitions

First, we remind that the term 'discrimination factor' (or 'fractionation factor'), as used by Caut *et al.* and recently also by others (e.g. Pearson *et al.*, 2003; Robbins *et al.*, 2005; Carleton *et al.* 2008; Fisk *et al.*, 2009; Wolf *et al.*, 2009) was originally defined in a different way in the bio- and geosciences (e.g. Craig, 1954; Farquhar *et al.*, 1989; Cerling & Harris, 1999; Mook, 2001). This is now opening opportunities for ambiguous use, a source of scientific error (Ioannidis, 2005). Originally and still in most cases the discrimination factor, abbreviated α (cf. Baertschi, 1952; 1953; Craig, 1954; Farquhar *et al.*, 1989; Mook, 2001), was defined as the ratio of the reaction constants k of the heavier ($^{heavy}k$) and the lighter isotope ($^{light}k$).

$$\alpha = {}^{heavy}k \, / \, {}^{light}k \qquad\qquad\qquad\qquad (A.1)$$

Eqn. A.1 is equivalent to the ratio R_S/R_P, where R denotes the abundance ratio of heavy to light isotopes and the indices S and P to indicate the substrate (diet) and the product (animal

tissue or body) of a reaction chain. Following the definition of δ ($\delta = (R_{sample}/R_{standard}) - 1$, where R_{sample} and $R_{standard}$ are the ratios of the heavy to the light isotopes in the sample and a standard; McKinney *et al.*, 1950) eqn. A.1 is also equivalent to the ratio $(1 + \delta_S) / (1 + \delta_P)$, where δ_S denotes the isotopic composition of the substrate and δ_P that of the product.

This definition of α is important as the rate law for chemical reactions implies that the reaction constants and hence their ratio α is independent of the isotopic composition of the reactant (i.e. the substrate of the reaction which is the diet in trophic relations) (IUPAC, 1997; Wright, 2004). The discrimination factor in its strict definition can hence not be influenced by the isotopic composition of the diet. Synonymous terms for α are 'isotope effect', 'fractionation factor' or 'enrichment factor' (Park & Epstein, 1960; O`Leary, 1981; Farquhar *et al.*, 1989; Cerling & Harris, 1999). As a ratio α can be used in multiplication operations with the isotopic composition of the diet (or more generally: the source) and is hence called factor and differs from discrimination.

Discrimination, Δ (*sensu* Blackmer & Bremner, 1977, Farquhar *et al.*, 1989) equals the difference between α and unity:

$$\Delta = \alpha - 1 \tag{A.2}$$

Eqn. A.2 is equivalent to

$$\Delta = \frac{\delta_S - \delta_P}{1 + \delta_P} \tag{A.3}$$

A synonymous term for Δ is 'enrichment' (Craig, 1954; Cerling & Harris, 1999).

The 'discrimination factor' as named by Caut *et al.* (here designated Δ') is the numerical difference between product and substrate isotope composition:

$$\Delta' = \delta_P - \delta_S \tag{A.4}$$

So, the 'discrimination factor' reported by Caut *et al.* should be termed 'diet-tissue shift' (or similar) (e.g. Ambrose, 2000; Darr & Hewitt, 2008; Auerswald *et al.*, 2009). This distinction is particularly important for diet-tissue discrimination studies with a wide range of diet isotopic composition, because discrimination (eqn. A.3) is independent of the isotopic composition of the diet, whereas the diet-tissue shift (eqn. A.4) is not (Fig. A.1). Although the

magnitude of the effect is small, it does affect the relationship between the diet-tissue shift and diet isotope composition presented by Caut *et al.*, since it reduces the (negative) slope.
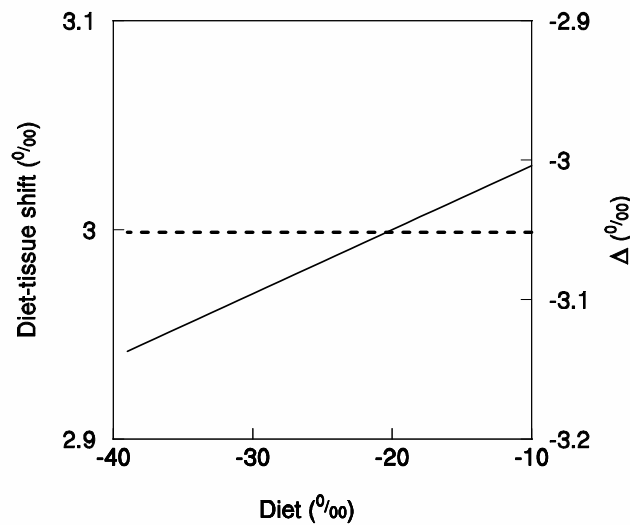
Fig. A.1 Diet-tissue shift (broken line, left axis) and diet-tissue discrimination Δ (solid line, right axis) as related to the isotopic composition of the diet. The calculations were made for the range of carbon isotope composition as given by Caut *et al.* (2009) and in assuming a true diet-tissue shift of 3‰ for a diet of -20‰ and no errors associated with the measurements.

