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**Spatio-temporal Variation of Carbon and Nitrogen Stable Isotope Composition along
Environmental Gradients in Inner Mongolia and Alpine Grasslands - Analyses of
Vegetation, Grazer Hair, Feces and Soil**

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ABSTRACT

Aims: The subject of the present study was the spatial and temporal variation of carbon and nitrogen stable isotope composition in Inner Mongolia and Alpine grasslands. The first aim was to assess the effect of altitude on the carbon and nitrogen isotope composition of ruminant grazer's hair and its relation to grassland vegetation. A further intent consisted in the recordation of the variation in $\delta^{13}\text{C}$ of the grassland of Inner Mongolia along environmental gradients and its relation on C_4 abundance and ^{13}C discrimination of C_3 plants. Lastly the temporal variation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the steppe of Inner Mongolia were determined in relation to changes in atmospheric CO_2 , climate and human impact.

Materials & Methods: Grassland vegetation, soil, feces and hair of ruminants were sampled along environmental gradients to analyze their isotope composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). In Inner Mongolia the percent contribution of C_4 plants to carbon pools was estimated from ^{13}C , considering effects of aridity on C isotope discrimination of C_3 plants. To reconstruct the temporal variation in C_4 abundance samples of datable woolen textiles were taken.

Results & Discussion: In Alpine grasslands $\delta^{13}\text{C}$ of hair and vegetation increased, while $\delta^{15}\text{N}$ decreased with altitude. The effect of altitude on hair ^{15}N is important for trophic relationships studies. The similarity of altitude effects on $\delta^{13}\text{C}$ of individual plant species, vegetation and hair indicates that the effect of altitude on species-level 'intrinsic water use efficiency' scales up linearly to the community and landscape level. Offsets between hair and vegetation ^{13}C or ^{15}N ('diet-hair shift') were altitude-independent. In Inner Mongolia $\delta^{13}\text{C}$ showed that percent C_4 in aboveground biomass varied between 0 and 100%, with about half of the variation occurring at the farm scale, and half at higher spatial scales. C_4 abundance was highest in the most arid zone and in the vicinity of towns. This 'town-effect' was related to decreased soil N concentration, but was not evident in ^{13}C of soil organic carbon (SOC), indicating that it developed in the last century. $\delta^{13}\text{C}$ of old woolen textiles assessed that average C_4 abundance increased within two steps from 2% (1928-57) to 9% (1958-97) and 25% (1998-2005). Predicted decreases in C_4 abundance due to rising CO_2 concentrations seems to be suppressed by a combination of rising regional temperature, increased human impact and short-term weather events, all favoring the spread of C_4 plants.

Conclusions: The present study demonstrates that grazer hair provides faithful spatially and temporally integrated records of grassland isotope composition, which are useful for ecosystem and environment reconstruction. First it could be shown that altitude affects $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of grassland in the same way at different scales of integration, while effects of altitude and related environmental variables on the diet-hair shift for ^{13}C and ^{15}N in ruminants are lacking. The second part of the study showed an expansion of C_4 plants in Inner Mongolia that may have resulted from overgrazing, disturbance and erosion, and regional warming. Variations in the C_4 abundance of the ecosystem in the Inner Mongolian steppe could be clearly related to processes at the regional scale, suppressing the effects of processes on a global scale.

ZUSAMMENFASSUNG

Zielsetzung: Die vorliegende Arbeit befasst sich mit der räumlichen und zeitlichen Veränderung der isotopischen Zusammensetzung von Kohlenstoff und Stickstoff in den Steppen der Inneren Mongolei und im Alpinen Grünland. Zunächst sollte der Einfluss der Höhenlage auf die isotopische Zusammensetzung von Kohlenstoff und Stickstoff in den Haaren von Wiederkäuern im Vergleich zur Vegetation des Weidelandes aufgezeigt werden. Ein weiteres Ziel bestand in der Erfassung der Variation der ^{13}C Signaturen in den Steppen der Inneren Mongolei entlang von Umweltgradienten im Zusammenhang mit der Abundanz der C_4 Pflanzen und der ^{13}C Diskriminierung von C_3 Pflanzen. Zuletzt wurden die zeitlichen Variationen der ^{13}C und ^{15}N Signaturen in der Inner Mongolischen Steppe im Zusammenhang mit Veränderungen im CO_2 Gehalt der Atmosphäre, des Klimas und des menschlichen Einflusses ermittelt.

Material und Methoden: Im Grünland wurde die Vegetation, der Boden sowie Kot und Haare von Wiederkäuern beprobt und die isotopische Zusammensetzung ($\delta^{13}\text{C}$ und $\delta^{15}\text{N}$) analysiert. In der Inneren Mongolei wurde der prozentuale Beitrag von C_4 Pflanzen zu Kohlenstoffpools anhand der ^{13}C Signaturen unter Berücksichtigung der ariditätsbedingten Einflüsse auf die isotopische Kohlenstoffdiskriminierung von C_3 Pflanzen bestimmt. Um die zeitliche Variation der Abundanz der C_4 Pflanzen zu rekonstruieren wurden datierbare Wolltextilien beprobt.

Ergebnisse und Diskussion: Im alpinen Grünland nahm $\delta^{13}\text{C}$ mit der Höhe zu, während $\delta^{15}\text{N}$ abnahm. Der Einfluss der Höhenlage auf die ^{15}N Signatur des Haares ist gerade für Trophiestudien bedeutsam. Der Höheneinfluss auf $\delta^{13}\text{C}$ ist sowohl für einzelne Pflanzenarten, auf Bestandesebene und für Haare von Weidetieren nahezu identisch, was darauf hinweist dass sich der Einfluss der Höhe auf die spezifische Wassernutzungseffizienz auf Artenebene linear auf die Pflanzengesellschafts- und Landschaftsebene überträgt. Verschiebungen zwischen den ^{13}C oder ^{15}N Signaturen von Haar und Vegetation ('Diet-Hair Shift') zeigten sich als höhenunabhängig. In der Inneren Mongolei zeigten die ^{13}C Signaturen, dass der prozentuale C_4 Anteil in der oberirdischen Biomasse zwischen 0 und 100% variiert, wobei die Hälfte der Variation auf Betriebsebene und die Hälfte in größeren räumlichen Skalen auftrat. Die Abundanz der C_4 Pflanzen war im aridesten Bereich und in der Nähe von Städten am höchsten. Dieser 'Town-Effect' stand im Zusammenhang mit verringerten Bodenstickstoffkonzentrationen, konnte durch die ^{13}C Signaturen des organischen Bodenkohlenstoffs (SOC) jedoch nicht bestätigt werden, was auf eine Entstehung im letzten Jahrhundert hindeutet. Die ^{13}C Signaturen alter Wolltextilien zeigten dass die mittlere Abundanz der C_4 Pflanzen in zwei Abschnitten von 2% (1928-57) auf 9% (1958-97) und 25% (1998-2005) anstieg. Vorhergesagte Abnahmen der C_4 Abundanz aufgrund steigender CO_2 Konzentrationen scheinen durch eine Kombination von Effekten die die Ausbreitung von C_4 Pflanzen begünstigen, wie steigenden Temperaturen innerhalb der Region, verstärkter menschlicher Einflussnahme und Witterungsereignissen, unterdrückt worden zu sein.

Schlussfolgerungen: Die vorliegende Arbeit zeigt, dass Haare von nichtselektierenden Weidegängern zuverlässige räumlich und zeitlich integrierende Aufzeichnungen der isotopischen Zusammensetzung des Grünlandes liefern, die zur Rekonstruktion von Ökosystem und Umwelt genutzt werden können. Zunächst konnte gezeigt werden, dass die Höhenlage die ^{13}C und ^{15}N Signaturen des Grünlandes auf unterschiedlichen Skalen der Integration in der selben Weise beeinflusst, wobei keinerlei Einflüsse der Höhenlage und damit verbundener Umweltvariablen auf den ‘Diet-Hair Shift’ der ^{13}C und ^{15}N Signaturen in Wiederkäuern auftraten. Der zweite Teil dieser Arbeit konnte eine Ausbreitung der C_4 Pflanzen in der Inneren Mongolei nachweisen, die auf Überweidung, Devastierung und Erosion sowie regionale Erwärmung zurückgeführt werden kann. Variationen im Vorkommen von C_4 Pflanzen im Ökosystem der Inner Mongolischen Steppe konnten eindeutig auf regional wirksame Prozesse, die den Einfluss globaler Prozesse unterdrücken, zurückgeführt werden.

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Chapter I. GENERAL INTRODUCTION

BIOGEOCHEMISTRY OF GRASSLAND REGIONS

Vegetation dynamics and cycles of carbon and nitrogen in temperate grasslands vary at different spatial and temporal scales. These variations are controlled by environmental and human impact. Therefore nutrient fluxes and species composition in temperate grasslands are the result of a multitude of influence factors and the history of their changes. Some of these changes have a global extension while others are restricted to regional or even local scale.

Carbon and nitrogen stable isotope signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) may add to the biogeochemical reconstruction of nutrient fluxes and vegetation dynamics, because they provide information on the nature and activity of participating processes (Dawson *et al.*, 2002). Stable isotopes offer the potential for quantitative statements on the movement of elements and organisms across landscapes and between components of the biosphere, and to reconstruct aspects of dietary, ecological and environmental history (West *et al.*, 2006). Hence their use is particularly suitable for ecosystem studies.

Studies in grassland ecosystems benefit from the use of mammalian herbivores tissues (such as bones, teeth, horn, hoof and hair) as they record and integrate the isotopic information of the grazed vegetation (Cerling & Harris, 1999). Recently hair has received particular interest as an object of isotope ecology, because it is formed continuously, is metabolically inactive after formation and thus has the potential to provide temporally resolved records (Jones *et al.*, 1981; Schwertl *et al.*, 2003a; Ayliffe *et al.*, 2004). Hence the use of grazer products, especially hair, can be used to retrieve isotopic information associated with spatial and temporal changes in grassland. Hair holds the potential to provide temporally resolved records of animal migration and dietary patterns (Cerling *et al.*, 2006), or of the effects of environmental changes/fluctuations on their habitat (Iacumin *et al.*, 2006; Schnyder *et al.*, 2006). However, to be useful as a recorder of vegetation isotope composition, there must be a known and sufficiently tight relationship between the isotopic composition of animal tissue and (grazed) vegetation.

SPATIO-TEMPORAL INTEGRATION OF CARBON AND NITROGEN POOLS

A difficulty in field studies of the isotopic 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. Also, at the level of single plants, the spatial integration of soil conditions is small, as it is limited by the expansion of the root system. 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, whereas both measures account for very high additional expenditure. An alternative to this is to take advantage of the ‘sampling’ activity of grazing livestock. Isotopic signals recorded in animal tissues provide a spatially integrated signal, where the scale of spatial integration is a function of the size of the species’ habitat/range. Such an integration may also benefit vegetation (palaeo-)ecological or biogeochemical studies at higher spatial (e.g. landscape or region) scales. Provided that animals do not select between photosynthetic types, and eventual post-ingestion fractionation of C and N isotopes is known, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of vegetation can be inferred from feces, wool or other animal tissue e.g. (Schnyder *et al.*, 2006). Feces of grazing animals integrate growing conditions of several days over the area grazed over the respective period, while grazer wool records the signature of grazed vegetation of the entire grazing ground (several ha-km²; depending on pasture productivity and management). Bulk samples of wool give an integrative signal over one up to several vegetation periods, depending on the availability of the respective samples. A higher temporal resolution can be achieved by analyzing single wool segments that can be assigned to certain periods (see below). Soil organic matter provides another archive which integrates vegetation isotopic signals over periods of decades to centuries (Boutton *et al.*, 1998; Ehleringer *et al.*, 2000).

USE OF HAIR AS ISOTOPIC RECORDER

Hair is a characteristic trait of mammalian skin with similar properties for the different species (Leblond, 1951). For a certain time, a single hair grows more or less continuously from the base of the hair follicle (Saitoh *et al.*, 1967). Mammalian hair predominantly consists of keratin, a sulphur-rich protein structure. During growth, keratin is formed in the basal few millimeters underneath the skin

(Leblond, 1951). Once the keratin structure is established, hair tissue is metabolically inactive (Montagna & van Scott, 1958). Every section of the hair shaft contains isotopic information from the time when it was produced. Thus, dynamic short-term dietary activities of mammals become permanently and sequentially recorded in hair-keratin. Hence, the use of hair in isotope ecology has several advantages as it remains stable after building (Jarrett, 1973), can be obtained non-invasively and with minimal disturbance from a living subject (Schwertl *et al.*, 2003a) and reflects the isotopic signature of the diet at a particular time (O'Connell & Hedges, 1999). Diet and animal movements at daily to weekly timescales become recorded in mammalian hair segments (Jones *et al.*, 1981; Hobson *et al.*, 1996; Ayliffe *et al.*, 2004). Diet changes recorded in hair can be used in addition as a proxy for environmental parameters, when feeding habits are related to climate parameters (Cerling *et al.*, 2004). Moreover, preserved hair, e.g. of human mummies, can be used for archeological diet reconstruction (White *et al.*, 1999). Iacumin *et al.* (2005) could even interpret the seasonal isotopic history of a mammoth, which was living about 42000 years BP, by the signature of its well-preserved hair.

Recently, hair has attracted particular interest because of its potential to resolve isotopic signals in time (Schwertl *et al.*, 2003a; Ayliffe *et al.*, 2004). However, to be useful, there must be a known systematic relationship between the isotopic composition of hair and grazed vegetation. Indeed, the C and N isotope composition of hair follows that of the diet (Jones *et al.*, 1981; Schwertl *et al.*, 2003a), albeit in a dampened fashion, due to the buffering by the digestive tract and body metabolic pools which exchange C and N with the substrate pools feeding hair growth (Hawkins, 1991; Ayliffe *et al.*, 2004). Also, hair is somewhat enriched with ^{13}C and ^{15}N relative to diet (diet-hair shift) (Sponheimer *et al.*, 2003a, 2003b). Notably, there is some variability in this shift which may have a biological basis. Thus, the diet-hair shift for ^{15}N in several mammalian herbivores (including cattle, horses, alpaca and goats) was up to 3‰ higher when animals were fed a diet with a high protein content (Sponheimer *et al.*, 2003b). Further, Cerling and Harris (1999) observed a variation of between 1.7 and 4.2‰ for the diet-keratin ^{13}C shift among a range of free-ranging and captive large ruminant herbivores. It is currently unknown if this variation has a (systematic) biological basis, or if it is entirely due to other factors such as experimental error or isotopic disequilibria between diet and hair.

ISOTOPIC SIGNATURE OF END-MEMBERS IN MIXING MODELS

In mixed diets several components contribute to the overall signal in the animal tissue. Feed components, which can be identified due to their isotopically wide separation, are products from C₃ and C₄ plants (Jones *et al.*, 1981). To conclude from an animal tissue on the components, the end-members, the signals in the animal tissue, which are produced by certain food components have to be known.

This approach faces major difficulties. One is the assumption that the trophic shift in the animal under focus is identical to the trophic shift obtained from end-members. This is not necessarily the case, because diet related variations may occur (van der Klift & Ponsard, 2003). Especially uniform diets used to determine end-members are not comparable to mixed feeding in all aspects, because they are often associated with differences in major and minor components like starch, fiber, protein or tannins, differences in digestibility or palatability and differences in physical properties. Furthermore, the keeping conditions in controlled studies may cause a different trophic shift than under free ranging conditions, for which the end-members are applied. It would hence be necessary to measure not only the end-members but different animals fed on a variety of diets under the same keeping conditions as the animal in focus. This can not be solved in controlled feeding experiments.

The second major limitation of the end-member approach is the assumption that the isotopic signature of the feed leading to the end-members is constant. The uptake of carbon by plants is combined with a discrimination against ¹³C, which depends on different basic conditions. However the isotopic signatures of C₃ and C₄ plants are not constant but depend to some degree on the conditions of growth. For C₃ plants the discrimination against ¹³C by the carboxylating enzyme Rubisco depends on photosynthesis *via* the ratio of intercellular to atmospheric CO₂ concentrations (c_i/c_a) (Farquhar *et al.*, 1982). This ratio reflects the relative magnitudes of net assimilation (A) and stomatal conductance (g) that relate to the CO₂ budget of a plant. Hence $\delta^{13}\text{C}$ is a useful indicator for the water use efficiency (WUE) of a plant, the ratio of carbon gain to water losses *via* stomatal conductance (Farquhar *et al.*, 1989). Thus, the variations in the discrimination of C₃ plants could be related to environmental parameters like soil moisture (Ehleringer & Cooper, 1988), humidity (Madhavan *et al.*, 1991), irradiance (Ehleringer *et al.*, 1986), temperature (Welker *et al.*, 1993), nitrogen availability (Condon *et*

al., 1992), salinity (Poss *et al.*, 2000) and atmospheric CO₂ concentration (Ehleringer & Cerling, 1995). Hence, spatial gradients in $\delta^{13}\text{C}$ of C₃ plants are reported across landscapes (Williams & Ehleringer, 1996) and with altitude and latitude (Körner *et al.*, 1991). In contrast to C₃ plants, variations in c_i/c_a of C₄ plants are relatively small (Farquhar, 1983).

These variations of the C₃ end-member contribute to the overall error in the estimation of the feed composition unless the end-members have been determined for a variety of different conditions occurring within a study area. Knowledge of these effects is hence a prerequisite for a more accurate use of mixing models. Given that such effects can be quantified and generalized within a pure C₃ environment like Alpine grassland and that it is known under which environmental conditions a certain animal tissue was produced, it may be possible to correct the $\delta^{13}\text{C}$ of the C₃ end-member in mixed C₃/C₄ environments for these effects. Thus, the estimation of the C₃/C₄ ratio from $\delta^{13}\text{C}$ in the hair of animals feeding in arid temperate grasslands like the Inner Mongolian steppe, where both photosynthetic types are common, could be improved by the use of a variable $\delta^{13}\text{C}$ for the C₃ end-member of the mixing model that co-varies with the spatial variation of its steering environmental variables. This is of importance as the Inner Mongolian steppe is part of one of the largest grassland biomes in the world, but little is known about its C₃/C₄ ratio and its spatial and temporal variation. In particular the C₃/C₄ ratio represents, due to its impacts on patterns of productivity, seasonality, digestibility or biogeochemistry, an important parameter of grassland ecology that needs to be recorded in consideration of its spatial and temporal patterns of variation.

AIMS

The following research was based on the hypothesis that hair of grazers provides an isotopic record of environmental and nutritional signals. The first aim of this study was to assess the effect of altitude on the carbon and nitrogen isotope composition of ruminant grazer's hair and its relation to grassland vegetation. This allowed to evaluate the use of hair isotope data for ecosystem reconstruction, animal nutritional ecology and biogeochemical studies in montane environments. It was of particular interest to test if the diet-hair shift for ^{13}C and ^{15}N is affected by altitude in domestic ruminant herbivores. Further the effect of altitude on community level $\delta^{13}\text{C}$ was assessed and compared with the known

plant species-level response. Moreover the effect of altitude on $\delta^{15}\text{N}$ of hair was determined and compared with local grassland vegetation and that in global data set (Chapter II.).

In a second step the $\delta^{13}\text{C}$ of the grassland of Inner Mongolia and its variation along gradients of temperature, precipitation and soil fertility was assessed and related on C_4 abundance and ^{13}C discrimination of C_3 plants. In particular, it was investigated how these relationships are reflected in carbon pools with distinct spatio-temporal integration (SOC, aboveground plant biomass, feces and wool) (Chapter III.).

Lastly the temporal variation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the steppe of Inner Mongolia were determined in relation to changes in atmospheric CO_2 , climate and human impact. Two explicit hypotheses were tested: (i) that changes in the carbon and nitrogen dynamics in the steppe of Inner Mongolia can be detected by isotopic analysis of woolen textiles, and (ii) these changes can be related to global changes in atmospheric CO_2 , or regional changes in temperature and land use in combination with drastic weather events (Chapter IV.).

Chapter II. ALTITUDE GRADIENTS OF GRASSLAND CARBON AND NITROGEN ISOTOPE COMPOSITION ARE RECORDED IN THE HAIR OF GRAZERS

ABSTRACT

Aim The hair of grazers provides an isotopic record of environmental and nutritional signals. Here, we assess the effect of altitude on the carbon and nitrogen isotope composition of ruminant grazer's hair and its relation to grassland vegetation, to evaluate the use of hair isotope data for ecosystem reconstruction, animal nutritional ecology and biogeochemical studies in montane environments.

Location European Alps.

Methods We sampled grassland vegetation (pure C_3) and the hair of ruminants along an altitude gradient (400-2500 m), and analyzed their isotope composition ($\delta^{13}C$ and $\delta^{15}N$). Results were compared with published effects of altitude on ^{13}C in C_3 plants at the species-level and on ^{15}N at the community-level. The study was complemented with a comparison of diet and hair isotope composition in ruminants held in confinement.

Results $\delta^{13}C$ of hair increased ($\sim 1.1\text{‰ km}^{-1}$) and $\delta^{15}N$ decreased ($\sim 1.1\text{‰ km}^{-1}$) with altitude. The same changes occurred in local grassland vegetation, and in regional to global grassland data sets. Offsets between hair and vegetation ^{13}C or ^{15}N ('diet-hair shift') were altitude-independent. Sheep (*Ovis aries*) and cattle (*Bos taurus*) exhibited a ^{13}C shift near +3‰, but that of goats (*Capra hircus*) was larger (+4.2‰) in alpine and confinement environments. The diet-hair shift for ^{15}N was more variable (+2.1 to +3.6‰).

Main conclusions Grazer hair provides faithful spatially and temporally integrated records of grassland isotope composition, which are useful for ecosystem and environment reconstruction. The effect of altitude on hair ^{15}N is important for trophic relationships studies: an altitude shift of 2000 m produced the same effect in hair- ^{15}N as would a shift from an animal tissue- to a plant-based diet. The similarity of altitude effects on $\delta^{13}C$ of individual plant species, vegetation and hair indicates that the effect of altitude on species-level 'intrinsic water use efficiency' scales up linearly to the community and landscape level.

INTRODUCTION

Altitude has profound effects on climatic and edaphic conditions and thus on primary production (Luo *et al.*, 2004) and degradation (Drewnik, 2006) processes, which imprint on the carbon and nitrogen isotope composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of plants and soils (Raich *et al.*, 1997; Austin & Vitousek, 1998). In C_3 plants, measurements at the species-level have shown that $\delta^{13}\text{C}$ increases with altitude (Körner *et al.*, 1988, 1991; Friend *et al.*, 1989; Morecroft & Woodward, 1990), meaning that the ratio of carbon gained to water lost in leaf gas exchange (intrinsic water use efficiency) increases with altitude (Farquhar *et al.*, 1989; Zhang *et al.*, 1993; Hultine & Marshall, 2000). In contrast, $\delta^{15}\text{N}$ in soil and plants decreases with altitude (Mariotti *et al.*, 1980; Schuur & Matson, 2001; Amundson *et al.*, 2003; Craine & Lee, 2003), suggesting decreasing volatile losses of N from ecosystems at higher altitudes (Högberg, 1997; Handley *et al.*, 1999; Tilsner *et al.*, 2003a, 2003b), which may be related to lower temperatures, lower pH and increasing precipitation (Handley *et al.*, 1999; Amundson *et al.*, 2003).

These isotope signals of vegetation should pass forward in the food chain and be recorded in hair, such as that of mammalian ruminant grazers. However, the relationships between altitudinal changes in carbon and nitrogen isotope composition of vegetation and that of herbivores has not been studied, although it is important for the interpretation of animal isotope data in biogeochemical reconstructions of alpine environments, or studies of the nutritional ecology and biogeography of animals. There are several uncertainties concerning the effect of altitude on hair carbon and nitrogen isotope signature and vegetation or diet. Most importantly, it is not known if the diet-hair isotopic shifts for ^{13}C and ^{15}N is constant with altitude, or if it is in itself affected by altitude. In general, hair is somewhat enriched in ^{13}C and ^{15}N relative to diet (De Niro & Epstein, 1978, 1981; Jones *et al.*, 1981; Nakamura *et al.*, 1982; Tieszen *et al.*, 1983; Cerling *et al.*, 1999; Schwertl *et al.*, 2003a; Sponheimer *et al.*, 2003a, 2003b), but there is significant variability in this (Iacumin *et al.*, 2006). It is currently unknown if this variation is related to diet quality or animal metabolism. One way of studying this question is to analyze the diet-hair shift along environmental, e.g. altitudinal, gradients, which might affect the shift *via* effects on both diet quality or animal maintenance metabolism.

Another uncertainty relates to the relationship between the effect of altitude on the $\delta^{13}\text{C}$ at the plant species-level (Körner *et al.*, 1988) and that on plant community-level, which has not been

studied. Since species differ in $\delta^{13}\text{C}$ (Körner *et al.*, 1988, 1991; Tsialtas *et al.*, 2001) and community composition changes with altitude (Ellenberg, 1978), it is unclear if the species-level effect translates linearly to the community and herbivore.

The present study sheds light on these questions. In particular: (i) it tests if the diet-hair shift for ^{13}C and ^{15}N is affected by altitude in domestic ruminant herbivores; (ii) it analyzes the effect of altitude on $\delta^{13}\text{C}$ of ruminant grazer hair and compares this with the known plant species-level response; and (iii) it determines the effect of altitude on $\delta^{15}\text{N}$ of hair and compares it with local grassland vegetation and that in a global data set. The study included sampling fresh herbage, hay and the hair of sheep (*Ovis aries*), goats (*Capra hircus*) and cattle (*Bos taurus*) along the entire altitudinal range of grassland in two per-humid regions in the Alps: the Bregenzerwald, in the Austrian federal state of Vorarlberg, and the Swiss canton of Grisons. Lastly, since the diet-hair ^{13}C and ^{15}N shifts in sheep have not been assessed before and the results of the regional study may be influenced by the occurrence of different breeds, we also analysed data from a confinement study with several breeds of sheep and goats.

MATERIALS AND METHODS

Field work

We chose a study area with a sufficiently large altitude range in which C_4 plants were absent from vegetation, and in which drought did not modify the ^{13}C response of grassland vegetation to altitude. These conditions are best met in the northern part of the European Alps, where the climate is humid to perhumid and large differences in altitude can be found. Breeding of sheep and goats is common in this area, and allowed sampling altitudes between 400 m and 2 500 m above sea level (a.s.l.). Hair was collected in the summer of 2003 in two neighbouring regions: the Bregenzerwald in the Austrian federal state of Vorarlberg and the Rhaetian Alps in the Swiss canton of Grisons (Figure II.1). In Grisons, a few samples were already collected in 2002.

In the Bregenzerwald, we collected the hair of 49 sheep and 32 goats from 31 farms. Most animals grazed high altitude pastures during the summer months (May to September), while low-altitude pastures were used for spring and autumn feeding and forage conservation (exclusively in the

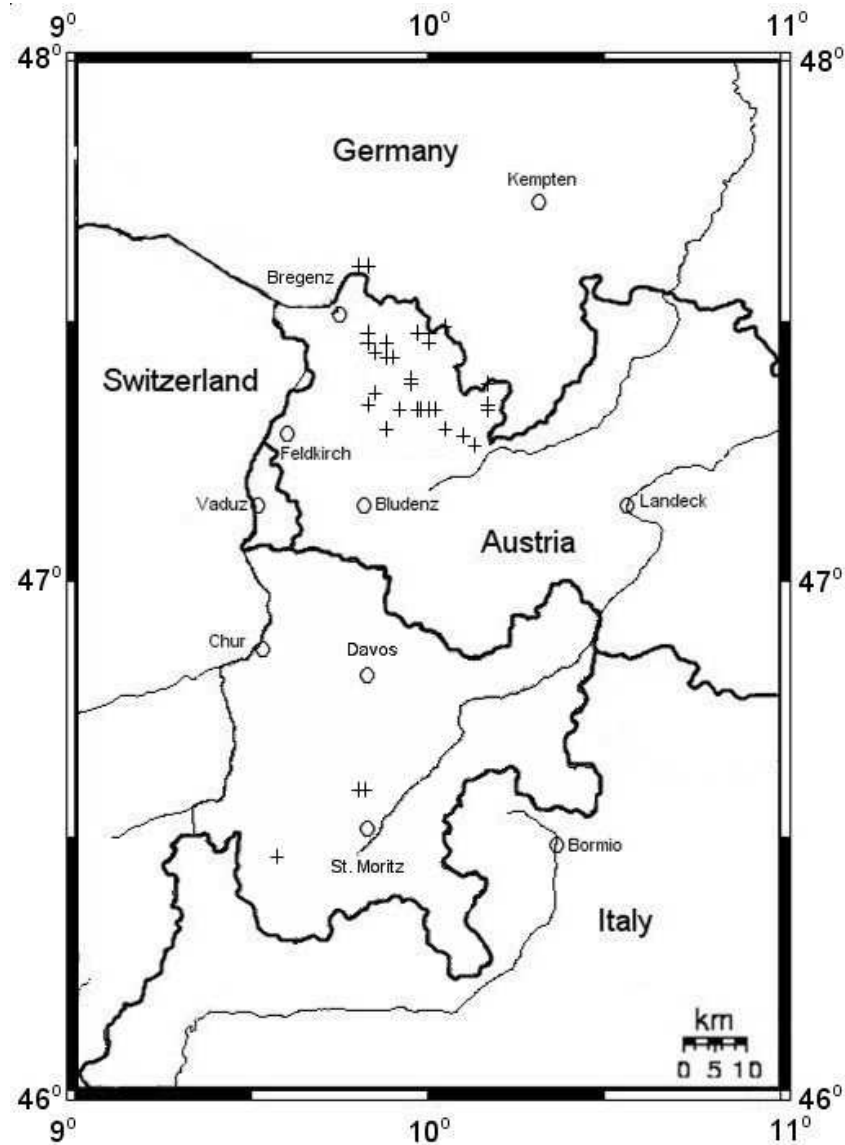


Figure II.1. Map of the study area in the European Alps. Samples derive from the Bregenzerwald in the Austrian federal state of Vorarlberg between 9°40' to 10°10' longitude and 47°30' to 47°45' latitude and from the Rhaetian Alps in the Swiss canton of Grisons between 9°30' to 9°50' longitude and 46°10' to 46°40' latitude. Crosses denote sampled sites.

form of hay) for the winter period. Sampled altitudes ranged from 500 to 1550 m above sea level (a.s.l.). Altitudinal ranges within individual pastures averaged 100 m, with a maximum of 300 m. For each pasture, a mean altitude was estimated. Animals did not receive any supplements while grazing pastures. In some cases the winter feed included a small amount (< 10% of the ration) of concentrate (58% wheat components, 10% barley, 15% other C₃ components, 15% maize flour, 2% minerals) in addition to hay, however, because of the small quantity, concentrate feeding had no measurable effect on the isotope signatures of hair grown in winter relative to that produced in summer (data not shown, but see below).

In Grisons, we sampled 12 sheep of different breeds in spring and autumn. We chose these animals for a repeated sampling because they were exposed to the largest difference in altitude between summer and winter. In summer, they grazed high-altitude pastures (2 500 m a.s.l.), while in winter, the animals were kept at about 400 m a.s.l. In addition, we sampled the tail switch hair of 12 cattle just fed on grass and hay, to verify if the altitude effect on hair isotope signatures was the same for cattle as it was for sheep and goats. The cattle hair samples were collected at two sites in the Bregenzerwald and three sites in Grisons, including Juf, the highest permanent settlement in Europe at 2100 m a.s.l.

Finally, we sampled 32 sheep and 3 goats held in confinement at a station of the Bavarian State Research Center for Agriculture in Grub, a few kilometres north of Munich (400 m a.s.l.). The sheep, which were all kept under the same conditions, included animals from four breeds: Merino (Merinolandschaf), White Mountain (Weißes Bergschaf), German Blackheaded Mutton (Schwarzkopf) and Suffolk. All animals received the same feed consisting of hay, grass silage and “Lämmerkorn” pellets (38% small grain, 22% sugar beet residues, 21% soy bean extract, 10% maize flour) during half a year prior to sampling. All components were simultaneously supplied in the feeding trough, which allowed for some selection.

Bulk samples of vegetation were taken on all sheep and goat pastures. This was done by hand-plucking, simulating the foraging action of a grazer (De Vries & Daleboudt, 1994). Hay samples from different harvests were obtained from each farm. Hair was collected by shaving a small piece of wool directly above the skin on the back of each sheep. The goat and cattle hair were plucked from the tail. Tail hairs are thicker than the regular guard hair and thus provided more mass per unit length for the isotope measurements.

Sample preparation

The vegetation samples were dried for one hour in a forced air oven at 95°C to eliminate biological activity and thereafter for 48 hours at 60°C. Dried samples were ball milled.

The wool and tail hair samples were treated using the procedures detailed by Schwertl *et al.* (2003a). Briefly, the hair was soaked in distilled water overnight to loosen dirt particles, cleaned in an

ultrasonic bath for 30 minutes, placed in a methanol/chloroform solution (66/33, v/v), and then placed on a shaker for two hours to remove the adipose. Thereafter samples were dried for 48 hours at 40°C. Two hairs of each goat sample were cut into 1 cm-long segments and the segments were placed in tin cups (5 x 9 mm). Each segment was analysed separately. In the case of the sheep wool, two replicates of narrow staples (locks of wool) were taken from each wool sample and also cut into segments of equal length. About 0.2-0.5 mg of each segment were then packed into tin cups.

Period-site assignment

It is evident that the tip part of each staple of wool was grown shortly after the time of last shearing, whereas the basal part was formed shortly before sampling. Accordingly, each hair segment along the staple could be assigned to the period in which it was grown, in assuming that hair growth rate was approximately constant (Schwertl *et al.*, 2003a; Schnyder *et al.*, 2006). This does not mean, however, that the substrates feeding hair growth are all derived directly from the diet that is ingested simultaneously with the formation of a piece of hair. The digestive tract and body metabolic pools have a buffering effect, so that the isotopic composition of hair only reflects the isotopic composition of a new diet when these pools have isotopically equilibrated with the new diet (Ayliffe *et al.*, 2004). Ayliffe *et al.* (2004) identified one of three pools, which contributed to hair growth of horses with a half-life time > 130 days. In studies with ruminants, equilibration time was much shorter. Jones *et al.* (1981) found that 74 days were required for complete equilibration of new hair growth with a new diet following a shift from a C₃ to a C₄ diet and *vice versa*. In another study with cattle, 85% of full equilibration was achieved in about 50 days following a shift from pasture to a hay- and silage-based diet (Schnyder *et al.*, 2006). In order to ascertain a (near-)complete equilibration we allowed for a minimum of 84 days of hair growth after a shift to a new pasture or to the winter stable. Hair grown during the intermediate equilibration period was eliminated from the analysis. Incomplete turnover of body pools would lessen the slope of hair isotope composition versus altitude. We found no evidence for such an effect.

Isotopic measurements

The samples were combusted in an elemental analyser (NA 1108; Carlo Erba, Milan) interfaced (ConFlo III; Finnigan MAT, Bremen) to an isotope ratio mass spectrometer (Delta Plus; Finnigan MAT). Carbon and nitrogen isotopic data are presented as $\delta^{13}\text{C}$ (‰) relative to the Vienna Pee Dee Belemnite standard and $\delta^{15}\text{N}$ (‰) relative to the air nitrogen standard:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000 \quad (\text{Eq. II.1})$$

where R is the ratio of $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ and X is the corresponding element (C or N).

All samples were measured against laboratory working standard gases, which were previously calibrated against IAEA secondary standards (IAEA NO3 for ^{15}N , accuracy of calibration $\pm 0.19\text{‰}$ SD, and IAEA CH6 for ^{13}C , accuracy of calibration $\pm 0.06\text{‰}$ SD). Solid internal laboratory standards (SILS), with a similar C/N ratio as the samples, were calibrated against these references. Hair was measured against protein powder (C/N: 3.3) and vegetation against wheat flour (C/N: 21.6). One SILS was measured after every tenth sample. The precision for sample repeats was better than 0.2‰ for $\delta^{13}\text{C}$ and better than 0.3‰ for $\delta^{15}\text{N}$.

Data analysis

For vegetation, a linear change of isotope signature with altitude leads to a linear model:

$$\delta_x = a + (b \times h), \quad (\text{Eq. II.2})$$

where a is the isotope signature at sea level (‰), b is the change of the isotope signature with altitude (‰ km⁻¹), and h is the height above sea level (km).

If there is no altitude effect on the diet-hair shift, the same model applies to animal tissues. The data of vegetation and animals grouped to regions, species or breeds can then be combined in a model containing dummy variables ($D1, D2, \dots$), which are 1 for samples of a specific group and zero for all other samples (Fox, 1997):

$$\delta_x = a + (b \times h) + (c \times D1) + (d \times D2) + \dots \quad (\text{Eq. II.3})$$

If $D1$ denotes sheep and $D2$ goats, the diet-hair shift for sheep and goats is given by c and d . In this way, the diet-hair shift can be estimated from data sets in which the vegetation and animal data are not necessarily paired for each altitude.

Where the overall slope b differs between groups this becomes evident as a statistically significant deviation between model (i.e. regression) estimates and measurements in the lower and upper half of the altitude range. This bias is to one side of the regression in the upper half and to the other side in the lower half of the altitude range. To examine this effect, the average deviations between model predictions and measurements ($\delta X_{pred.} - \delta X_{meas.}$) for the upper and lower half of the altitude range were computed for each group and compared statistically.

Selection among components of feed offered on pasture and in the stable could not be excluded. This could potentially cause a systematic deviation between the mean isotopic composition of all feed on offer and the feed actually ingested by animals. Such a mechanism would affect the estimates of c and d . Since such effects could not be excluded with certainty, we termed c and d ‘apparent’ (rather than ‘true’) diet-hair shifts.