Possible sources of bias

In our opinion, the analysis of Caut *et al.* is affected by two sources of bias: one is a mathematical/statistical artefact, the other is an experimental bias. As we show, both sources of bias (unfortunately) work in the same direction, mutually amplifying the spurious effect of diet isotope composition on the diet-tissue shift.

Mathematical artefact

Any measured value of diet isotope composition is an estimate, which is termed 'random variable' in statistics. Any random variable is composed of its true value (μ) and a random error (ε), which can be treated by statistical procedures. The same applies to parameters derived from random variables like the mean. In addition, bias may occur, which cannot be statistically treated by averaging and other procedures. Assuming no bias for the moment, the measured value of diet isotope composition thus is $\delta_S = \mu_S + \varepsilon_S$, and $\delta_P = \mu_P + \varepsilon_P$ for the tissue isotope composition.

There are many sources of error, including the errors of sampling and measurement, which contribute to ε_S and ε_P. When regressing the difference between measured values of diet and

tissue isotope composition (i.e. a 'measured' diet-tissue shift: $\delta_P - \delta_S$) against the measured value of diet isotope composition (δ_S), as done by Caut *et al.*, then the error term of the independent variable (ε_S) appears in the independent and the dependent variable, causing a erroneous (artificial) relationship between the two variables (Fig. A.2), which is termed spurious correlation in statistics (Pearson, 1897; Kenney, 1982; Kanaroglou, 1996).
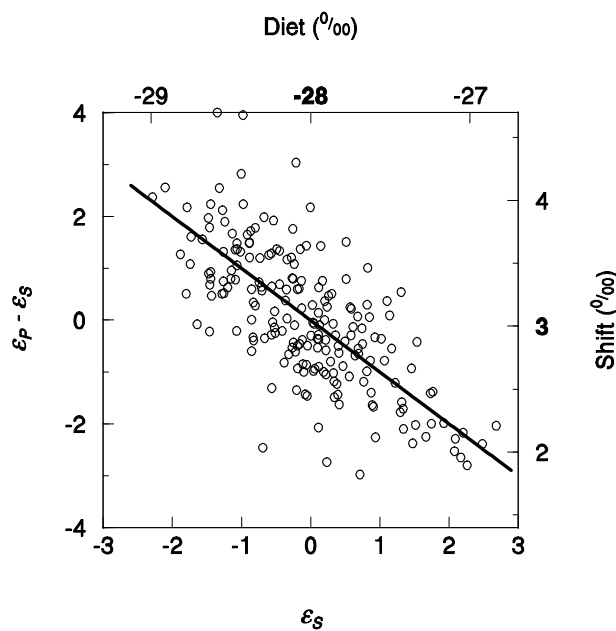
Fig. A.2 Shift between two random numbers (ε_S and ε_P equivalent to the errors ε of the diet and the tissue) depending on random number ε_S; both random numbers were generated for a true mean of 0 and a true standard deviation of 1; n=200; the line has a slope of -1.
The top axis and the right axis exemplify the effect of the error terms ε for a true isotopic value of -28‰ of the diet and a true shift of 3‰ assuming standard deviations for the measurements of diet and tissue of 0.4‰.

Therefore, regression analysis and related statistical procedures require that the independent and the dependent variable are independent measurements (Fox, 1997; Sachs & Hedderich, 2006). This is not the case when the dependent variable includes the independent. So, the regression of diet-tissue shift against diet isotope composition is invalid and invalidates any further statistical evaluation. In support of their approach, Caut *et al.* cited the Hills (1978) paper, but this does not treat this particular statistical issue, and it does not contain arguments in support of their approach.

The reason for this statistical rule is illustrated in Fig. A.2 by using a biologically meaningful example: it shows the artefact, which results when the error of the independent variable is included in the dependent variable in a regression analyses. The analysis is based on one pair of samples, a diet and an animal tissue, for which the true isotopic values are known: μ_S -28‰ and μ_P -25‰ (thus, the diet-tissue shift is 3‰). The total error (measurement, sampling and

other) was assumed to yield a standard deviation of 0.4‰ for both diet and tissue data. From this we drew 200 random pairs of 'measured' diet and tissue isotope values, and plotted the 'measured' diet-tissue shift against diet isotope composition (Fig. A.2). Subsequently we performed a regression analysis. This yielded a strong dependence of the diet tissue-shift on diet isotope composition. As is apparent, the artefact resulted solely from including the random error of the independent variable in both the independent and the dependent variable. Based on statistical reasoning and the example given above, we suspect that the effect of diet isotope composition on the diet-tissue shift, as reported by Caut *et al.*, is mainly a mathematical/statistical artefact. Of course, the data set of Caut et al. also contains true variation of diet isotope composition, but this does not fix the error. In our ideal example: if we avoided the artefact by regressing the 'measured' values of tissue against the 'measured' values of diet (not shown), then the slope of the relationship between the two was one. This meant that estimates of diet and tissue isotope composition varied in parallel, and the error was randomly distributed around the regression. So, there was no effect of diet isotope composition on discrimination, consistent with the initial specifications.

Kenney (1982) and Kanaroglou (1996) have shown formally for the case where the dependent $(\delta_P - \delta_S)$ is a difference with the independent (δ_S) that the equation of the correlation coefficient r becomes:

$$r_{\delta S, \delta P - \delta S} = \frac{\frac{s_{\delta P}}{s_{\delta S}} \times r_{\delta S, \delta P} - 1}{\sqrt{1 + \left(\frac{s_{\delta P}}{s_{\delta S}}\right)^2 - 2 \times \frac{s_{\delta P}}{s_{\delta S}} \times r_{\delta S, \delta P}}} \qquad (A.5)$$

where s denotes the standard deviation of δ_P and δ_S. For our example, where we assumed no correlation between δ_P and δ_S and an equal s for δ_P and δ_S, eqn. A.5 predicts $r_{\delta S, \delta P - \delta S} = 0.707$, which matches what we found ($r_{\delta S, \delta P - \delta S} = 0.708$). Eqn. A.5 can then be used to predict and remove the spurious component (r_s) of the correlation coefficient $r_{\delta S, \delta P - \delta S}$ (Kenney, 1982; Kanaroglou, 1996):

$$r_s = \frac{-1}{\sqrt{1 + \left(\frac{s_{\delta P}}{s_{\delta S}}\right)^2}} \qquad (A.6)$$

Applying eqn. A.6 to the data sets of Caut *et al*. turns all previously significant correlations insignificant (Table A.1) proving that the effect found by Caut *et al*. is entirely spurious in their data set. The sign of the correlation coefficient even changed from negative to positive after correction, which agrees with the prediction in Fig. A.1 that the calculation of a shift instead of discrimination should cause a small positive effect.

Table A.1 Correlation coefficients between the diet-tissue shift and the isotopic composition of the diet ($r_{\delta S, \delta S - \delta P}$) as calculated by Caut et al. (2009) and correlation coefficients corrected for the spurious component according to Kenney (1982) (r_{corr}) for the nitrogen and carbon istotopic composition and four classes of animals.

	Class	n	$r_{\delta S, \delta S - \delta P}$	r_{corr}	Change in significance from $r_{\delta S, \delta S - \delta P}$ to r_{corr}
$\delta^{13}C$	Mammals	96	- 0.69	- 0.01	$P < 0.05 \rightarrow$ n.s.
	Bird	61	0.04	0.25	n.s. $\rightarrow$ n.s.
	Fish	40	- 0.50	0.17	$P < 0.05 \rightarrow$ n.s.
	Invertebrates	90	- 0.29	0.20	$P < 0.05 \rightarrow$ n.s.
$\delta^{15}N$	Mammals	79	- 0.39	0.20	$P < 0.05 \rightarrow$ n.s.
	Bird	53	0.04	0.38	n.s. $\rightarrow P < 0.05$
	Fish	37	- 0.59	0.24	$P < 0.05 \rightarrow$ n.s.
	Invertebrates	74	- 0.59	0.14	$P < 0.05 \rightarrow$ n.s.