RESULTS

Field survey at different altitudes

Hay produced in 2002 and fresh herbage grown in 2003 did not differ in their isotopic signatures (Figure II.2). $\delta^{13}\text{C}$ in hay from different cuts of the same pasture varied by less than 0.2‰. The $\delta^{13}\text{C}$ of vegetation and hair increased by 1.1‰ km⁻¹ (± 0.1 ‰ SE) altitude (Figure II.2, Table II.1). Slopes of the regression for vegetation and hair did not differ, as any significant difference in the mean deviations between regression estimates and measurements were observed in the upper and lower half of the altitude range in any of the groups (Table II.2). Hence, altitude did not affect the apparent diet-hair shift.

The apparent diet-hair shift for $\delta^{13}\text{C}$ was +3.0‰ (± 0.2 ‰ SE) for cattle and +3.2‰ (± 0.2 ‰ SE) for sheep; cattle and sheep not being significantly different at $P < 0.05$. However, the shift was greater for goats (+4.2‰, ± 0.2 ‰ SE). Both, in the case of sheep and in the case of goats, the diet-hair shift for ^{13}C on pasture matched the values found in the confinement study (see below). The regression with altitude and animal type explained 93% of the variation in $\delta^{13}\text{C}$.

$\delta^{15}\text{N}$ also exhibited an altitude gradient and decreased by 1.1‰ km⁻¹ (± 0.3 ‰ SE) (Figure II.3, Table II.1). Again, no significant difference in the mean deviations between regression estimates and

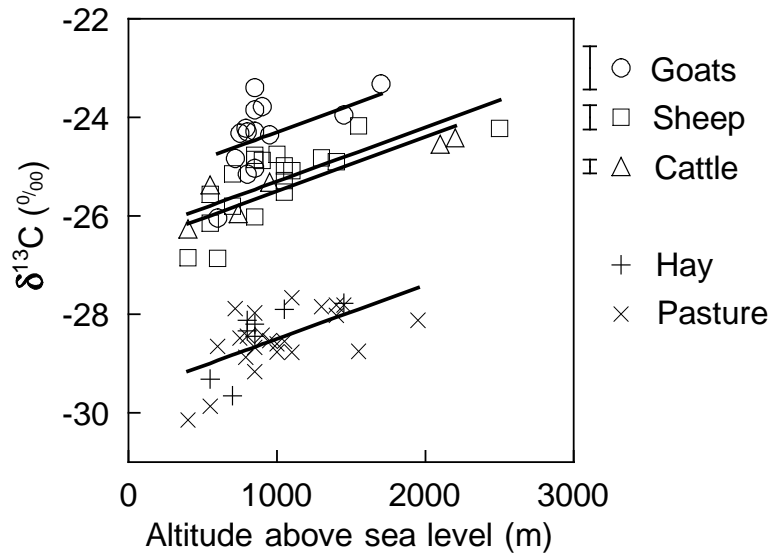


Figure II.2. Average $\delta^{13}\text{C}$ of the hair of sheep, goats and cattle and bulk samples of vegetation (fresh pasture and hay) along altitude in the Alps. Each animal data point is the mean of an individual farm. Between two and 12 animals (mean = 3) were sampled on each farm. Bars display the mean 95% confidence interval for the data points. The solid line is a linear regression with altitude including a species-specific diet-hair shift.

Table II.1. Coefficients, standard error (SE) and significance P for the dummy regressions in Fig. 2 (^{13}C) and Fig. 3 (^{15}N).

Coefficient	$\delta^{13}\text{C}$	SE	P	$\delta^{15}\text{N}$	SE	P
Slope	0.0011	0.0002	<0.0001	-0.0011	0.0003	<0.01
Intercept	-29.5	0.2	<0.0001	2.6	0.4	<0.0001
Shift sheep	3.2	0.2	<0.0001	3.6	0.3	<0.0001
Shift goats	4.2	0.2	<0.0001	3.1	0.4	<0.0001
Shift cattle	3.0	0.2	<0.0001	3.2	0.4	<0.0001

Table II.2. Mean deviation of data from the regression line of Figures 2 and 3 for the lower and upper half of the altitudinal range (significance column: n.s. = not significant at $P < 0.05$).

Group	^{13}C (‰)			^{15}N (‰)		
	mean deviation			Mean deviation		
	lower	upper	Significance	lower	upper	Significance
	half	half		half	half	
Vegetation	0.01	0.02	n.s.	-0.17	0.16	n.s.
Sheep	-0.06	0.07	n.s.	-0.11	0.11	n.s.
Goats	-0.05	0.12	n.s.	-0.06	0.07	n.s.
Cattle	0.11	-0.09	n.s.	0.30	-0.30	n.s.

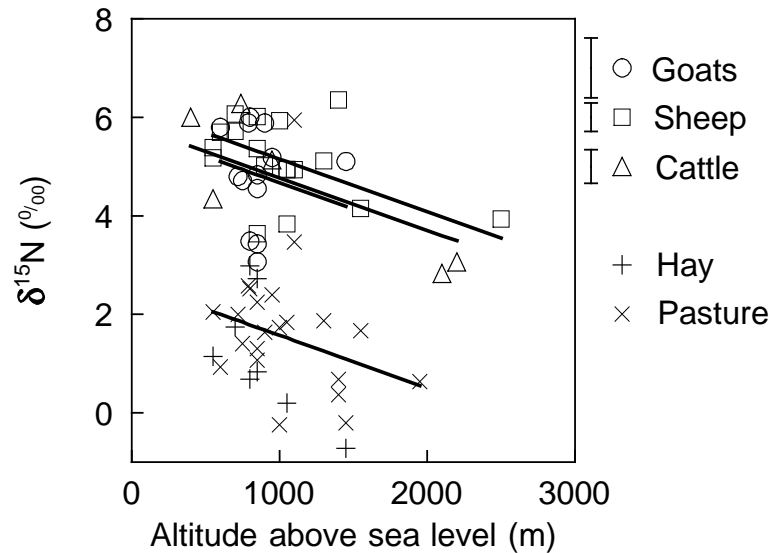


Figure II.3. Average $\delta^{15}\text{N}$ of the hair of sheep, goats and cattle and of bulk samples of vegetation (fresh pasture and hay) along altitude in the Alps. Each animal data point is the mean of an individual farm. Between two and 12 animals (mean = 3) were sampled on each farm. Bars display the mean 95% confidence interval for the data points. The solid line is a linear regression with altitude including a species-specific diet-hair shift.

measurements in the upper and lower half of the altitude ranges occurred in any group of samples (Table II.2), indicating that altitude did not modify the diet-hair shift. The diet-hair shift for ^{15}N was larger than in the confinement experiment (see below), namely 3.6‰ (± 0.4 ‰ SE) for sheep, 3.2‰ (± 0.5 ‰ SE) for cattle and 3.1‰ (SE ± 0.4 ‰) for goats. Again, altitude and animal type explained a large part of the variation ($r^2=0.74$), but the unexplained variation was larger than that for ^{13}C , indicating that the ^{15}N signature may be strongly influenced by local effects.

Diet-hair shift in the confinement experiment

The feed offered in the confinement experiment included three components which differed in their $\delta^{13}\text{C}$ (hay -29.3, silage -30.3, concentrate pellets -30.2) and $\delta^{15}\text{N}$ (hay 3.4‰, silage 5.5‰, concentrate pellets 1.9‰). Supposing that animals selected freely among components, the range of possible combinations of C and N isotope signatures in their diet fell inside a triangle, in which the C and N isotope signature of individual feed components defined the coordinates of the vertices (Figure II.4). If feed selection was the only cause for differences in hair isotope signatures between animals, the hair signatures should all fall inside a triangle of the same shape and orientation, but displaced relative to the diet triangle by the diet-hair shifts for ^{13}C and ^{15}N . Indeed, the C and N isotope signature of the hair of sheep mostly fell inside such a triangle. Most data points were located near the hay and silage

vertices, and none close to that of concentrate, showing that all animals ingested a hay- and silage-dominated diet, due to the small amount of concentrate in the ration. Overall, a diet-hair shift of +3.2‰ for ^{13}C and +2.1‰ for ^{15}N gave the best fit to the sheep data (Figure II.4). There was no evidence for differences among breeds in this relationship ($P > 0.05$ in a t-test).

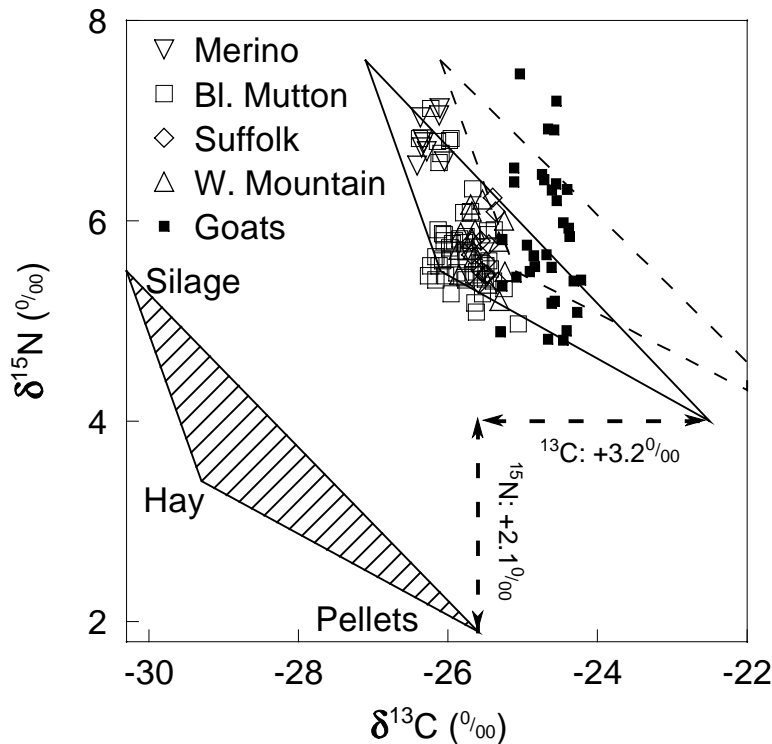


Figure II.4. Nitrogen and carbon isotopic signatures ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$) of the feed (hatched area) and hair of sheep and goats. Data points are 1 cm-long hair segments from different breeds of sheep ($n = 123$; Merino: 9 animals; Blackheaded Mutton: 11 animals; Suffolk: 3 animals; White Mountain: 3 animals) and goats ($n = 36$; Boer: 3 animals). Animals were kept in confinement and received a diet including three components (silage, hay and concentrate pellets). The hatched area gives the possible range of combinations of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in ingested feed that could be achieved by selective feeding. The unhatched solid-line triangle reflects a diet-hair shift of 3.2‰ for ^{13}C and 2.1‰ for ^{15}N (sheep data), the broken-line triangle denotes a shift of 4.2 and 2.1‰ (goat data).

The goat hair ^{13}C versus ^{15}N relationship differed significantly from that of the sheep ($P > 0.05$), although the variation among goats was much larger particularly for ^{15}N (Figure II.4). Overall, a diet-hair shift of 4.2‰ (± 0.7 SD) for ^{13}C and 2.1‰ (± 0.8) for ^{15}N provided the best fit for the goat data.

DISCUSSION

Altitude has no effect on the diet-hair shift of ^{13}C and ^{15}N

We found no evidence for an effect of altitude (or related environmental variables: temperature and precipitation) on the apparent diet-hair shift for ^{13}C or ^{15}N . This suggests that any metabolic stress or dietary change associated with exposure to altitude had no effect on the shift.

To our knowledge there are no other reports of altitude effects on diet-hair or other diet-tissue shifts for ^{13}C or ^{15}N , limiting opportunities for discussion. Also, in principle, lack of evidence for an altitude effect renders futile any discussion of ecological or physiological mechanisms that might underlie it. Still, absence of an effect is in agreement with the data of Cerling & Harris (1999), which provide no indication of any effects of environment or diet on carbon isotope fractionation between diet and bioapatite among a wide range of ungulate mammals living in contrasting environments. Moreover, just recently, Murphy & Bowman (2006) reported on the diet-bone collagen ^{15}N shift in kangaroo along a gradient of aridity in Australia. They found no effect of the environmental gradient on the shift.

The diet-hair shift for ^{13}C in sheep and cattle in this study falls in the range of values reported by others for domestic ruminant animals: 1.8 to 3.4‰ (mean 2.6‰ \pm 0.6‰ SD) (Table II.3). Also the data, including those of goats, agree with the data of Cerling & Harris (1999), who compared the diet-keratin shift in eight wild ruminant mammals by analyses of horn, hoof and hair: 1.7 to 4.2‰ (mean 3.1‰ \pm 0.7‰ SD). While the study of Cerling & Harris provided little indication of systematic differences in the diet-keratin shift, our study suggests a higher apparent diet-hair shift in goats than in cattle. Although we cannot exclude the possibility that this effect was associated with some inter-specific difference in feed selection, our observation is in agreement with that of Sponheimer *et al.* (2003a), who also observed a higher diet-hair shift in goats (and camelids) than in cattle. To our knowledge domestic sheep have not been studied before, but our study suggests that their diet-hair shift is close to that of cattle, and less than that of goats. Knowledge of the diet-hair shift in sheep is potentially useful for biogeochemical investigations and environmental reconstructions because of the great importance of this species in the use of grassland in many regions of the world.

Table II.3. Diet-hair shift of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (‰) for large herbivores under different feeding conditions as reported in literature and in this study (\pm denotes the standard error). Information on feeding includes keeping conditions, photosynthetic type of forage (C_3/C_4) and experimentally varied protein content.

Animal	Feeding	^{13}C (‰)	^{15}N (‰)	Reference
Horse	C_3 , confinement, low protein	2.7		Ayliffe <i>et al.</i> , 2004
Horse	C_3 , confinement, high protein	3.3		Ayliffe <i>et al.</i> , 2004
Horse	Confinement, low protein		2.0	Sponheimer <i>et al.</i> , 2003b
Horse	Confinement, high protein		4.5	Sponheimer <i>et al.</i> , 2003b
Cattle	C_4 , pasture	1.8		Jones <i>et al.</i> , 1981
Cattle	C_4 , confinement	1.8		Jones <i>et al.</i> , 1981
Cattle	C_4 , pasture	1.9		Minson <i>et al.</i> , 1975
Cattle	C_4 , confinement	2.0		Schwertl <i>et al.</i> , 2005
Cattle	C_4 , confinement	2.6		Sponheimer <i>et al.</i> , 2003a
Cattle	C_3 , pasture	2.7		Minson <i>et al.</i> , 1975
Cattle	C_3 , pasture	2.7		Schwertl <i>et al.</i> , 2005
Cattle	C_3 , pasture	3.1		Minson <i>et al.</i> , 1975
Cattle	Confinement, low protein		2.5	Sponheimer <i>et al.</i> , 2003b
Cattle	Confinement		3.2	Sutoh <i>et al.</i> , 1987
Cattle	Confinement, high protein		4.0	Sponheimer <i>et al.</i> , 2003b
Cattle	Pastures at 6 altitude levels	3.0 ± 0.2	3.2 ± 0.5	This study
Alpaca	C_3 , confinement	3.1		Sponheimer <i>et al.</i> , 2003a
Alpaca	Confinement, low protein		3.5	Sponheimer <i>et al.</i> , 2003b
Alpaca	Confinement, high protein		6.5	Sponheimer <i>et al.</i> , 2003b
Llama	C_3 , confinement	3.4		Sponheimer <i>et al.</i> , 2003a
Goat	C_3 , confinement	3.2		Sponheimer <i>et al.</i> , 2003a
Goat	Confinement, low protein		2.5	Sponheimer <i>et al.</i> , 2003b
Goat	Confinement, high protein		5.0	Sponheimer <i>et al.</i> , 2003b
Goat	Confinement, mixed diet	4.2 ± 0.7	2.1 ± 0.8	This study
Goat	Pastures at 14 altitude levels	4.2 ± 0.2	3.1 ± 0.4	This study
Sheep	Confinement, mixed diet	3.2 ± 0.3	2.1 ± 0.5	This study
Sheep	Pastures at 19 altitude levels	3.2 ± 0.2	3.6 ± 0.3	This study

The range of apparent diet-hair shifts for $\delta^{15}\text{N}$ found in this study (+2.1 to +3.6‰) is within the range reported for large mammalian herbivores (cf. e.g. Sponheimer *et al.*, 2003b). Again, there was evidence for a difference between goats and sheep in diet-hair shifts for animals from the altitude gradient, but this was not confirmed in the confinement experiment. Again, this could be related to

some inter-specific difference in diet selection in confinement and on pasture. Sponheimer *et al.* (2003b) have demonstrated significant differences in the diet-hair shift for ^{15}N between different ruminant herbivores on both low- and high-protein diets. Moreover, they found a significant effect of protein content on the shift, with high protein content causing a larger shift. Yet, we failed to detect a relationship between protein content of the diet and the diet-hair shift in our study: although protein content in forage varied between 80 and 240 g kg⁻¹ dry weight, this did not correlate with variation in the diet hair shift in sheep or goats ($r^2=0.01$). Certainly, the physiological mechanisms underlying variation in the diet-hair ^{15}N -shift are complex (e.g. compare Sponheimer *et al.*, 2003b; Cherel *et al.*, 2005; Balter *et al.*, 2006; Murphy & Bowman, 2006), not well understood, and require further experimental study.

Altitude affects the ^{15}N of grazer hair via an effect on vegetation ^{15}N

Our data reveal a very significant effect of altitude on hair ^{15}N , which was a direct result of the effect of altitude on vegetation ^{15}N . Since the diet-hair shift for ^{15}N was not affected by altitude, the hair ^{15}N signature directly reflected that of vegetation. This also meant that the vegetation ^{15}N signature could be estimated from the ^{15}N of hair by simply correcting for the diet-hair ^{15}N shift. The data so obtained agree well with a global data set of grassland vegetation- ^{15}N that was compiled from literature (Figure II.5a). Altitude influenced ^{15}N in the same way in the global and our alpine data set, although unexplained scatter was evident in both: on average, $\delta^{15}\text{N}$ decreased by 1.6‰ km⁻¹, but this explained only one-third of the total variation. Notably, similar scatter was present in our vegetation samples, the hair samples and the global data set, although the three data sets reflect different spatial and temporal scales of integration.

As the altitude-related ^{15}N signal is passed forward in the food chain, it is immediately relevant for the interpretation of isotope data in animal nutritional ecology studies in montane environments: for example, an upward shift of 1000 m in the altitude range of an omnivorous species would have a similar effect on hair $\delta^{15}\text{N}$ as a shift from a predominantly animal tissue-based diet to a predominantly plant-based diet (i.e. ~50% of one trophic level step). Thus far, the effects of altitude on $\delta^{15}\text{N}$ of diet/prey have generally not been considered explicitly in nutritional ecology studies. Yet, in a

study of male and female black and grizzly bear trophic relationships, Hobson *et al.* (2000) noted that the lower $\delta^{15}\text{N}$ in the hair of female grizzly bears, which was tentatively interpreted in terms of a lower contribution of animal tissues to their diet, could in fact also be related to its higher altitude habitat.

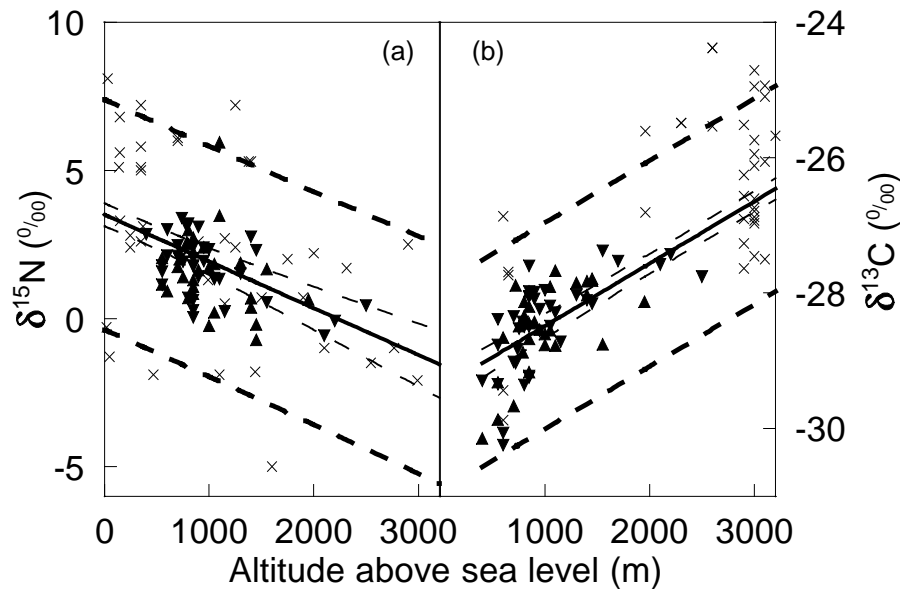


Figure II.5. Change in (a) $\delta^{15}\text{N}$ and (b) $\delta^{13}\text{C}$ of vegetation with altitude from the literature (crosses) and this study (triangles). Literature data in (a) present a global data set of grassland vegetation (including steppes, savannas, and temperate grassland) compiled from data by Mariotti *et al.* (1980), Handley *et al.* (1999), Jacot *et al.* (2000 a,b) and Amundson *et al.* (2003). Literature data in (b) display single species from an Alpine data set of Körner *et al.* (1988, 1991). Literature data were corrected to 2003 using the change in the carbon isotopic composition of atmospheric CO_2 . Data from this study include fresh pasture and hay samples (upright triangles) and vegetation estimated from hair analysis corrected for the animal species-specific diet-hair isotopic shift (downward triangles). The thick lines show the regression for all data (a: $r^2=0.20$; b: $r^2=0.59$), the dashed thin lines denote the 95% confidence interval for the regressions and the dashed thick lines denote the 95% confidence interval for the samples.

The present data also provide a hint on how altitude- and trophic position-related effects on hair (or other animal tissue) isotope composition may be distinguished: both ^{13}C and ^{15}N tend to become enriched with each step along the trophic chain, whereas altitude causes an increase in ^{13}C but a decrease in ^{15}N . The resulting isotopic segregation may well be useful for nutritional ecological studies in montane environments.

One asset of the present study is that it allowed a long-term assignment of animals to certain altitudes. Such an assignment is more difficult with wild mammalian herbivores, which move freely across a wider range of altitudes, and habitats (and food sources) differing in ^{15}N . These movements

inevitably cause isotopic disequilibria between food ingestion and hair growth, yielding another level of complexity in data interpretation. Such disequilibria are particularly long-lasting in large-bodied animals which have slowly turning over metabolic pools (Ayliffe *et al.*, 2004; Balter *et al.*, 2006). Yet, chronologies of diet isotope composition can be derived by hair segmental analysis (Schwertl *et al.*, 2003a) and pool modelling (Ayliffe *et al.*, 2004), and used for the reconstruction of migration patterns (Cerling *et al.*, 2006).

The effect of altitude on grazer hair and plant community ^{13}C is the same as the ‘average’ plant species’ response

The effect of altitude on community-level ^{13}C obtained in this study (1.1‰ km^{-1}) was very similar to the average species-level responses observed by others in studies encompassing a wide range of plant types and climate zones [1.2‰ km^{-1} ($\pm 0.3\text{‰}$) for 23 different C_3 species; compiled from Körner *et al.*, 1988; Friend *et al.*, 1989; Vitousek *et al.*, 1990; Cordell *et al.*, 1999; Hultine & Marshall, 2000; Kogami *et al.*, 2001; we excluded reports from bogs or data sets in which there was clear evidence for drought effects on the altitude gradient. Where several data sets for the same species were available we took the average from the different data sets]. In particular, the community-level response of $\delta^{13}\text{C}$ to altitude (Figure II.2) was also close to the 0.9‰ km^{-1} of the forbs and grasses collected in the Alps by Körner *et al.* (1988, 1991) (Figure II.5b).

Agreement of the plant species- and community-level response to altitude is not necessarily evident because the range of reported species responses is large (-0.9‰ to 2.7‰ km^{-1}), species within a community can differ in $\delta^{13}\text{C}$ by as much as 4‰ (Körner *et al.*, 1991; Tsialtas *et al.*, 2001; see also Figure II.5b), and species composition changes with altitude (Ellenberg, 1978). The great similitude of community-level and average species responses to altitude suggests that the ‘average’ species-level response to altitude scales up linearly to the community-level. This would imply that intrinsic water use efficiency increases along altitudinal gradients in the same way at the species and the community level. Furthermore, the hair ^{13}C data suggest that this response also scales up linearly to higher spatial scale such as that of the landscape or entire grazing grounds.

CONCLUSIONS

This study demonstrates that altitude affects $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of grassland in the same way at different scales of integration. Importantly, we provide direct empirical evidence for the lack of an effect of altitude and related environmental variables on the diet-hair shift for ^{13}C and ^{15}N in sheep, goats and cattle. This means that grazer hair provides faithful isotopic records of grassland vegetation, and thus can be used for ecosystem reconstruction and retrospective biogeochemical analyses of grassland in montane (and other) environments. Sheep, goats and cattle are extensively used to graze grassland, one of the largest biomes of the world, and their hair grows near-continuously, integrating the isotopic signal of the grazed vegetation over large areas (several hectares to many square kilometres) and long periods (months to years), generating, as it were, landscape- and regional-scale isotopic biogeographic maps, that wait to be read.

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Chapter III. LARGE VARIATION OF ISOTOPIC COMPOSITION AMONG CARBON POOLS IN INNER MONGOLIA GRASSLAND – PATTERNS AND CONTROLS

ABSTRACT

C_3 and C_4 plants have different effects on biogeochemical cycles of carbon, water and nutrients, and they produce distinct isotope ($\delta^{13}\text{C}$) signals which are important for biogeochemical modeling. The variability and uncertainty of ^{13}C signals is potentially large in environments which support the existence of both photosynthetic types, such as the grassland of Central East Asia. Here, we report results from a multi-year (2000-2005), regional-scale (approx. 200 000 km^2) study of the ^{13}C composition of the Inner Mongolia steppe. The analysis was based on sampling of carbon pools with distinct spatio-temporal integration: soil organic carbon (SOC; m^2 , >decades), plant community aboveground biomass (m^2 , months), and feces (hectares, months) and wool (km^2 , year) of sheep and goats. The percent contribution of C_4 plants to carbon pools was estimated from ^{13}C , considering effects of aridity on C isotope discrimination of C_3 plants. The percent C_4 in aboveground biomass varied between 0 and 100%, with about half of the variation occurring at the farm scale, and half at higher spatial scales. The $\delta^{13}\text{C}$ of vegetation was less negative than that of SOC, indicating C_4 expansion in the last century. C_4 abundance was highest in the most arid zone (near the Gobi desert) and in the vicinity of towns. This ‘town-effect’ was related to decreased soil N concentration, but was not evident in ^{13}C of SOC, indicating that it developed in the last century. Expansion of C_4 plants (mainly annual ruderal dicots) may have resulted from overgrazing, disturbance and erosion, and regional warming.

INTRODUCTION

The carbon isotope composition ($\delta^{13}\text{C}$) of plants and soil organic carbon (SOC) hold important information on carbon fluxes and linked biogeochemical cycles (Schimel, 1995; Ehleringer *et al.*, 2000). Among all biomes the variability of the ^{13}C signal is greatest in grassland. This is primarily related to the presence of variable proportions of C_3 and C_4 photosynthetic types (Bird & Pousai, 1997; Tieszen *et al.*, 1997; Collatz *et al.*, 1998) and the large contrast in carbon isotope discrimination (Δ) between them (Farquhar *et al.*, 1989). Variation in the $\text{C}_3\text{:C}_4$ ratio has wide biogeochemical and land use implications: it affects the magnitude and seasonal distribution of biomass production, soil C storage, water use and nutrient cycling (Bird & Pousai, 1997; Tieszen *et al.*, 1997; Epstein *et al.*, 1998; Sage & Kubien, 2003; Semmartin *et al.*, 2004). Hence, as it indicates the $\text{C}_3\text{:C}_4$ ratio, the $\delta^{13}\text{C}$ is a useful indicator of vital functions of grassland. However there exist very few regional-scale investigations of $\text{C}_3\text{:C}_4$ mixed grassland ^{13}C signatures.

Yet, there is abundant evidence that the current distribution of C_4 plants is primarily controlled by growing season temperature (Ehleringer *et al.*, 1997) and that this is related to the higher effective quantum yield of CO_2 fixation (Ehleringer & Bjorkman, 1977) and higher maximum photosynthetic rate of C_4 's at high temperature (Sage & Kubien, 2003). But the seasonal distribution of precipitation, aridity, soil fertility, and disturbance (for instance by overgrazing) may exert secondary, modifying effects. Predominance of summer rainfalls benefits the C_4 more than the C_3 , whereas predominance of precipitation in the cool season benefits C_3 growth. C_4 dicots predominate in hot arid, saline or highly disturbed habitats (Ehleringer *et al.*, 1997). Nitrogen loading can cause a replacement of C_4 grasses by C_3 grasses (Wedin & Tilman, 1996). It should be expected that such secondary controls have their strongest effect on $\text{C}_3\text{:C}_4$ abundance in those regions which have a growing season mean temperature that is near the $\text{C}_3\text{:C}_4$ transition-temperature. The steppe of Inner Mongolia exhibits such a climate, with average temperature near the $\text{C}_3\text{:C}_4$ transition-temperature during the summer months when most of the annual precipitation falls. Grassland utilization is heavy, with overgrazing leading to erosion and consequent declines in soil fertility in many areas (Li *et al.*, 2003; Su *et al.*, 2005). There are several reports indicating increases in the number and abundance of C_4 species with aridity in grasslands of North Western China and Mongolia (Pyankov *et al.*, 2000; Wang, 2004), although

conflicting evidence has also been presented (Wang, 2003; Ni, 2003). Promotion of the spread of annual C_4 grasses and C_4 dicots by desertification in Inner Mongolia was reported by Wang (2002c). But so far, there has been no assessment of the overall effect of these (putative) secondary drivers on C_4 expansion and the related ^{13}C signal at regional scale. This is the subject of this study.

Although the $\text{C}_3:\text{C}_4$ ratio is the main factor affecting $\delta^{13}\text{C}$ of grassland, variation in ^{13}C discrimination of C_3 plants may contribute. Thus, the $\delta^{13}\text{C}$ of C_3 plants often decreases with precipitation, particularly in (semi-)arid regions (e.g. Schulze *et al.*, 1998; Liu *et al.*, 2005). Yet, absence of a response to a precipitation gradient in arid regions has also been observed (e.g. Schulze *et al.*, 1996b). This factor must be considered when estimating the $\text{C}_3:\text{C}_4$ ratio from C isotope composition.

The aim of this work was (i) to assess the $\delta^{13}\text{C}$ of the grassland of Inner Mongolia and its variation along gradients of vegetation period temperature, precipitation and soil fertility, and how these depend on C_4 abundance and ^{13}C discrimination of C_3 plants, and (ii) to investigate how these relationships are reflected in carbon pools with distinct spatio-temporal integration (SOC, aboveground plant biomass, feces and wool). It is shown that C_4 plants account for approx. 26% of the (area-weighted) biomass production of investigated Inner Mongolia grassland, but that community-level variation in percentage C_4 is almost full scale (0-100%), and related to climatic and soil nitrogen gradients, and a hitherto undescribed ‘town-effect’.

MATERIALS AND METHODS

Study area

The study area was situated between $111^{\circ}38'$ and $117^{\circ}49'$ E (approx. 500 km), and $41^{\circ}48'$ and $45^{\circ}46'$ N (approx. 450 km) in the Autonomous Region of Inner Mongolia in the Peoples Republic of China. Sampled altitudes ranged from 800-1500 m above sea level (a.s.l.). Climatic data for the region were obtained from Climate Source Inc., Corvallis, Oregon. Mean annual precipitation increases from 100 mm in the western part to 400 mm in the eastern part of the region (Figure III.1). Most of the precipitation (65-75%) falls during summer (June-August). The spatial distribution of temperature varies more, following altitudinal and latitudinal trends. Mean annual temperature and mean

temperature of the vegetation period (May-September) vary from 0-6 °C and 14-19 °C, respectively. The soils in the eastern part are mostly Haplic Kastanozems and Chernozems on loesslike substrates, whereas Regosols and Luvic Kastanozems are more common in the western part. Almost the whole area is used for small ruminant livestock production (mainly sheep and cashmere goats). 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.

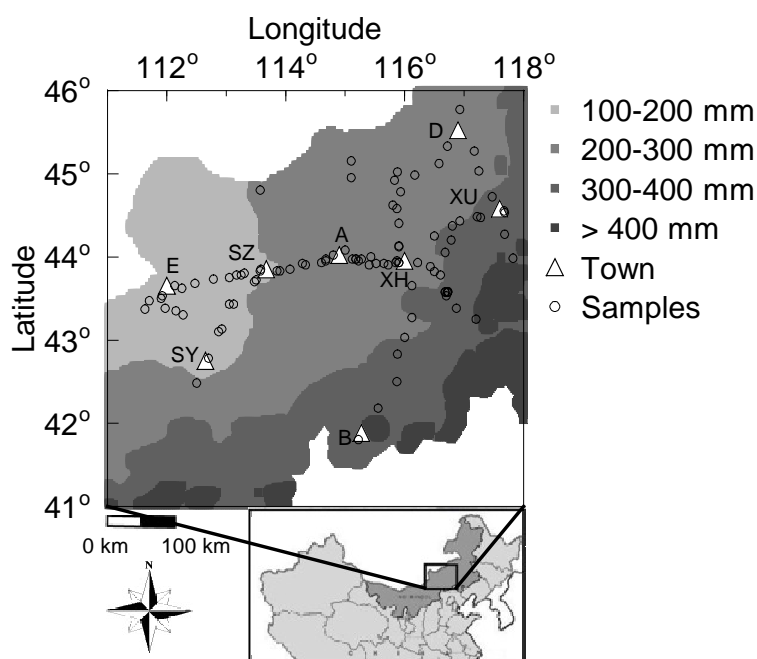


Figure III.1. Inner Mongolia, P.R. China (small map) and study area (large map) with spatial variation of mean annual precipitation (MAP) averaging 1961-1990, taken from a high resolution map (approx. 2.2 x 1.6 km) obtained from Climate Source Inc., Corvallis, Oregon. Circles display sampling sites. Towns are A = Abaga Qi, B = Baochang, D = Dong Ujimqin Qi, E = Erenhot, SY = Sonid Youqi, SZ = Sonid Zuoqi, XH = Xilinhot, XU = Xi Ujimqin Qi.

Sampling

Samples were collected in August/September 2003, August/September 2004, and July 2005. Bulk samples of aboveground plant biomass were collected within approx. 100 m² on 92 sites across the region (2003, 2004 and 2005). At some of the sites (n=19) the dominant and co-dominant species

(contributing >5% to total aboveground biomass) were sampled separately, with the aim of obtaining separate estimates of the $\delta^{13}\text{C}$ of the C_3 and C_4 components of plant communities (2003, 2004 and 2005). Together, these sites encompassed the entire aridity gradient in the region. On average seven C_3 species (range 1 to 11) and two C_4 species (0 to 4) were collected per site.

Soil was sampled to a depth of 20 cm within 1 m² on 56 sites (2003 and 2004). Feces were collected within 100 m² on 32 sites in 2004. Wool from 234 sheep and 25 goats dating from 1998 – 2005 was sampled on 69 sites (2003 and 2004). Most wool samples (n = 232) were obtained from local herders and originated from a recent shearing, which generally takes place in June. Some samples (n = 27) were collected from animals during the sampling trips.

Sample Preparation

All samples were air dried during collection in the field. In addition plant, soil and feces samples were dried prior to milling: plant samples 48 hours at 60 °C, feces and soil samples 48 hours at 40 °C. Soil was sieved (2 mm). Soil, plant and feces samples were ground to homogeneity with a ball mill. Carbonates were removed from soil (Harris *et al.*, 2001) for C isotope analysis, but untreated soil samples were used for nitrogen analysis. 0.7-0.8 mg plant material or feces in tin cups (5 x 9 mm) and 9.9-10.1 mg soil in silver cups were analyzed for isotope ratio. The wool samples were cleaned by the procedure of Schwertl *et al.* (2003a). For measurements, two replicates of narrow staples were taken from each wool sample and cut into 1 cm-long segments. About 0.2-0.4 mg of each segment were then packed into tin cups for isotope analysis. In some cases (n = 120) the staples were cut into cm pieces, which were analyzed separately to detect changes over the year.

Isotope and elemental analysis

The carbon isotope composition and carbon and nitrogen content of each sample was determined with 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 isotopic data are presented as $\delta^{13}\text{C}$, with $\delta^{13}\text{C} = (\text{R}_{\text{sample}}/\text{R}_{\text{standard}}) - 1$, where R is the $^{13}\text{C}/^{12}\text{C}$ 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 vegetation, feces and soil 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 0.10‰ (SD) for feces, 0.36‰ for vegetation and 0.50‰ for de-carbonated soil samples.

Climate data

For each site we defined the GPS xyz coordinates. Climate data averaging 1961-1990 were taken from a high resolution map (approx. 2.2 x 1.6 km) obtained from Climate Source Inc., Corvallis, Oregon.

Estimation of C_4 fraction

The percent contribution of C_4 plants (P_{C4}) to aboveground biomass at each site was estimated from the $\delta^{13}\text{C}$ of vegetation using a two-member mixing model:

$$P_{C4} = [(\delta_V - \delta_{C3}) / (\delta_{C4} - \delta_{C3})] 100, \quad (\text{Eq. III.1})$$

where δ_V denotes vegetation, and δ_{C3} and δ_{C4} are the end-members of the mixing model (local C_4 and C_3 vegetation, respectively). Regionalized estimates of δ_{C3} and δ_{C4} were obtained from ^{13}C analyses of the C_3 and C_4 vegetation samples (see Results).