Eqn. A.5 implicitly predicts that the range and the strength of the relationship in Fig. A.2 depend on the errors of the two measured variables, which may not be the same. If the error for the tissue is lower than that for the diet, then the relation in Fig. A.2 becomes tighter (higher r^2). If tissue measurements have no error (which could approximately be true where measurements of the same tissue are repeated many times) then the r² equals one. Conversely, if the error for the tissue is larger than for the diet, then r^2 is smaller than in Fig. A.2. In general, we assume that it is quite difficult to maintain a diet with constant isotopic composition for a sufficiently long period of time (see Experimental bias). Thus, achieving an isotopic equilibrium between diet and tissue and subsampling of diet is more demanding than sampling of tissue, generating larger errors for diet.

Experimental bias

An animal body comprises several compartments, which can be assigned to one of three functional groups, which are relevant to isotopic exchange kinetics: (i) metabolic pools which are supplied by digesta, (ii) stores which exchange carbon or nitrogen compounds with metabolic pools (thus showing turnover), and (iii) inert ('dead') tissues which receive carbon

or nitrogen from metabolic pools during formation, but do not exchange with them (no turnover) (Fig. A.3). While metabolic pools respond rapidly to a change in the isotopic composition of the diet, they do not reach isotopic equilibrium until stores are fully equilibrated (Ayliffe *et al.*, 2004). Stores include liver, muscle, bone collagen and fat stores, which differ in turnover times (Telang *et al.*, 2002; Ojeda-Avila, *et* al., 2003; Ayliffe *et al.*, 2004; Sponheimer *et al.*, 2006; Cerling *et al.*, 2007; Zazzo *et al.*, 2007). 'Dead' tissues include hair, feathers or chitin skeletons, which do not equilibrate with metabolic pools and stores (Montagna & Ellis, 1958; O'Connell & Hedges, 1999; Roth & Hobson, 2000). The presence of 'dead' pools makes it impossible to achieve isotopic equilibrium in experiments with whole-body analysis except if they are renewed by shedding or moulting. The presence of stores also hampers the assessment of true diet-tissue discrimination, as they can have half-lives which are orders of magnitude higher than those of metabolic pools, which complicates their detection (Ayliffe *et al.*, 2004). This problem, failure to equilibrate isotopically, causes an experimental bias.
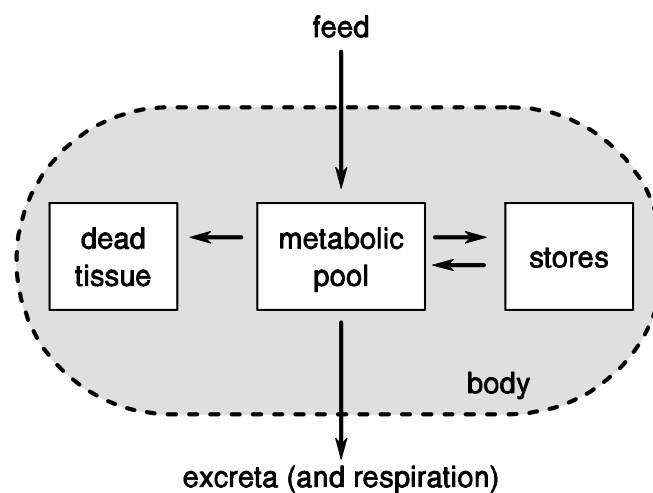
Fig. A.3 Conceptual model of an animal as composed of three functional compartments: (i) a metabolic pool, (ii) stores which exchange matter with the metabolic pool, and (iii) inert ('dead') tissue, which unidirectionally receives matter from the metabolic pool during formation.

Unfortunately, the mathematical artefact and experimental bias caused by the isotopic memory, work in the same direction, strengthening the bias. The main difference between the two sources of errors is in the value of the slope. Concerning the mathematical artefact: here the slope is always -1 for the diet-tissue shift (or close to 1 for discrimination) provided that there is no real variation in the isotopic composition of the diet (Fig. A.2). But, the slope decreases with increasing variation of the diet. That is, the bias gets smaller if diet isotope

composition varies much. Theoretically, the slope becomes nil, if the variation of diet isotope composition is infinite.