In principle, percent C_4 can also be estimated from feces or hair/wool considering possible isotopic offsets with respect to vegetation: in studies with pure or mixed C_3 or C_4 diets the $\delta^{13}\text{C}$ of hair was 2 to 3‰ enriched with ^{13}C relative to diet, while feces were depleted by -0.4 to -2.0‰ (Jones *et al.*, 1981; Sponheimer *et al.*, 2003a). This offset, δ_d , ($\delta_d = \delta_{\text{diet}} - \delta_X$, where X stands for hair or feces) is termed ‘diet-hair shift’ or ‘diet-feces shift’ (as appropriate), and results from ^{13}C fractionation during digestion or metabolism of animals (De Niro & Epstein, 1978). The $\delta^{13}\text{C}$ of vegetation can be estimated as $\delta_V = \delta_X + \delta_d$, if the shift is known. So far, however, there are no published estimates of these shifts for sheep. In consequence, we assessed the vegetation-hair ($n = 58$) and vegetation-feces

shifts ($n = 30$) by paired sampling across the region. In the case of hair we compared the $\delta^{13}\text{C}$ of vegetation with that of the most-recently grown 1 cm-long wool segment.

Percent C_4 may also be estimated from $\delta^{13}\text{C}$ of SOC. However, it must be considered that SOC reflects the $\delta^{13}\text{C}$ of some former time vegetation which grew in the presence of CO_2 with a less negative $\delta^{13}\text{C}$, and that decomposition of organic matter in soils may be associated with ^{13}C enrichment. In consequence, for ecosystems with a constant composition of plant functional types, one should expect that SOC is enriched in ^{13}C by up to several ‰ relative to standing aboveground biomass. Although we acknowledge uncertainties concerning (i) the age of SOC in the region, and (ii) the magnitude of ^{13}C -enrichment during SOC decomposition (Ehleringer *et al.*, 2000; Santruckova *et al.*, 2000), we derived tentative estimates of former C_4 abundance from $\delta^{13}\text{C}$ of SOC by assuming that the total shift was 1.55‰ for a SOC age of 50 years corresponding to the $\delta^{13}\text{C}$ decrease of atmospheric CO_2 by 1.55‰ in the last 50 years (NOAA, 2006). The radio carbon age at a continuously grazed site within the central part of the research area was estimated to be younger than 60 yr (Dr. Angelika Kölbl; pers. comm.). For a SOC age of 100 yr the total shift would be 1.78‰ and for an age of 25 yr it would be 1.39‰. In the estimations, we neglected potential additional ^{13}C -enrichment during decomposition of plant material in soil (Ehleringer *et al.*, 2000; Santruckova *et al.*, 2000), which led to conservative (i.e. low-end) estimates of percent C_4 from $\delta^{13}\text{C}$ of SOC.

Statistical relationship between $\delta^{13}\text{C}$ and environmental parameters

The effect of environmental variables (temperature, precipitation and soil nitrogen content) on $\delta^{13}\text{C}$ of vegetation, feces, hair, and SOC was analyzed by regressions containing dummy variables (Fox, 1997). For vegetation a linear change of signature with an environmental parameter leads to a linear model following Equation II.2 [$\delta^{13}\text{C} = a + (b \times c)$], where a is the intercept ^{13}C signature (‰), b is the change of the signature with temperature (‰ °C⁻¹), precipitation (‰ mm⁻¹) or soil nitrogen content (‰ %⁻¹), and c is the value of the environmental parameter [temperature (°C), precipitation (mm) or soil nitrogen content (%)]. As long as there is no environmental effect on the shifts, the same model should apply for all examined materials. The data of vegetation and other sample types can then be combined in a model containing dummy variables (D1, D2,...), which are 1 for samples of a specific group and

zero for all other samples. Following Equation II.3 [$\delta^{13}\text{C} = a + (b \times c) + (d \times \text{D1}) + (e \times \text{D2}) + \dots$], the shift for wool and feces compared to vegetation is given by d and e , if D1 denotes wool and D2 feces.

Where the overall slope b does not apply to a group this is evident as a statistically significant deviation between the data of this group and the regression to one side for the upper half and a deviation to the other side for the lower half of the environmental gradient. To examine this effect the average deviations $\text{av.}(\delta^{13}\text{C}_{\text{pred.}} - \delta^{13}\text{C}_{\text{meas.}})$ for the upper and lower half within each group were computed and statistically compared.

To eliminate the interfering influence of extremely deviating data on single sites reflecting farm-scale effects rather than environmental effects, we used robust regressions for the calculation of environmental gradients. A least trimmed square robust regression (LTS) introduced by Rousseeuw (1984) was used. After deleting detected outliers following an outlier and leverage point diagnostic, a least square regression, termed final weighted least square regression (FWLS), was computed and used to determine environmental gradients from our data set.

Geostatistical analysis

To quantify the spatial gradients of isotopic signatures and C_4 abundance we conducted geostatistical analyses using GS+ for Windows (Version 3.1) and SAS (Version 9.1). Semi-variograms were constructed as a measure of the coherence of spatial phenomena (Webster & Oliver, 2004), whereas spatial variations were calculated separately for different years (1998-2005), to eliminate the influence of inter-annual variations on the overall spatial variation. Based on the semivariograms we constructed maps by ordinary kriging,

RESULTS

The $\delta^{13}\text{C}$ of carbon pools

The isotopic composition of carbon pools varied greatly in the region, but the range differed strongly between types of pools: the $\delta^{13}\text{C}$ of whole vegetation varied between -15‰ (5th percentile) and -26‰ (95th percentile of the $\delta^{13}\text{C}$ -distribution), whereas the site-means for wool ranged between -17‰ and -22‰ , feces between -18‰ and -27‰ , and SOC between -21‰ and -25‰ (Figure III.2).

Remarkably, the range of $\delta^{13}\text{C}$ in wool was quite similar for 1-cm long segments (reflecting growth of approx. 2 months) of wool of individual animals, and the site-mean (which included the wool from several animals pooled over several years for one site).

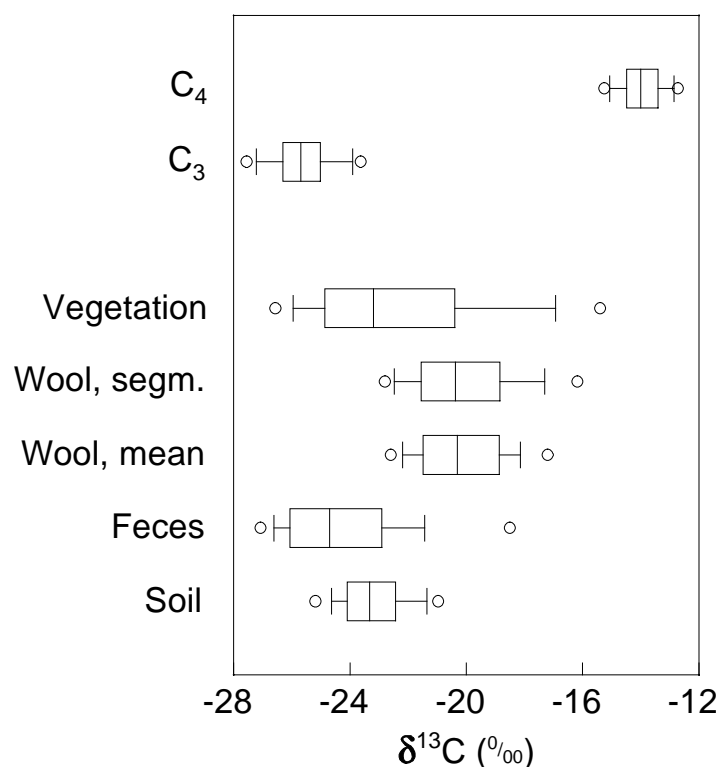


Figure III.2. Carbon isotope composition ($\delta^{13}\text{C}$) of carbon pools in Inner Mongolia grassland: C₄ component of vegetation (16 sites), C₃ component of vegetation (24 sites), whole vegetation (92 sites), 1-cm long segments of wool staples (943 samples from 69 sites), site mean of wool (69 sites), feces (32 sites), and soil (56 sites). Plots give the median and the 5, 10, 25, 75, 90 and 95 percentiles of $\delta^{13}\text{C}$ distributions.

The C₃ and C₄ components of vegetation had a mean $\delta^{13}\text{C}$ of -25.7‰ and -13.9‰ , respectively. Although the $\delta^{13}\text{C}$ of the C₃ and C₄ components also exhibited some variation, this was three to four times smaller than that of whole vegetation (Figure III.2) indicating a variation in the C₃:C₄ ratio (see below).

Whole vegetation, wool and feces differed markedly in their median $\delta^{13}\text{C}$ (Figure III.2). These differences corresponded almost exactly to the isotopic shifts which were observed in paired samplings: on average, wool was enriched by 2.6‰ , and feces were depleted by 1.7‰ relative to vegetation sampled at the same location (Table III.1). There were no significant differences in hair- $\delta^{13}\text{C}$ between sheep and goats grazing the same pastures ($P > 0.05$, data not shown).

Table III.1. Shifts of $\delta^{13}\text{C}$ between (i) vegetation and hair, (ii) vegetation and feces, and (iii) vegetation (including vegetation estimated from hair and feces data) and SOC from paired samplings. Mean, 95% confidence interval of mean (CI), number of sampling locations (n).

Type of shift	Mean (‰)	CI (‰)	n
Vegetation – hair	+2.6	0.8	58
Vegetation – feces	–1.7	0.8	29
Vegetation – SOC	–0.7	0.5	124

Remarkably, the $\delta^{13}\text{C}$ of SOC was 0.7‰ more negative than that of vegetation ($P < 0.05$). This suggests that C_4 abundance has increased in the past.

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

The $\delta^{13}\text{C}$ of carbon pools correlated with mean July temperature, mean annual precipitation, and soil nitrogen content (Table III.2). The strength of this correlation was similar for all environmental parameters. However, the effects were not independent, since there were also correlations between the environmental parameters: in particular, mean July temperature was closely correlated with mean annual precipitation ($r = -0.80$, $P < 0.001$), but soil nitrogen content was also correlated with temperature ($r = -0.48$, $P < 0.001$), and with mean annual precipitation ($r = 0.58$, $P < 0.001$) (data not shown). As a result, the effects of environmental variables on $\delta^{13}\text{C}$ of vegetation could not be separated, and each response function of $\delta^{13}\text{C}$ to an environmental variable included direct and indirect effects.

Table III.2. Effect of environmental variables on $\delta^{13}\text{C}$ of Inner Mongolia grassland carbon pools: mean July temperature (MJulT), mean annual precipitation (MAP), and soil nitrogen content (SNC) quantified by a final weighted least squares regression.

Parameter	Range	$\delta^{13}\text{C}$ -response	SE (‰)	r^2	P
MJulT	18 – 23 °C	+0.65‰/°C	± 0.08	0.60	0.001
MAP	120 – 370 mm	–0.011‰/mm	± 0.002	0.58	0.001
SNC	0.2 – 3.2 mg/g N in dry soil	–0.8‰/(mg/g)	± 0.1	0.62	0.001

Outliers, identified by outlier tests, were removed from the data set before estimating the response of $\delta^{13}\text{C}$ to environmental variables. The number of outliers was quite large in the vegetation

data (16 out of 92 data points), but outliers were also present in the feces (2 of 32) and wool data (1 of 67). In all instances the ‘outliers’ had a comparatively high $\delta^{13}\text{C}$, indicating particularly high contributions of C_4 plants to carbon pools at some sites. These sites were mostly located in the vicinity of towns (see below).

The response of $\delta^{13}\text{C}$ to the environmental variables was the same for all carbon pools with no significant differences in the mean deviations between observations and the dummy regression estimates in the upper and lower half of the regression. This was true for the different carbon pools and the regressors temperature, precipitation and soil nitrogen (Table III.3).

Table III.3. Mean deviation of $\delta^{13}\text{C}$ (‰) for the lower and upper half of the slope in a dummy regression of carbon pools and mean July temperature, MJulT, mean annual precipitation, MAP, or soil nitrogen content, SNC, (significance column indicates a significant deviation between both halves; n.s. indicates that the overall slope also applies for the individual pool).

Pool	MJulT				MAP			SNC			
	n	Lower half	Upper half	Signifi- cance	Lower half	Upper half	Signifi- cance	n	Lower half	Upper half	Signifi- cance
Vegetation	92	-0.11	0.20	n.s.	-0.06	0.02	n.s.	56	0.19	-0.22	n.s.
Feces	32	-0.05	0.14	n.s.	0.15	-0.19	n.s.	14	0.20	-0.24	n.s.
Wool	69	0.19	-0.11	n.s.	0.02	-0.06	n.s.	54	0.02	-0.03	n.s.
Soil	56	0.02	0.07	n.s.	0.02	-0.07	n.s.	56	-0.13	0.09	n.s.

$\delta^{13}\text{C}$ increased by 0.7‰/°C mean July temperature, and decreased by 1.1‰ for each 100 mm increment in mean annual precipitation (Table III.2). Correlations with July, vegetation period or mean annual precipitation (MAP) showed no difference in the significance of the relationship, since these parameters were related strongly to each other. Also, $\delta^{13}\text{C}$ decreased with soil nitrogen content. Soil nitrogen content was low throughout the study area (0.02 – 0.34%) and tended to decrease along the precipitation gradient from the southeast to the western part of the region.

Effect of aridity on $\delta^{13}\text{C}$ of the C_3 and C_4 component of vegetation

An ordinary least squares regression estimated that $\delta^{13}\text{C}$ of the C_3 component of vegetation decreased by 0.008‰/mm MAP ($r^2 = 0.29$, $P < 0.01$). In contrast to the C_3 , the $\delta^{13}\text{C}$ of the C_4 component exhibited no systematic relationship with any environmental variable ($P \gg 0.05$).

C_4 abundance

C_4 abundance was estimated with Eq. III.1, using a constant value for δ_{C_4} (−13.9‰) and a precipitation-dependent estimate of δ_{C_3} . The latter was obtained as:

$$\delta_{\text{C}_3} = \delta_{\text{Base}} + (m \Delta_{\text{MAP}}), \quad (\text{Eq. III.2})$$

where δ_{Base} is the $\delta^{13}\text{C}$ of the C_3 component of a community at a MAP of 250 mm (−25.7‰), Δ_{MAP} (mm) is the site-specific deviation of MAP from the base situation, and m is the change of δ_{C_3} per unit change of MAP (0.008‰/mm) as found above. As MAP correlated with temperature and soil nitrogen, eventual effects of these factors on δ_{C_3} were also (in the main) accounted for by m . We also estimated C_4 abundance from ^{13}C of feces and wool samples considering the isotopic shift between vegetation and hair or feces (Table III.1).

On average of all sites C_4 plants accounted for 26% of aboveground plant biomass, but C_4 abundance varied widely between sites (0 to 100%). Variation in C_4 abundance was tightly related to $\delta^{13}\text{C}$ and – therefore – exhibited very similar relationships with mean July temperature, MAP and soil nitrogen content. C_4 abundance increased by 5.5%/°C mean July temperature ($P < 0.001$) (Figure III.3), decreased by 8.6% for each 100 mm increase in MAP ($P < 0.001$) and decreased by 8.2% for each % increase in soil nitrogen content ($P < 0.003$; data not shown). Although all these relationships were highly significant, the scatter was large in all of them (for example see Figure III.3).

The regional mean C_4 abundance estimated from vegetation, feces and wool was very similar (28%, 25%, and 24%), but that estimated from SOC assuming a SOC age of 50 yr was much lower (<10%). Again, this indicates that the abundance of C_4 's has increased in the last century.

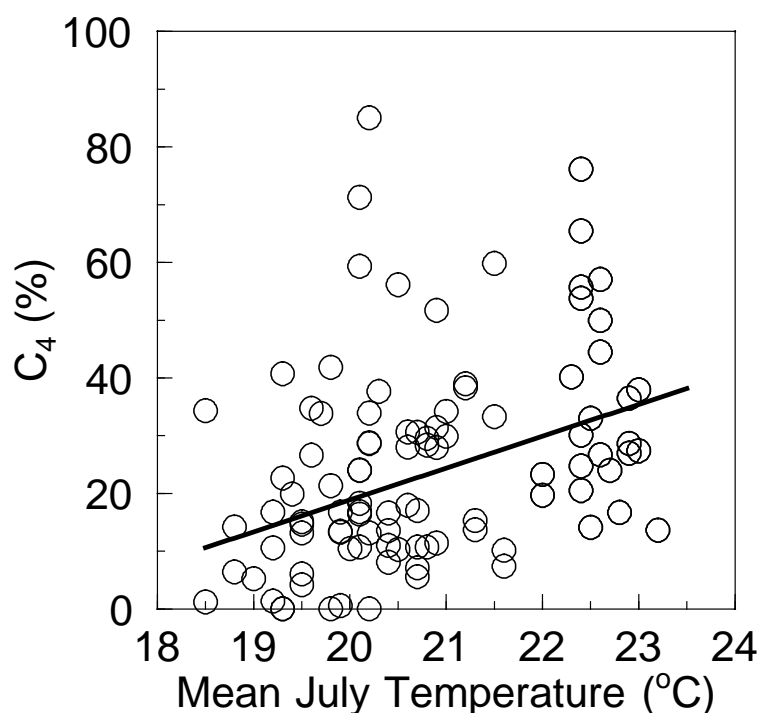


Figure III.3. Relationship between current-day percent C_4 in aboveground biomass and mean July temperature (1961-1990) in a final weighted least square regression. Site-specific percent C_4 was estimated from the ^{13}C of vegetation, wool and feces samples collected at each site ($n = 99$).

Geographic variation of C_4 abundance

A geostatistical analysis of C_4 abundance yielded a semivariogram following a linear-to-sill model (Figure III.4). The nugget effect accounted for 41% of the total sill, which was reached at a lag of 1.7° . Maps of current-day C_4 abundance as reflected in $\delta^{13}\text{C}$ of vegetation, feces and wool and former days C_4 abundance as reflected in $\delta^{13}\text{C}$ of SOC were generated by ordinary kriging. The current-day map (Figure III.5a) demonstrated an increase of C_4 abundance from 10% in the south-eastern part to about 30% in the western part of the region near the Gobi desert. This spatial gradient corresponded broadly to the direction of temperature, precipitation and soil nitrogen gradients.

Besides these large scale gradients, the C_4 abundance was higher in the vicinity of three towns within the shortgrass and desert steppe (Abag Qi, Sonid Zuoqi, Erenhot; MAP < 275 mm) than further away (Figure III.5a). This ‘town-effect’ was substantiated by a significant negative power function between percent C_4 and distance to the nearest town in the short-grass and desert steppe (Figure III.6) indicating that C_4 doubled when approaching a town.