Concerning the experimental bias: the slope (bias) is equal to the proportion of non-equilibrated body material in non-equilibrium feeding studies. Accordingly, as a special case, the slope is -1 for the diet-tissue shift immediately after a diet switch, when diet and tissue have not started to equilibrate at all.

Clearly, studies with constant chemical and isotopic composition of feed are mainly affected by the mathematical artefact. Conversly, studies with varying feed isotope composition suffer mainly from the memory effect.

As explained above, we can not be certain about the exact magnitude of experimental bias in the report of Caut *et al*., as a reinterpretation would require knowledge of the exact kinetics of isotopic composition of metabolic, storage and dead pools of all animals in the review. But these data are usually not reported in the original studies. Yet, in the Caut *et al*. review about 10% of the studies used diet-switches and these are particularly sensitive to isotopic memory effects. Such studies occupy the extremes of the range of diet isotope compositions and therefore have a strong leverage effect on the regression. In addition, about 30% were field studies, which are affected naturally by variation in diet isotope composition.

The problem can only be overcome by studies in which isotopically (and chemically) uniform feed is provided from birth to death, similar to the classical experiments by De Niro & Epstein (1978; 1981). Where maternal material constitutes a significant proportion of body mass, controlled and constant feeding must start with the mothers as in the experiments by Hobson & Clark (1992). If diet-tissue shifts are to be based on metabolic pool samples, then the constancy of diet isotope composition should be ensured (and documented) by diet sampling over a multiple of the turnover time of relevant body stores. Still, there may be special situations, such as essential amino acids which are derived from only one feed source (e.g. Fantle *et al*., 1999).

The danger of memory effects in the estimation of feed-tissue discrimination can be reduced by avoiding whole body analysis, and focusing on a metabolic pool like blood serum (Roth & Hobson, 2000; Felicetti *et al*., 2003; Hilderbrand *et al*., 1996; Darr & Hewitt, 2008) or recent products of metabolic pools, such as new hair growth (Hobson *et al.,* 2000; Cerling *et al*., 2006; Schnyder *et al*., 2006; Mützel *et al*., 2009; Zazzo *et al*., 2007; 2008). A high resolution record of changes in diet isotope composition can be obtained by analysis of the longitudinal variation of isotope composition along a hair (Ayliffe *et al*., 2004). Such analyses have

revealed the existence and kinetic properties of body stores and can predict the experimental bias which results from isotopic memory by stores.

Conclusions

We agree with Caut *et al.* that understanding and estimating discrimination in diet consumer relationships is a very important issue in trophic network studies and diet reconstructions. Avoiding mathematical and experimental artefacts in the estimation of diet-tissue discrimination is essential to identify (and quantify) true effects of diet on discrimination. We argue that the 'Diet-Dependent Discrimination Factor' method (as proposed by Caut *et al.*) results from these artefacts and becomes unnecessary when these errors are avoided. True effects may result from other factors, such as diet protein content and amino acid composition (Sponheimer *et al.*, 2003b; Gaye-Siesegger *et al.*, 2004), diet selection (Garcia *et al.*, 2003), differential digestibility of diet components in mixed rations (Caswell *et al.*, 1973; Fantle *et al.*, 1999) or routing (Fantle *et al.*, 1999; Dalerum & Angerbjörn, 2005).

References

Ambrose SH (2000) Controlled Diet and Climate Experiments on Nitrogen Isotope Ratios of Rats. *Biogeochemical Approaches to Paleodietary Analysis*, (eds S.H. Ambrose & M.A. Katzenberg), pp. 243-259. Kluwer Academic Publishers, New York.

Auerswald K, Wittmer MHOM, Männel TT, Bai YF, Schäufele R, Schnyder H (2009) Large regional-scale variation in C3/C4 distribution pattern of Inner Mongolia steppe is revealed by grazer wool carbon isotope composition. *Biogeosciences*, **6**, 795-805.

Ayliffe LK, Cerling TE, Robinson T *et al*. (2004) Turnover of carbon isotopes in tail hair and breath CO_2 of horses fed an isotopically varied diet. *Oecologia,* **193**, 11-22.

Baertschi P (1952) Die Fraktionierung der Kohlenstoffisotopen bei der Absorption von Kohlendioxyd. *Helvetica Chimica Acta*, **35**, 1030-1036.

Baertschi P (1953) Die Fraktionierung der natürlichen Kohlenstoffisotopen im Kohlendioxydstoffwechsel grüner Pflanzen. *Helvetica Chimica Acta*, **36**, 773-781.

Blackmer AM, Bremner JM (1977) Nitrogen isotope discrimination in denitrification of nitrate in soils. *Soil Biology & Biochemistry*, **9**, 73-77.

Carleton SA, Kelly L, Anderson-Sprecher R, Del Rio CM (2008) Should we use one-, or multi-compartment models to describe C-13 incorporation into animal tissues? *Rapid Communications in Mass Spectrometry*, **22**, 3008-3014.

Caswell H, Reed F, Stephens SN, Werner PA (1973) Photosynthetic pathways and selective herbivory: A hypothesis. *American Naturalist,* **107**, 465-480.

Caut S, Angulo E, Courchamp F (2008) Discrimination factors (Δ^{15}N, Δ^{13}C) in an omnivorous consumer: effect of diet isotopic ratio. *Functional Ecology,* **22**, 255-263.

Caut S, Angulo E, Courchamp F (2009) Variation in discrimination factors (Δ^{15}N and Δ^{13}C): the effect of diet isotopic values and applications for diet reconstruction. *Journal of Applied Ecology,* **46**, 443-453.

Cerling TE, Harris JM (1999) Carbon isotope fractionation between diet and bioapatite in ungulate mammals and implications for ecological and paleoecological studies. *Oecologia*, **120**, 347-363.

Cerling TE Wittemyer G, Rasmussen HB, Vollrath F, Cerling CE, Robinson TJ, Douglas-Hamilton I (2006) Stable isotopes in elephant hair document migration patterns and

diet changes. *Proceedings National Academy Sciences United States America*, **103**, 371-373.

Cerling TE, Ayliffe LK, Dearing MD, Ehleringer JR, Passey BH, Podlesak DW, Torregrossa AM, West AG (2007) Determining biological tissue turnover using stable isotopes: the reaction progress variable. *Oecologia*, **151**, 175-189.

Craig H. (1954) Carbon 13 in plants and the relationships between carbon 13 and carbon 14 variations in nature. *The Journal of Geology,* **62**, 115-149.

Dalerum F, Angerbjörn A (2005) Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. *Oecologia,* **144**, 647-658.

Darr RL, Hewitt DG (2008) Stable isotope trophic shifts in white-tailed deer. *Journal of Wildlife Management*, **72**, 1525-1531.

De Niro MJ, Epstein S (1978) Influence of diet on distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta*, **42**, 495-506.

De Niro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta*, **45**, 341-351.

Fantle MS, Dittel AI, Schwalm SM, Epifanio CE, Fogel ML (1999). A food web analysis of the juvenile blue crab, *Callinectes sapidus*, using stable isotopes in whole animals and individual amino acids. *Oecologia,* **120**, 416-426.

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology,* **40**, 503-537.

Felicetti LA, Schwartz CC, Rye RO, Haroldson MA, Gunther KA, Phillips DL, Robbins CT (2003) Use of sulfur and nitrogen stable isotopes to determine the importance of whitebark pine nuts to Yellowstone grizzly bears. *Canadian Journal of Zoology*, **81**, 763-770.

Fisk AT, Sash K, Maerz J, Palmer W, Carroll JP, MacNeil MA (2009) Metabolic turnover rates of carbon and nitrogen stable isotopes in captive juvenile snakes. *Rapid Communications in Mass Spectrometry*, **23**, 319-326.

Fox J (1997) *Applied regression, linear models, and related methods*. SAGE Publications, Thousand Oaks.

Garcia F, Carrere P, Soussana JF, Baumont R (2003) The ability of sheep at different stocking rates to maintain the quality and quantity of their diet during the grazing season. *Journal of Agricultural Science,* **140**, 113-124.