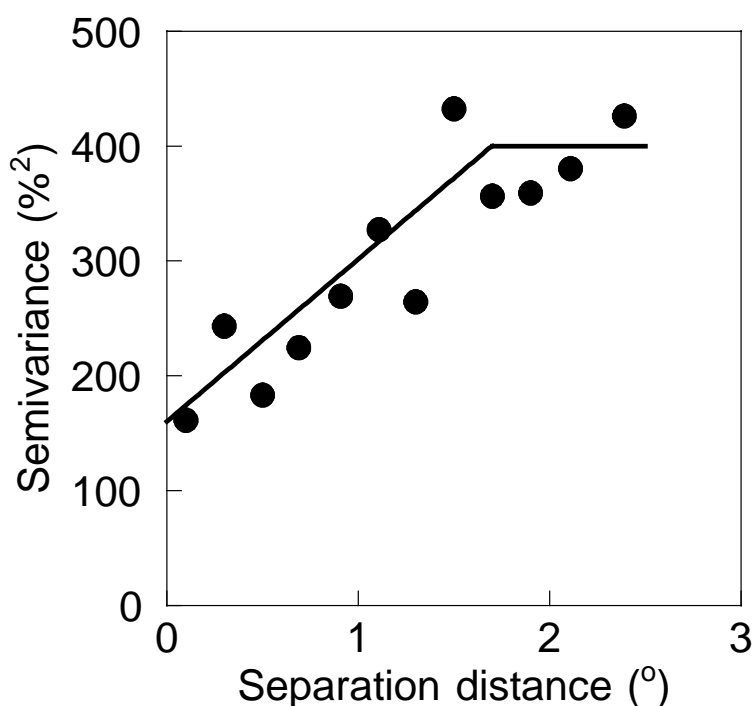


Figure III.4. Empirical (circles) and theoretical (line) semivariogram of percent C_4 in above-ground biomass (in %) calculated from isotopic signatures of vegetation, wool and feces. The theoretical semivariogram is a linear-to-sill model (nugget = $160\%^2$; sill = $400\%^2$; range = 1.7° ; $r^2 = 0.78$). Semivariances were calculated separately for different years (1998-2005) and pooled, to eliminate the influence of inter-annual variations on the overall spatial variation.

The map of C_4 abundance generated from $\delta^{13}\text{C}$ of SOC (assuming a mean age of 50 years for SOC) suggested similar spatial trends of C_4 abundance, albeit at lower absolute levels (Figure III.5b). Interestingly, the $\delta^{13}\text{C}$ of SOC indicated no ‘town-effect’.

Discussion

Isotopic shifts between carbon pools

In ecosystems with a constant composition of plant functional types the SOC is generally enriched in ^{13}C by up to several ‰ relative to standing aboveground biomass (or plant litter), since it derives from former vegetation which assimilated CO_2 with a less negative $\delta^{13}\text{C}$ (‘terrestrial Suess-effect’). In some cases a ^{13}C enrichment during decomposition of soil organic matter is even added to this effect e.g. (Ehleringer *et al.*, 2000; Santruckova *et al.*, 2000), but evidence for the opposite has also been reported (Boutton *et al.*, 1998). Although the magnitude of the ‘constant vegetation’-SOC shift is uncertain (since it depends on the age of SOC, and the magnitude of fractionation during humification which are both unknown), it is highly unlikely that it would produce an overall negative $\delta^{13}\text{C}$ shift. The present

observation of a negative ^{13}C shift therefore indicates that C_4 plants have expanded recently in the region. This observation disagrees with predictions of Collatz *et al.* (1998) for this region, which estimated an expansion of C_3 in response to the industrial-time increase of CO_2 . The observed shift towards more C_4 's may have resulted from regional warming ($+2\text{ }^\circ\text{C}$ increase in mean annual temperature in the last 30 years; (Beijing Climate Center, 2005), and increased disturbance (see below).

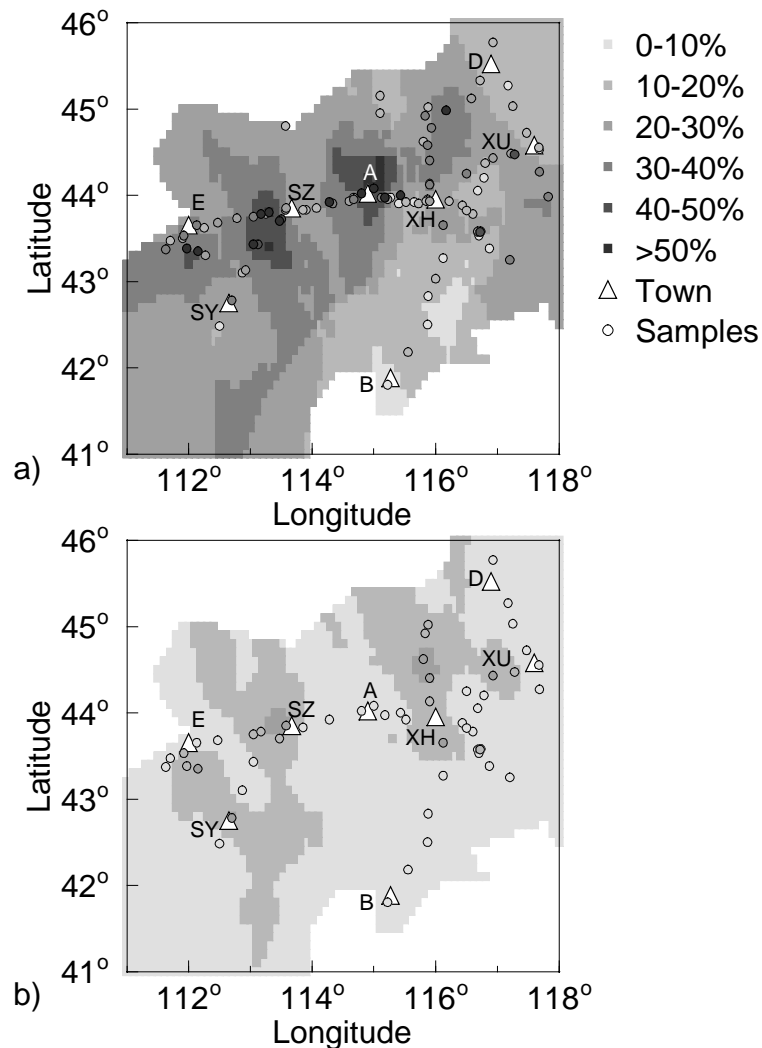


Figure III.5. Percent C_4 regionalized by kriging of C_4 percentage calculated from isotopic signatures of (a) vegetation, wool and feces and (b) soil organic carbon SOC. $\delta^{13}\text{C}$ of SOC was corrected to 2005 using the change in the carbon isotopic composition of atmospheric CO_2 assuming an average age of 50 years for soil carbon. Circles display sampling sites and the respective C_4 percentage. Towns are A = Abag Qi, B = Baochang / Taibus Qi, D = Dong Ujimqin Qi, E = Erenhot, SY = Sonid Youqi, SZ = Sonid Zuoqi, XH = Xilinhot, XU = Xi Ujimqin Qi.

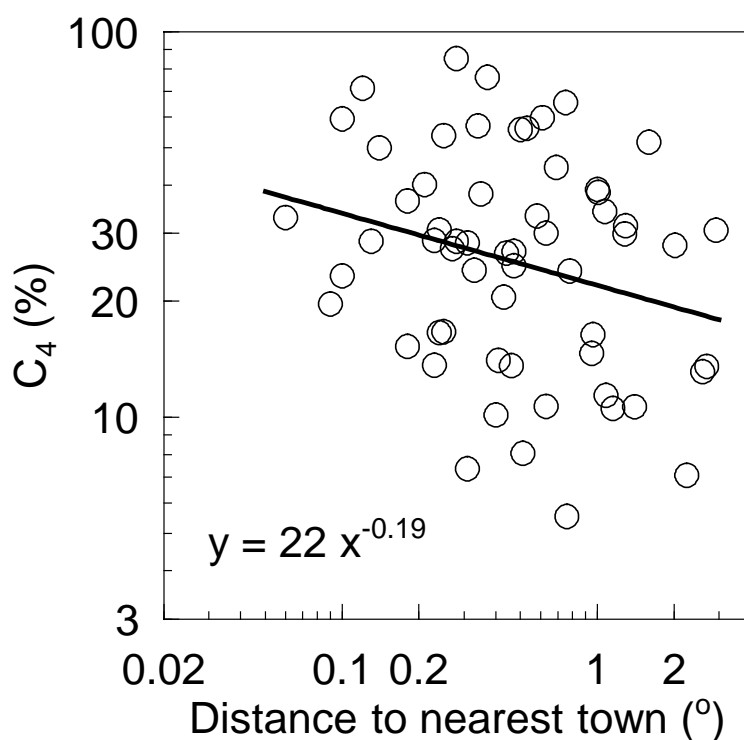


Figure III.6. Average percent C_4 in biomass calculated from the isotopic signatures of vegetation, wool and feces, dependent on the distance to the nearest town. Only sites within the more arid part of the study area (MAP < 275 mm; short-grass and desert steppe) are included (n = 60).

Within the overgrazed steppe of Inner Mongolia livestock consumes a large proportion of the annual net primary production of the C_3 : C_4 mixed grassland of Inner Mongolia (Sneath, 1998).

Preferential grazing of one metabolic type (C_3 relative to C_4 , or *vice versa*) is one mechanism by which grazers can affect vegetation composition (Caswell *et al.*, 1973), and hence carbon pools, fluxes and their isotopic composition. Yet, the present data indicate that there was no systematic preferential grazing of one photosynthetic type. This was suggested by the fact that the observed vegetation-wool (+2.6‰), and vegetation-feces (−1.7‰) shifts were virtually the same as shifts with pure C_3 or C_4 diets (average diet-hair shift: +2.6‰ ± 0.6‰ SD in the studies of (Minson *et al.*, 1975; Jones *et al.*, 1981; Sponheimer *et al.*, 2003a; Ayliffe *et al.*, 2004; Schwertl *et al.*, 2005), average diet-feces shift for ruminants: −1.2‰ ± 0.6‰ SD in the studies of (Jones *et al.*, 1981; Sponheimer *et al.*, 2003a). If animals had grazed preferentially the C_3 component of vegetation (thus consuming biomass with a more negative $\delta^{13}\text{C}$ than that of the whole vegetation), then the vegetation-hair shift must have been smaller, and the vegetation-feces shift more negative. Preferential grazing of the C_4 component would have the opposite effect. Yet, no such effects were observed. Absence of selective grazing may be

related to the high stocking rate in the region, which generates a high grazing pressure and minimizes opportunities for selection. Also, there may be no systematic difference in palatability between the metabolic types (both groups including palatable and unpalatable species; but see (Barbehenn *et al.*, 2004). Indeed, we found no effect of C_4 abundance on nitrogen content of aboveground biomass (data not shown). Further, the congruence of vegetation-hair and vegetation-feces shifts of mixed $\text{C}_3\text{:C}_4$ grassland with those observed in pure C_3 and pure C_4 systems also suggests that the ingested C_3 and C_4 components had a similar digestibility. If, for instance, the C_4 was much less digestible than the C_3 , then feces should be enriched with C_4 -derived biomass, whereas the hair should be depleted. This would result in a smaller (apparent) vegetation-hair shift and a larger vegetation-feces shift, but this did not occur. One might argue that effects of digestibility and selection on isotopic shifts could cancel each other, leading to wrong conclusions. However, this would require that animals select the less digestible component, which runs counter empirical knowledge of grazing ecology (van Soest, 1994). Also, such a compensation could only show up in either wool or feces but not in both at the same time.

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

This study reveals a high variability of $\delta^{13}\text{C}$ in Inner Mongolia grassland at various scales: plant community, farm and region. To a great extent, the structure of this variation was captured by sampling pools with contrasting spatio-temporal integration. Individual vegetation samples give the mean isotopic composition of aboveground biomass grown in a period of weeks to months in an area of approx. 100 m². Feces provide a similar temporal integration (months), but reflect biomass grazed in a period of a few days (Sponheimer *et al.*, 2003c), corresponding to an area of several hectares to km². Whole wool integrates the isotopic signal of feed ingested between two successive shearings (one year) on an area that encompasses the grazing grounds and the haymaking areas of an entire farm (several km²). The pooled wool samples integrated the isotopic signal of the grazed biomass over interannual variations (up to five consecutive years per sites). Since wool grows continuously, it also integrated the isotopic signal of all feed ingested in one year, thus also integrating seasonal variation in ¹³C.

The variation in site-mean wool ^{13}C was about half that observed in vegetation samples. This implies that about half of the total variation in $\delta^{13}\text{C}$ at the plant community level was related to within-farm spatial variation at time scales less than 5 years. Conversely, half of the variation must have been related to longer-term, larger scale patterns in the region, such as climatic gradients and the town effect (see below).

That farm-scale spatial variation accounted for about half of the total variation of $\delta^{13}\text{C}$ in the region was also indicated by the nugget effect which was about half of the total sill of the semivariogram (Figure III.4). Landscape-level heterogeneity in (micro)climate, edaphic conditions and disturbance may all have contributed to variation at the local scale. In particular, farm-scale disturbance gradients probably represent a very large proportion of the regional-scale range of disturbance: disturbance is very high near night paddocks and water stations, and low on hay meadows. Expansion of C_4 's in response to disturbance (by over-stocking or traffic) has been observed in several studies in this region (Li *et al.*, 2006). Interestingly, however, inter-seasonal and intra-seasonal variation in $\delta^{13}\text{C}$ appeared to have only a small influence, as was indicated by the great similarity of $\delta^{13}\text{C}$ variation in wool segments and whole wool samples bulked over several years (Figure III.2).

Regional variation in C_3/C_4 composition is related to environmental gradients and a 'town-effect'

Although growing-season temperature is recognized as the dominant global control of current C_4 distribution (Ehleringer *et al.*, 1997), it had only a weak effect on community-level percent C_4 in Inner Mongolia grassland, explaining only 18% of the total variation in C_4 abundance. Absence of a dominant temperature effect was likely related to several factors: (i) regional variation in growing season temperature was relatively small ($\sim 5^\circ\text{C}$ total range), (ii) growing season temperature was close to or within the reported range of C_3 - C_4 transition temperature (compare with Table 2 in Ehleringer *et al.*, 1997), (iii) there is a large variability in quantum yields among the C_4 subtypes (including grasses and dicots) that are present in the regional flora, implying a range of different C_3 : C_4 transition temperatures and (iv) there are other environmental controls on C_4 abundance, including a hitherto undescribed 'town-effect', disturbance, soil fertility and aridity.

The observed soil nitrogen gradient may be partly due to desertification effects related to overgrazing. Stocking rates of the grassland of Inner Mongolia have increased almost 10-fold between 1949 and 1989 (SBIMAR, 1999), causing overgrazing in vast regions and consequent desertification (Sneath, 1998). Soil nitrogen concentrations below 0.2% appeared in the more arid parts of the study as well as near towns ('town-effect'). These sites exhibited a low vegetation cover and a stone cover on the soil surface. These soils are suspected to have suffered heavy wind erosion causing a depletion of organic matter from the soil (Li *et al.*, 2003). Decreased soil nitrogen may be caused by losses of top soil through erosion by wind or heavy rain. Susceptibility to erosion is greatly increased by overgrazing and other sources of disturbance such as vehicle tracks. Low nitrogen (Wedin & Tilman, 1996) and disturbance may both promote increases in C_4 abundance. Disturbance related to overgrazing (Wang, 2002a) or vehicles (Li *et al.*, 2006), was observed to promote the spread of C_4 plants in East Asian grasslands, including Inner Mongolia steppe ecosystems. Disturbance by overstocking and traffic may also be enhanced in the vicinity of large settlements, contributing to the 'town effect'. As it is related to stocking rates, which have greatly increased in the second half of the 20th century, disturbance may also be a main driver of the recent regional-scale increase of C_4 's.

ACKNOWLEDGEMENTS

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Chapter IV. TEMPORAL TRENDS IN CARBON AND NITROGEN ISOTOPE SIGNATURES OF THE INNER MONGOLIAN STEPPE: EFFECTS OF GLOBAL OR REGIONAL CHANGE?

ABSTRACT

The earth of the 21. century underlies a distinct global change including variations in climate and atmospheric CO₂ concentration that influence the competitiveness between C₃ and C₄ plants in temperate grasslands. Indeed temporal variations in C₄ abundance related to global changes may experience a modification by regional climate or local human activity. Fractions of C₃/C₄ plants to overall phytomass can be detected by their contrasting ¹³C discrimination. ^δ¹⁵N of plants is an indicator of the nitrogen cycle, with elevated ^δ¹⁵N indicating N-losses. Here we quantify the temporal variation of ^δ¹³C and ^δ¹⁵N in woolen textiles obtained within an area extending over 300 000 km² in the steppe of Inner Mongolia. Thus we could assess the variation in C₄ abundance in relation to global changes in atmospheric CO₂ or climate and regional changes in land use. This work states that local woolen textiles are an appropriate tool for ecosystem studies dealing with temporal variations, as they record and conserve the isotopic information of the grazed pastures. Moreover we could assess that average discrimination Δ_{hair} between air-C and wool-C of single decades increased by 0.7‰ per 100 mm mean annual precipitation indicating an increase in the ¹³C discrimination of C₃ plants and a decrease in the abundance of C₄ plants. Absolute Δ_{hair} for different decades decreased after 1960 (- 0.9‰) and 2000 (- 2.9‰). ^δ¹⁵N of single decades increased by 1.0‰ per 100 mm mean annual precipitation, reflecting increased gaseous nitrogen losses with decreasing precipitation. ^δ¹⁵N exhibited no temporal variation, indicating constant humidity conditions for periods lacking climate data. Average C₄ abundance increased within two steps from 2% (1928-57) to 9% (1958-97) and 25% (1998-2005). Simultaneous increasing scatter indicates increasing system instability. No significant trends in C₄ abundance and scatter occurred within any period. These findings contradict predicted decreases in C₄ abundance due to rising CO₂ concentrations. This effect seems to be suppressed by a combination of rising regional temperature, increased human impact and short-term weather events, all

favoring the spread of C₄ plants. A geostatistical analysis assessed that spatial patchiness increased for the period 1963-98, while period 1999-2005 exhibited a distinct increase in C₄ abundance around towns in the dessert steppe that seemed to result from enhanced disturbance/overgrazing. This work substantiates an increase in C₄ abundance in the Inner Mongolian steppe that can be ascribed to regional changes in land-use combined with drastic weather events and exceptionally over-regional changes in temperature suppressing global effects related to atmospheric CO₂ concentration.

INTRODUCTION

The earth of the 21. century is subject to a substantial human alteration including transformations that occur on a worldwide scale or exhibit sufficient cumulative effects to have worldwide impact, leading to a global change (Field *et al.*, 1992; Vitousek *et al.*, 1997; Walther *et al.*, 2002). Human impact has transformed 30-50% of land surface (Vitousek *et al.*, 1986) and influences strongly the carbon and nitrogen fluxes on a global scale. Atmospheric CO₂ concentration increased by about 30% since the beginning of the industrial revolution (Vitousek, 1994) and today more nitrogen is fixed by human activity than by all natural terrestrial sources combined (Galloway *et al.*, 1995). Rising CO₂ concentrations enhance the greenhouse effect and affect climate and will lead to further climate change in the prospective century (Intergovernmental Panel on Climate Change, 2007).

In the huge steppe ecosystem of East Asia C₃ and C₄ plants coexist. Changes in the atmospheric CO₂ concentrations influence the competition between C₃ and C₄ plants favoring C₃ plants at higher concentrations (Ehleringer *et al.*, 1991). Based on the quantum yield of photosynthesis Collatz *et al.* (1998) modeled the global C₄ abundance for recent and pre-industrial times and predicted a reduction of C₄ contents for most temperate grasslands due to rising CO₂ concentrations. According to their model the effect of rising CO₂ is the most influencing factor for the variation in C₄ abundance despite simultaneously climatic changes occurred within the steppe of East Asia (Beijing Climate Center, 2005), that could affect the C₃/C₄ distribution within this area. The influence of these global changes on the C₃/C₄ distribution can be modified by local human activity. Changes in land use that lead to overgrazing and severe degradation of the highly sensible steppe ecosystem may promote the spread of C₄ plants (Pyankov *et al.*, 2000) counteracting the rise in atmospheric CO₂. However little is

known about environmental or human induced changes in the Central Asian steppe, as the whole area is thinly settled, exhibits poor socioeconomic structures and was politically isolated for ages.

Carbon and nitrogen stable isotope signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of system pools provide a quantitative method to follow the movement of elements and organisms across landscapes and between components of the biosphere on a global or regional scale (Fung *et al.*, 1997; Martinelli *et al.*, 1999). Differences in $\delta^{13}\text{C}$ signatures of the ecosystem give evidence on variations in the proportions of C_4 and C_3 plants that differ in their ^{13}C discrimination, which generates a $\delta^{13}\text{C}$ difference of about 16‰ between them (Farquhar *et al.*, 1989). However, the ^{13}C signature of C_3 plants is also sensitive to water stress (Ehleringer & Cooper, 1988) and altitude (Körner *et al.*, 1988), which may cause variation up to 2-3‰.

The $\delta^{15}\text{N}$ of plants relates to the openness of the nitrogen cycle, with elevated $\delta^{15}\text{N}$ indicating losses of nitrogen from the system (Högberg & Johannisson, 1993). This effect results from ^{15}N discrimination in processes leading to nitrogen losses, and is especially true for volatilization of NH_3 and N_2O (Högberg, 1997). Several studies have shown, that arid ecosystems exhibit high gaseous losses of nitrogen e.g. (West & Skujins, 1977; Klubek & Skujins, 1981), though volatilization increases with decreasing precipitation on a global scale, leading to higher $\delta^{15}\text{N}$ signatures (Amundson *et al.*, 2003). Precipitation gradients for $\delta^{15}\text{N}$ were found for different ecosystems, whereas the slope differed due to regional conditions affecting nitrogen fluxes e.g. (Heaton, 1987; Austin & Sala, 1999). Changes in climatic conditions and land use should influence the nitrogen cycle of an ecosystem and affect its $\delta^{15}\text{N}$ signatures due to changes in microclimate and soil nitrogen status.

In grassland studies integrated isotopic information can be collected from mammalian grazers' tissues which incorporate the isotopic composition of diet collected from large grazing areas over extended periods (Cerling & Harris 1999). Recently, hair has attracted particular interest because of the potential to resolve isotopic signals in time (Schwertl *et al.*, 2003a). However, to be useful, there must be a known systematic relationship between the isotopic composition of hair and of grazed vegetation. Indeed, the C and N isotope composition of hair follows that of the diet but exhibits an enrichment (Tieszen *et al.*, 1983), further called diet-hair shift, that underlies a certain range of variation (see Chapter II). In the steppe of Inner Mongolia the shift between vegetation and wool of

sheep and goats amounts to 2.6‰ for $\delta^{13}\text{C}$ (see Chapter III). While grassland has no permanent natural archive with temporal resolution, wool will be stored in form of woolen products at least for a certain time. Though temporal trends should be recorded and filed in ruminant wool, an analysis of the composition of stable isotopes of carbon and nitrogen in hand-crafted woolen textiles from the last century, that could be assigned to certain periods and locations, should allow to indicate change the carbon and nitrogen dynamics within the respective period.

Hence we determine the temporal variation of carbon and nitrogen isotope signatures in the steppe of Inner Mongolia in relation to changes in atmospheric CO_2 , climate and human impact. We prove if changes in the carbon and nitrogen dynamics in the steppe of Inner Mongolia can be detected by isotopic analysis of woolen textiles and try to identify causal relations to global changes in atmospheric CO_2 or climate or regional changes of land use. Moreover we show that overgrazing as effect of regional extension and over-regional temperature anomalies had a much higher impact on the variation in C_4 abundance than effects of global extension.

Hence we will use the wool archive to resolve spatially and temporally the changes in vegetation which are reflected in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and relate this to global climate and regional land use changes.

HISTORICAL OVERVIEW OF LAND USE PRACTICE

During the last century Inner Mongolia has seen large changes in land use as a result of socio-economic conditions (Sneath, 1998). The number of livestock increased between 1949-1986 from 7.7 Mio. to 32.0 Mio. sheep units (Shan, 1996) with a larger increase in the number of sheep (510%) than goats (330%) (Erdenijab, 1996). Between 1986-1998 another increase up to 70.3 Mio. sheep units occurred (SBIMAR, 1999). This increased grazing pressure. Studies suggest that more than 75% of pastures in some regions have been degraded (Sneath, 1998), which in turn increased the pressure on the remaining pastures. On the other hand data from the 1930s indicate that Inner Mongolia supported about the same quantity of livestock at that time (70 Mio. sheep units) as it had in 1990 (Chang, 1933; Inner Mongolian Territorial Resources Compilation Committee, 1987; Sneath, 2000) which may indicate that the observed overgrazing is not directly caused by an increased stocking rate but by a decreased bearing capacity e.g. due to keeping conditions. Case studies found the highest levels of

degradation in districts with the lowest livestock mobility (Sneath, 2002). A low livestock mobility prevents that grazing can be adapted to the high inter-annual and regional variability in rainfall and subsequently in primary production, which is typical for Inner Mongolia. E.g., the long-term variation coefficient of annual precipitation at meteorological stations in inner Mongolia is 30-40% indicating the large inter-annual variability and the correlation in annual precipitation between neighboring stations mostly yields $r^2 < 0.5$ demonstrating the large spatial variation.

Historically, most of this region was inhabited by Mongolian mobile pastoralists, who lived in felt tents and moved with their herds to different seasonal pastures. The herding practice was based on general access to the bounded pasture territories. Animals were herded by monastery serfs or banner princes' serfs. These institutions were replaced in the 1950s, and the pastoralists were collectivized in 1957, loosing most of their pastoral mobility (Humphrey & Sneath, 1995). Thousands of square kilometers of land were damaged by attempts to plow steppe land for crop cultivation in the late 1950s and the 1960s (Erdenijab, 1996). These and other policies have reduced or eliminated large-scale pastoral movements between seasonal pastures and increased the tendency toward year-round grazing of livestock in specific areas.

The collectives of pastoralists were dissolved again in the early 1980s, and individual pastoralists gained ownership of livestock. After 1990 the pasture land was divided into individual allocations for each herding household. The strong rise in China's economic market after 1995 enhanced the demand for agricultural products and simultaneously improved the flow of goods within and into Inner Mongolia. Feeding supplement became accessible which allowed to get more animals through the winter but also to decrease grazing pressure in early spring.

MATERIALS AND METHODS

Study area

The study area comprises the semiarid to arid grassland within the East Asian Steppe where climatic conditions allow a coexistence of C_3 and C_4 plants. This area is situated between $111^{\circ}38'$ and $117^{\circ}49'$ E (approx. 500 km) and $41^{\circ}48'$ and $45^{\circ}46'$ N (approx. 450 km) in the Autonomous Region of Inner Mongolia in the Peoples Republic of China. Altitude ranges from 800 to 1500 m a.s.l. Climatic data

for the region were obtained from Climate Source Inc., Corvallis, Oregon. Mean annual precipitation (MAP) exhibits a spatial gradient from 100 mm in the western part up to 400 mm in the eastern part of the region (see Figure III.1). Most of the precipitation (65-75%) falls during summer (June-August). Soil nitrogen contents exhibit a similar spatial pattern as MAP (see Chapter III.). The spatial distribution of temperature is more variable, following altitudinal and latitudinal trends. Mean annual temperature and mean temperature of the vegetation period (May-September) vary from 0-6°C and 14-19°C respectively. The soils in the eastern part area mostly Haplic Kastanozems and Chernozems on loesslike substrates, whereas Regosols and Luvic Kastanozems are more common in the western part (FAO, 1997). Almost the whole area is used for small ruminant livestock production (sheep and cashmere goats). On most sites animals graze from spring till winter depending on snow cover and availability of herbage. Some herders operate a semi-nomadic production system with seasonal pastures farther away from their primary residence. In overgrazed areas grazing is suspended during winter and/or early spring to allow for recovery of vegetation. Animals do not receive any 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. On most sites grazing qualification and confinement feeding was not practiced prior to 2000.

Sampling

Samples were collected in August/September 2003 and 2004. Soil was sampled to a depth of about 10-15 cm on 56 sites. 414 wool samples originating from the period 1928-2005 were collected at 99 sites. As the animals are sheared only once a year in June their wool samples mostly contain the isotopic signal of the vegetation of the previous year and were dated accordingly.

Recent wool samples were taken from dated fleece sheared by the herders or were directly obtained during sampling trips, while older wool was provided from the inventory of the farmers. Moreover at every site samples were taken from woolen material (clothes, mats, tents etc.) if it concerned self-made by wool of the respective farmer's animals. Only those materials were sampled for which the temporal origin of the sample could be obtained.. However, errors in time assignment are likely and may amount to of +/- 5 yr especially for older samples.

Sample Preparation

All samples were air dried during collection in the field. In addition soil samples were dried 48 hours at 40°C prior to milling. Soil was sieved with a mesh size of 2 mm. Then samples were ground to homogeneity with a ball mill. Inorganic C was removed from the soil prior to isotopic measurement using an HCl fumigation method as described by Harris *et al.* (2001).

The wool samples were cleaned by the procedure of Schwertl *et al.* (2003a). In brief, samples were soaked in distilled water over night to loosen dirt particles, placed in an ultrasonic water bath for 30 minutes, transferred to a solution of two parts Methanol and one part Chloroform, and then placed on a shaker for two hours to remove the adipose. The clean wool was dried for 48 hours at 40 °C in a forced drought drying oven. If the staple structure of wool was still intact two replicates of narrow staples were taken from each wool sample and cut into 1 cm-long segments. Individual segments were analyzed separately. About 0.2-0.4 mg of each segment were then packed into tin cups (5 * 9 mm).

Isotope and elemental analysis

The carbon and nitrogen isotope composition of each sample was determined with an elemental analyser (NA 1110; Carlo Erba, Milan) interfaced (ConFlo III; Finnigan MAT, Bremen) to an isotope ratio mass spectrometer (Delta Plus; Finnigan MAT). Isotopic data are presented as $\delta^{13}\text{C}$ relative to the Vienna Pee Dee Belemnite standard and $\delta^{15}\text{N}$ relative to air nitrogen as: $\delta X = (R_{\text{sample}}/R_{\text{standard}}) - 1$, where δX is $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ and R is the $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ ratio respectively. Each sample was measured against laboratory working standard gases (CO_2 and N_2), which were previously calibrated against IAEA secondary standards (IAEA CH6 and NO3, accuracy of calibration $\pm 0.06\text{‰}$ and $\pm 0.19\text{‰}$). After every tenth sample a solid internal lab standard (SILS) with similar C/N ratio as sample material (fine ground wheat flour for soil samples, protein powder for wool) was run as a control. The SILS were previously calibrated against international standards (IAEA-CH6 and –NO3).

Climate data

For each site we defined the GPS xyz coordinates. Climate data averaging 1961-1990 were taken from a high resolution map (approx. 2.2 x 1.6 km) obtained from Climate Source Inc., Corvallis, Oregon.

Estimation of C₄ fraction

Due to the increase of fossil CO₂ in the atmosphere the $\delta^{13}\text{C}$ signature of atmospheric CO₂ decreases over the years according to the ‘Suess Effect’ (Keeling, 1979). This decrease is also recorded in the vegetation and the animals grazing on it and requires a correction for the calculation of C₄ abundance from $\delta^{13}\text{C}$ signatures of different points in time. Therefore we used the discrimination Δ_{hair} between air and animal hair, which remains independent of the trend in air $\delta^{13}\text{C}$, to estimate the C₄ abundance for different points in time. Discrimination can generally be calculated with the following equation (Farquhar & Richards 1984):

$$\Delta = (\delta^{13}\text{C}_{\text{source}} - \delta^{13}\text{C}_{\text{product}}) / (1000 + \delta^{13}\text{C}_{\text{product}}) * 1000 \quad (\text{Eq. IV.1})$$

For Δ_{hair} the wool is the product while air as source was determined for every point in time by two empirically obtained functions based on measured values of several gauging stations and ice cores (Figure IV.1).

To obtain the C₄ abundance from Δ_{hair} , a simple two components mixing model was used. End members are given by the discrimination of pure C₃ (Δ_{C3}) and C₄ (Δ_{C4}) vegetation and the diet-hair shift. Δ_{C4} was 5.6‰; $\pm 0.3\%$ CI determined from 23 samples obtained in 2004 in the Steppe of Inner Mongolia. Δ_{C3} was 17.7‰ for the average MAP (250 mm) of the study area but varies with MAP (Chapter III) according to:

$$\Delta_{\text{C3}} = 17.7\text{‰} + 0.008\text{‰} \times \text{MAP} \quad (\text{Eq. IV.2})$$

The diet-hair shift within the study area ($+2.6\text{‰}$ $\delta^{13}\text{C} \approx -2.6\text{‰}$ Δ) was constant along environmental gradients (see Chapter III). The proportion of C₄-C in samples (P_{C4} , %) was then estimated as:

$$P_{\text{C4}} (\text{‰}) = 100 * ((\Delta_{\text{hair}} + 2.6) - \Delta_{\text{C3}}) / (\Delta_{\text{C4}} - \Delta_{\text{C3}}). \quad (\text{Eq. IV.3})$$

P_{C4} was also calculated from $\delta^{13}C$ of SOC by assuming that the total shift between vegetation and soil was 1.55‰ for a SOC age of 50 years corresponding to the $\delta^{13}C$ decrease of atmospheric CO_2 by 1.55‰ in the last 50 years (Figure IV.1). For detailed discussion of estimated soil age see Chapter III.

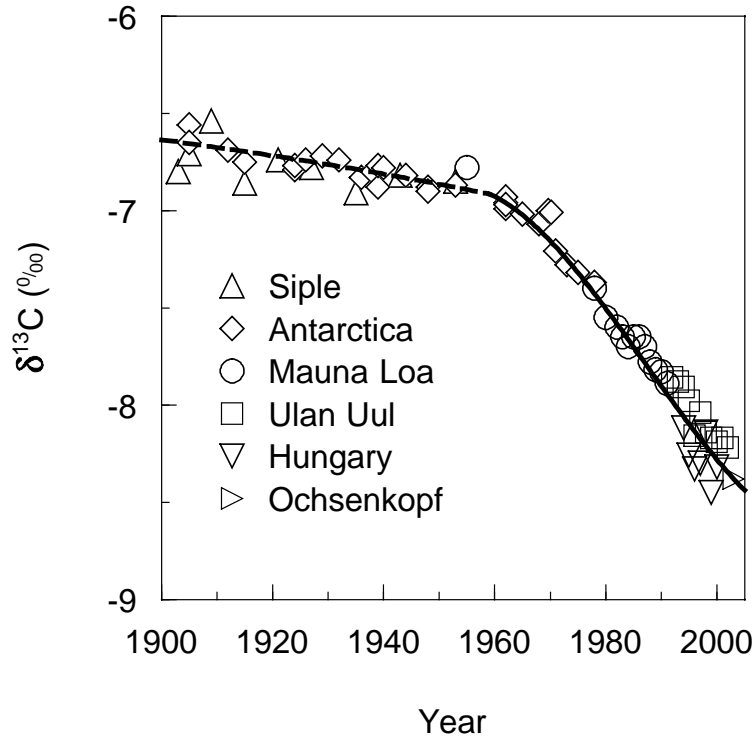


Figure IV.1. Temporal trend of air $\delta^{13}C$ based on isotopic measurements from two ice-cores (Siple, Antarctica) and 4 gauging stations. The dashed line displays the decrease in signature from 1900-1958 ($y = -26.52 x^3 + 136.24 x^2 - 234.28 x + 128.56$; $x = \text{kyr}$) while the solid line displays the decrease from 1959-2005 ($y = 11707.12 x^3 - 69789.99 x^2 + 38640.1 x + 91785.48$; $x = \text{kyr}$). Data are obtained from (Keeling, 1979; Friedli *et al.*, 1986; Conway *et al.*, 1994; Gat *et al.*, 2001; NOAA, 2006).

Statistical evaluation of period-specific variations in Δ

The Δ of different samples varies along the MAP gradient and also with age. Preliminary analysis had shown that the variation with time did not follow continuous functions but varied between periods. For any period a linear change of Δ with MAP leads to a linear model following Equation II.2 [$\delta^{13}C = a + (b \times c)$], where a is the intercept, b is the slope of Δ with MAP (‰ mm^{-1}), and c is MAP (mm). As long as there is no effect of precipitation on the temporal variation in Δ , the same model should apply for the other examined periods. To account for both sources of variation simultaneously, Δ was statistically evaluated using a regression containing dummy variables (Fox, 1997). The data were grouped according to periods and assigned to dummy variables (D1, D2,...) which are 1 for samples

of a specific period and zero for all other periods. Following Equation II.3 [$\delta^{13}\text{C} = a + (b \times c) + (d \times \text{D1}) + (e \times \text{D2}) + \dots$], the difference in Δ for period 1 and period 2 compared to a reference period is given by d and e , if D1 denotes period 1 and D2 period 2.

Where the overall slope b does not apply to a period this becomes evident as a statistically significant deviation between the data of this group and the regression to one side for the upper half and a deviation to the other side for the lower half of the precipitation gradient. To examine this effect the average deviations $\text{av.}(\Delta_{\text{pred.}} - \Delta_{\text{meas.}})$ for the upper and lower half within each group were computed and statistically compared.

Geostatistical analysis

In addition to MAP other environmental and land use variables may influence C_4 distribution. Due to the multitude of influences and their mutual interactions, it is impossible to examine these influences by regression techniques. All spatial influences, however, will cause spatial autocorrelation. Applying GS+ for Windows (Version 3.1) and SAS (Version 9.1), semi-variograms and ordinary kriging were used to quantify the degree and range of autocorrelation and to display the resulting spatial pattern.

RESULTS

Temporal variation of isotopic signatures

Δ_{hair} increased highly significantly ($p < 0.001$) with MAP for old and recent wool samples (Figure IV.2.). Average Δ_{hair} of recent samples was lower compared to old samples. There is no general shift towards lower Δ_{hair} but some samples with extraordinarily low Δ_{hair} appear among recent samples, while the upper range of Δ_{hair} is similar in old and recent wool. Furthermore, the relation to MAP becomes steeper for recent samples indicating that the temporal change in average Δ_{hair} , varies in space.