Gaye-Siessegger J, Focken U, Abel HJ, Becker K (2004) Individual protein balance strongly influences $\delta^{15}N$ and $\delta^{13}C$ values in Nile tilapia, *Oreochromis niloticus. Naturwissenschaften,* **91**, 90-93.

Hilderbrand GV, Farley SD, Robbins CT, Hanley TA, Titus K, Serheen C. (1996) Use of stable isotopes to determine diets of living and extinct bears. *Canadian Journal of Zoology*, **74**, 2080–2088.

Hills M (1978) On ratios – a response to Atchley, Gaskins, and Anderson. *Systematic Zoology,* **27**, 61-62.

Hobson KA, Clark RG (1992) Assessing avian diets using stable isotopes. 2. Factors influencing diet-tissue fractionation. *Condor*, **94**, 189-197.

Hobson KA, McLellan BN, Woods JG (2000) Using stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes to infer trophic relationships among black and grizzly bears in the upper Columbia River basin, British Columbia. *Canadian Journal of Zoology*, **78**, 1332-1339.

Hwang YT, Millar JS, Longstaffe FJ (2007) Do $\delta^{15}N$ and $\delta^{13}C$ values of feces reflect the isotopic composition of diets in small mammals? *Canadian Journal of Zoology,* **85**, 388-396.

Ioannidis JPA (2005) Why most published research findings are false. *PLoS Medicine*, **2**, 696-701.

IUPAC (1997) Compendium of Chemical Terminology 2nd Edition (eds A.D.McNaught & A.Wilkinson) Blackwell Scientific Publications.

Kanaroglou PS (1996) On spurious correlation in geographical problems. *The Canadian Geographer*, **40**, 194-202.

Kenney BC (1982) Beware of spurious self-correlations! *Water Resources Research*, **18**, 1041-1048.

Männel TT, Auerswald K, Schnyder H (2007) Altitudinal gradients of grassland carbon and nitrogen isotope composition are recorded in the hair of grazers. *Global Ecology and Biogeography,* **16**, 583-592.

McCutchan JH, Lewis WM, Kendall C, McGrath CC (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, **102**, 378-390.

McKinney CR, McCrea JM, Epstein S, Allen HA, Urey HC (1950) Improvements in mass spectrometers for the measurement of small differences in isotope abundance ratios. *Review of Scientific Instruments*, **21**, 724-730.

Montagna W, Ellis RA (1958) *The biology of hair growth.* Academic Press, New York.

Mook WG (2001) *Environmental isotopes in the hydrological cycle, Principles and applications. Vol. I: Introduction – theory, methods.* IAEA, Vienna. www.iaea.org/programmes/ripc/ih/volumes/volume2.htm, 2001.

Mützel E, Lehn C, Peschel O, Hölzl S, Rossmann A (2009): Assignment of unknown persons to their geographical origin by determination of stable isotopes in hair samples. *International Journal of Legal Medicine*, **123,** 35-40.

O'Connell TC, Hedges REM (1999) Isotopic comparison of hair and bone: Archaeological analyses. *Journal of Archaeological Science*, **26**, 661-665.

O'Leary MH (1981) Carbon isotope fractionation in plants. *Phytochemistry*, **20**, 553-567.

Ojeda-Avila T, Woods HA, Raguso RA (2003) Effects of dietary variation on growth, composition, and maturation of *Manduca sexta* (Sphingidae: Lepidoptera). *Journal of Insect Physiology*, **49**, 293-306.

Park R, Epstein S (1960). Carbon isotope fractionation during photosynthesis. *Geochimica et Cosmochimica Acta*, **21**, 110-126.

Pearson K (1897) Mathematical contributions to the theory of evolution – On a form of spurious correlation which may arise when indices are used in the measurement of organs. *Proceedings of the Royal Society of London*, **60**, 489-455.

Pearson SF, Levey DJ, Greenberg CH, Del Rio CM (2003) Effects of elemental composition on the incorporationof dietary nitrogen and carbon isotopic signatures in an omnivorous songbird. *Oecologia*, **135**, 516-523.

Robbins CT, Felicetti LA, Sponheimer M (2005) The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds. *Oecologia*, **144**, 534-540.

Roth JD, Hobson KA (2000) Stable carbon and nitrogen isotopic fractionation between diet and tissue of captive red fox: implications for dietary reconstruction. *Canadian Journal of Zoology*, **78**, 848–852.

Sachs L, Hedderich J (2006) *Angewandte Statistik – Methodensammlung mit R.* 12 ed., Springer, Berlin, Germany.

Schnyder H, Schwertl M, Auerswald K, Schäufele R (2006) Hair of grazing cattle provides an integrated measure of the effects of site conditions and inter-annual weather variability on $\delta^{13}C$ of temperate humid grassland. *Global Change Biology*, **12**, 1315-1329.

Sponheimer M, Robinson T, Ayliffe LK *et al*. (2003a) An experimental study of carbon-isotope fractionation between diet, hair, and feces of mammalian herbivores. *Canadian Journal of Zoology,* **8**, 871-876.

Sponheimer M, Robinson T, Ayliffe LK *et al*. (2003b) Nitrogen isotopes in mammalian herbivores: hair $\delta^{15}N$ values from a controlled feeding study. *International Journal of Osteoarchaeology,* **12**, 80-87.

Sponheimer M, Robinson TF, Cerling TE, Tegland L, Roeder BL, Ayliffe LK, Dearing MD, Ehleringer JR (2006). Turnover of stable carbon isotopes in the muscle, liver, and breath CO2 of alpacas (Lama pacos). *Rapid Communications in Mass Spectrometry*, **20**, 1395-1399.

Telang A, Buck NA, Wheeler DE (2002) Response of storage protein levels to variation in dietary protein levels. *Journal of Insect Physiology*, **48**, 1021–1029.

Wolf N, Carleton SA, Del Rio CM (2009) Ten years of experimental animal isotope ecology. *Functional Ecology*, **23**, 17-26.

Wright MR (2004) An introduction to chemical kinetics. John Wiley & Sons, New York.

Zazzo A, Harrison SM, Bahar B, Moloney AP, Monahan FJ, Scrimgeour CM, Schmidt O (2007) Experimental determination of dietary carbon turnover in bovine hair and hoof. *Canadian Journal of Zoology*, **85,** 1239-1248.

Zazzo A, Moloney AP, Monahan FJ, Scrimgeour CM, Schmidt O (2008) Effect of age and food intake on dietary carbon turnover recorded in sheep wool. *Rapid Communications in Mass Spectrometry,* **22**, 2937-2945.

Candidates' contribution and publications

Chapter II

Wittmer *et al*. (2008) Biogeosciences

The candidate partially took the samples (2006 sampling campaign), aggregated all meteorological data, carried out the statistical and geostatistical analysis, wrote the first draft of the manuscript and composed all graphs. The candidate handled the reviewers' comments and wrote the reply.

Chapter III

Wittmer *et al*. (2010a) Basic and Applied Ecology

The candidate took samples (partially in 2007), carried out all statistical analysis, wrote the first draft of the manuscript and composed all graphs. The candidate handled the reviewers' comments and wrote the reply.

Chapter IV

Tentative author-list: Wittmer MHOM, Auerswald K, Schönbach P, Bai YF, Schnyder H

The candidate took samples (partially in 2007), carried out all statistical analysis, wrote the first draft of the manuscript and composed all graphs.

Chapter V

Auerswald *et al*. (2009) Biogeosciences

The candidate carried out the sampling campaign in 2007, aggregated the meteorological data, and performed the statistical and geostatistical analysis. The candidate participated in the writing of the manuscript and composed all graphs.

Chapter VI

Wittmer *et al*. (2010b) Global Change Biology

The candidate carried out the sampling campaign in 2007, aggregated all meteorological data, performed the statistical and geostatistical analyses, wrote the first draft of the manuscript and composed all graphs. The candidate handled the reviewers' comments and wrote the reply.

Chapter VII

Tentative author-list: Wittmer MHOM, Auerswald K, Bai YF, Schnyder H

The candidate carried out the sampling campaign in 2007, aggregated all meteorological and agricultural data, performed the statistical and geostatistical analyses, wrote the first draft of the manuscript and composed all graphs.

Chapter VIII

Tentative author-list: Wittmer MHOM, Auerswald K, Schnyder H

The candidate carried out all statistical analysis, wrote the first draft of the manuscript and composed all graphs.

Annex

Auerswald *et al*. (2010) Journal of Applied Ecology

The candidate initiated the study, performed the literature search, the statistical analysis and contributed to the first draft and the revision of the manuscript.