Analyzing the change in time with higher temporal resolution may be biased by the influence of MAP if the range of MAP is not homogeneously covered by the samples within a certain period of time. To avoid this bias, the Δ_{hair} was corrected for MAP prior to a detailed analysis of the change in time. The slope of new samples (0.009‰/mm) was used for the correction as old samples cover a huge

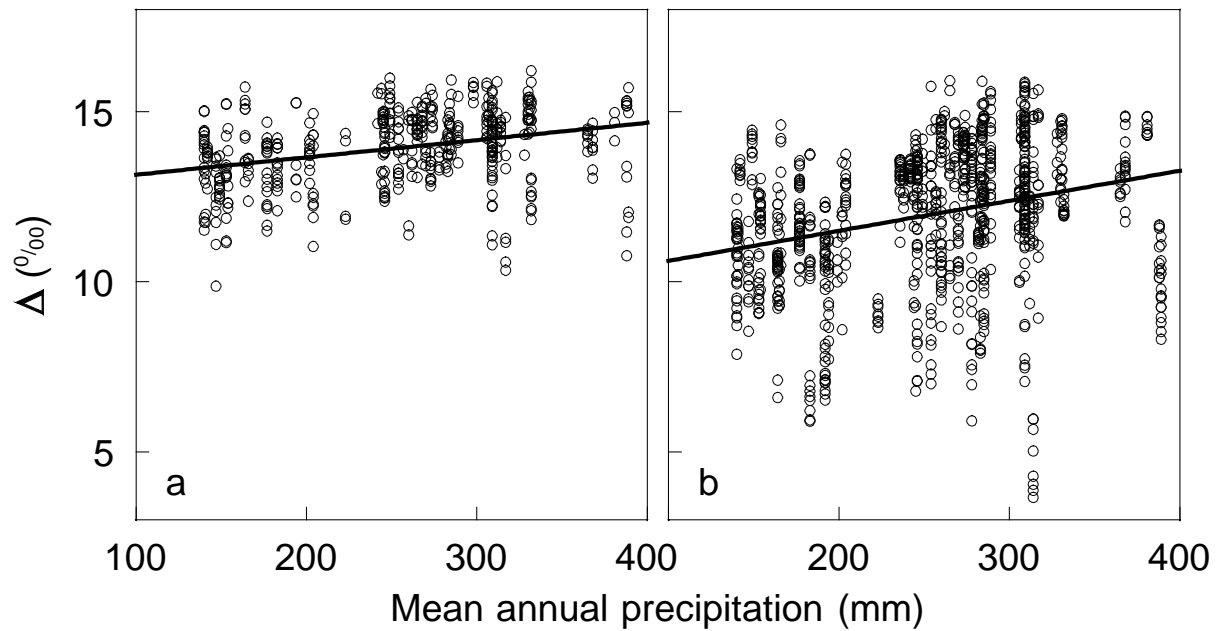


Figure IV.2. Variation in Δ_{hair} with mean annual precipitation for (a) old wool samples (1928-97; $n = 459$; $y = 0.0051x + 12.6$) and (b) recent wool samples (1998-2005; $n = 948$; $y = 0.0088x + 9.7$).

period in which temporal variations in Δ_{hair} lead to a lowering of the gradient in MAP compared to precipitation gradients with higher temporal resolution (data not shown). Furthermore, the number of samples decreased strongly with increasing age (e.g., 1009 samples within 1996-2005 but only 4 samples within 1926-1935). For normally distributed data one can expect that the range of measured values is much larger for recent than for old samples, which is only caused by this discrepancy in sample numbers even without a change in the variability of the population. To analyze the temporal trend and to avoid the bias by the sample number, the following approach was applied. Averages of 10-yr moving windows were calculated, which ascertains that enough samples exist in any period of time ($n_{\text{min}} = 11$, $n_{\text{max}} = 1009$) with still an annual resolution. To allow comparing the averages, their 95% CI is calculated and additionally the SD is given to quantify changes in variability (Figure IV.3.). Surprisingly, there was no continuous trend in temporal Δ_{hair} that changed significantly at two breakpoints, 1957 and 1997, with no trend in between breakpoints leading to three periods differing in average Δ_{hair} (Table IV.1.). The trend in the moving average after 1997 is mainly an artifact of the moving average and mostly disappears, when using a shorter time window (see r^2 in Table IV.1.).

Correlating the uncorrected Δ_{hair} with MAP again yields highly significant ($p < 0.001$) correlations for all three periods (Table IV.2.). The slope was not statistically different among all

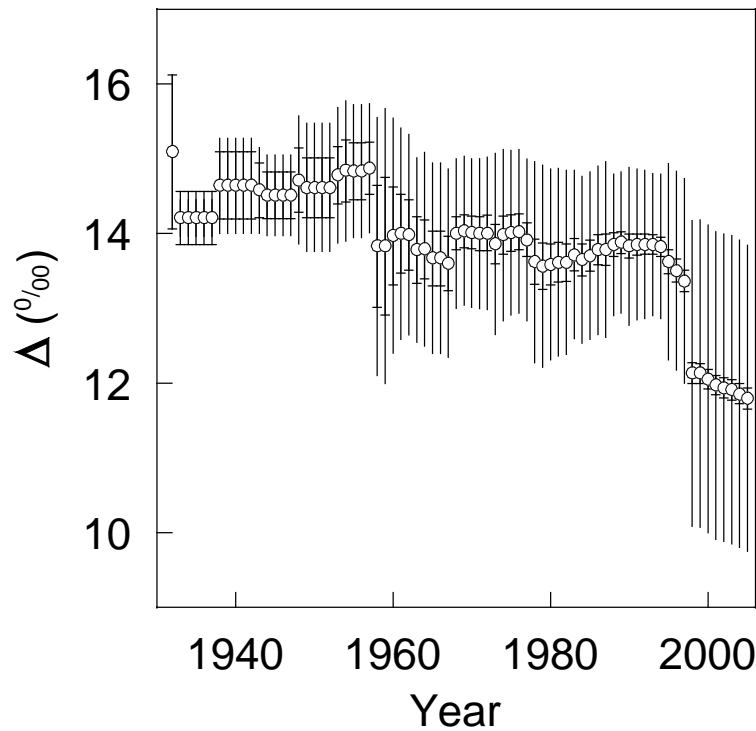


Figure IV.3. Moving average of Δ_{hair} for 10-years periods, based on Δ_{hair} of single samples ($n = 1407$). Δ_{hair} of single samples (Δ_{sample}) was adjusted to eliminate the influence of MAP on Δ by the equation: $\Delta_{\text{hair}} = \Delta_{\text{sample}} + (m \Delta_{\text{MAP}})$, where Δ_{hair} is the Δ at a MAP of 250 mm, Δ_{MAP} (mm) is the site-specific deviation of MAP from the base situation (250 mm), and m is the change of Δ_{hair} per unit change of MAP (0.009‰/mm) as found above. Error bars give the 95% CI (with end line) and SD (without end line).

Table IV.1. Statistical characteristics of Δ_{hair} for three distinct periods and r^2 of the temporal trend within the periods (in contrast to Figure IV.3, Δ_{hair} is not corrected for MAP because a sufficient number of samples exist in any period to cover evenly the whole range of MAP).

Period	n	Average	SD	95% CI	r^2
1920-57	30	14.6	0.9	0.3	0.00
1958-97	429	13.9	1.1	0.1	0.03
1998-2005	948	12.0	2.1	0.1	0.06

Table IV.2. Mean absolute deviation between measurement and the dummy regression of Δ_{hair} versus MAP for different periods and mean deviation for the lower and upper half of the slopes (significance column indicates a significant deviation between both halves). Different letters within the columns denote significant differences at $p < 0.05$.

Period	Δ (‰) vs. MAP					
	abs. deviation			mean deviation		
	n	mean	Significance	lower half	upper half	Significance
1920-57	30	0.65	A	0.10	-0.12	n.s.
1958-97	429	0.82	B	0.08	-0.14	n.s.
1998-2005	948	1.59	C	-0.23	0.16	n.s.

periods, as no significant difference in the mean deviations between regression estimates and measurements were observed in the upper and lower half of the precipitation range in any of the groups. Besides the absolute discrimination, its variation increased with time, especially in the youngest period, which had an absolute deviation from the regression of 1.59‰, which is almost 3 times larger than the deviation occurring in the oldest period.

As precipitation and temperature influence the nitrogen isotope composition of an ecosystem, $\delta^{15}\text{N}$ signatures can act as additional indicator for climatic variability where climate data are lacking. $\delta^{15}\text{N}$ of wool samples exhibited a precipitation gradient ($r^2 = 0.27$; $p < 0.001$) and decreased by 1.16‰ (± 0.05 ‰ SE) per 100mm MAP. Again, no significant difference in the mean deviations between regression estimates and measurements in the upper and lower half of the precipitation range occurred in any period (Table IV.3.), indicating that the discrimination against $\delta^{15}\text{N}$ along the environmental gradient was not modified with time. In contrast to the Δ_{hair} there was no shift in the $\delta^{15}\text{N}$ signatures between periods. The scatter along the gradient increased significantly with time. Climate data for the observed area are available from 1957-2005, while data gaps occur between 1964-73 (NNDC, 2006). Though most recent years exhibited comparatively low precipitation, no clear temporal trends in average precipitation and $\delta^{15}\text{N}$ occurred from 1957-2005 (Figure IV.4). As nitrogen isotope signatures even exhibit no trend for the period 1928-2005, it can be assumed that precipitation on average also remained constant over the whole observed period.

Table IV.3. Mean absolute deviation for the regression of $\delta^{15}\text{N}$ in wool versus MAP for different periods and mean deviation for the lower and upper half of the slopes (significance column indicates a significant deviation between both halves). Differences in the group-specific intercept of the regression give the change in $\delta^{15}\text{N}$ compared to the reference period (1920-57). Different letters within the column denote significant differences at $p < 0.05$.

Period	$\delta^{15}\text{N}$ (‰) vs. precipitation						Change in $\delta^{15}\text{N}$ (‰) compared to period 1920-57	
	abs. deviation			mean deviation			Significance	
	n	mean	Significance	lower half	upper half	Significance		
1920-57	30	0.67	A	0.12	-0.03	n.s.	0.00	A
1958-97	429	0.87	B	0.15	0.02	n.s.	-0.36	A
1998-2005	948	0.98	C	0.05	-0.02	n.s.	-0.12	A

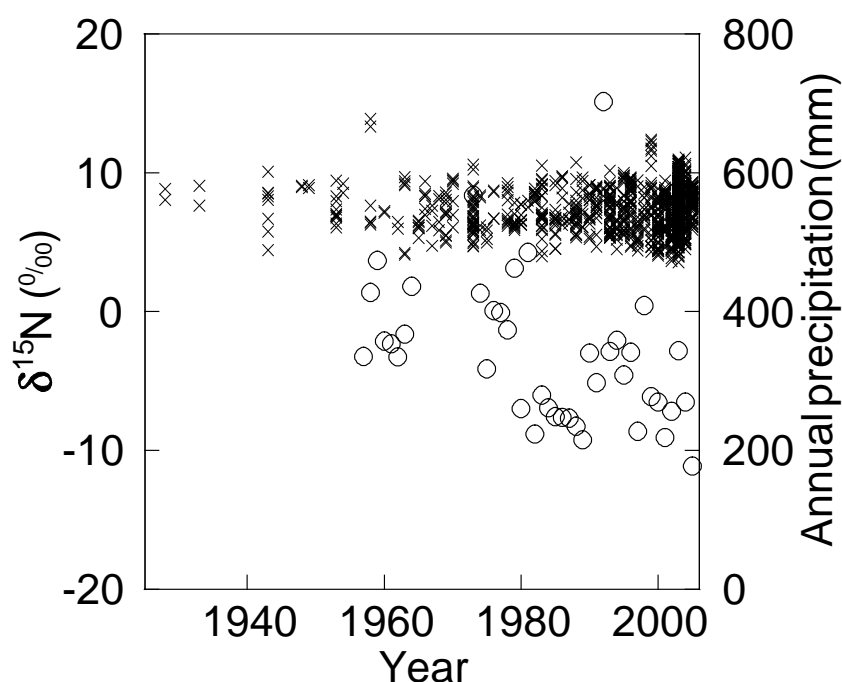


Figure IV.4. $\delta^{15}\text{N}$ of wool samples ($n = 1407$) and annual precipitation (circles) averaged over 9 climate stations (Abag Qi, Bailing Miao, Duolun, Erenhot, Jurh, Mandal, Uliastai, Xilinhot, Xi Ujimqin Qi; (NNDC, 2006) in the range of the sampled area.

Temporal variation of calculated C_4 abundance

The calculated C_4 abundance exhibited a temporal increase that occurred in two steps consistent with the change in average Δ_{hair} (Figure IV.5.). Average C_4 abundance increased from 2% ($\pm 2\%$ CI) to 9% ($\pm 1\%$ CI) after 1957. A second increase occurred after 1997, reaching a new plateau at 25% ($\pm 1\%$ CI). Average C_4 abundance and mean deviation differed significantly between these periods, whereas no significant trend was visible within the periods (Table IV.4.). SOC gave virtually *average* P_{C_4} of 8‰ assuming a mean retention time for carbon in the soil pool of 50 years.

A geostatistical analysis of C_4 abundance yielded semivariograms following linear-to-sill models for the periods 1958-97, 1998-2005 and the soil data (Figure IV.6). No spatial trend occurred for the period 1920-57. Semivariances increased with each period, while semivariances of soil data exceeded that of the first two periods but fell below that of the newest period. Maps of C_4 abundance obtained by kriging (Figure IV.7.) exhibited a C_4 abundance of 0-10% throughout the whole study area

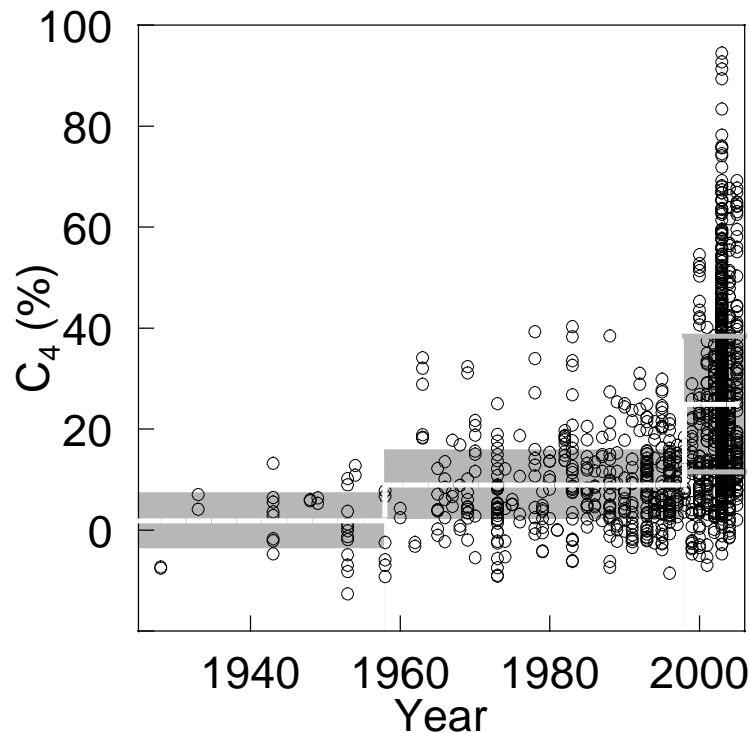


Figure IV.5. Change in C_4 abundance (calculated from Δ) with time. Open symbols indicate single samples ($n = 1407$), dotted lines average values for distinct periods and shaded areas the mean absolute deviation for the respective period.

Table IV.4. Average calculated C_4 abundance, mean absolute deviation and mean deviation for the lower and upper half for three periods differing in C_4 abundance and scatter (significance column indicates a significant deviation between both halves). Different letters within the column denote significant differences at $p < 0.05$.

Period	C_4 abundance (%)							
	Average abundance			Abs. deviation		Mean deviation		
	n	Mean	Significance	Mean	Significance	Lower half	Upper half	Significance
1920-57	30	2	A	5	A	0.6	-0.6	n.s.
1958-97	429	9	B	7	B	0.1	-0.1	n.s.
1998-2005	948	25	C	13	C	-0.6	0.6	n.s.

for the period 1920-57 almost without any spatial pattern (pure nugget effect in the semivariogram). Only at the western margin of the sampled area an average C_4 abundance between 10-20% occurred during this period. During 1958-97 C_4 abundance ranged between 0-20% while patchiness increased without clear pattern. In contrast the most recent period (1998-2005) displayed a clear pattern with C_4 abundance increasing from 10% in the eastern part to about 30% in the western part of the study area towards the Gobi desert corresponding to the spatial variation of temperature, precipitation and the

soil nitrogen. Areas around the three large towns within the shortgrass/dessert steppe (< 275 mm MAP) (Abaga Qi, Sonid Zuoqi, Erenhot) exhibited higher values (partly exceeding 50%) than surrounding areas with similar environmental conditions. Kriging of C_4 abundance calculated from $\delta^{13}C$ of SOC assuming a mean carbon age of 50 years gives evidence for a similar spatial trend with a C_4 abundance ranging from 0 - 25% (Figure IV.7.). However SOC provides no indication for the town-effect.

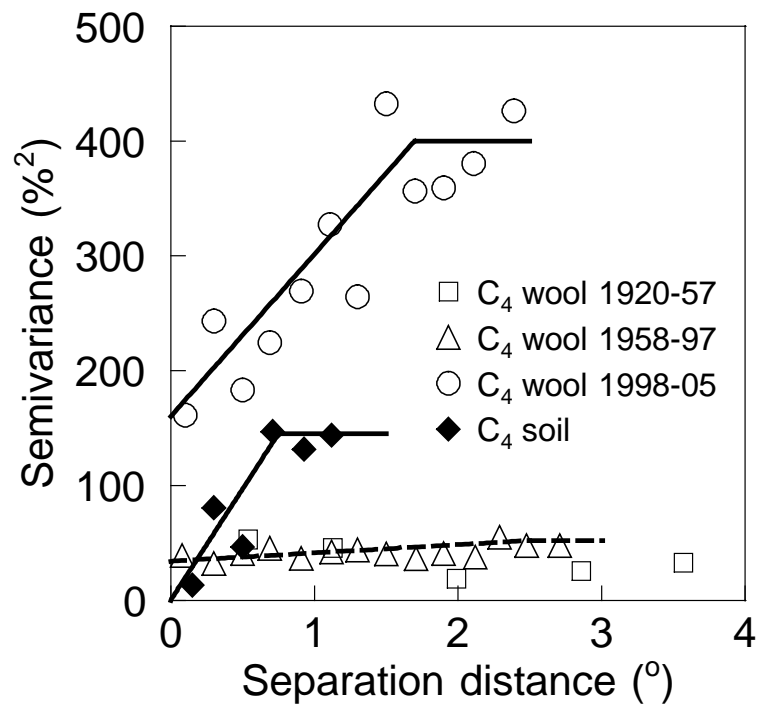


Figure IV.6. Empirical (symbols) and theoretical (lines) semivariograms of percent C_4 in above-ground biomass (in %) calculated from isotopic signatures of wool of three periods and soil. The theoretical semivariograms are linear-to-sill models. No spatial trend occurred for the period 1920-57.

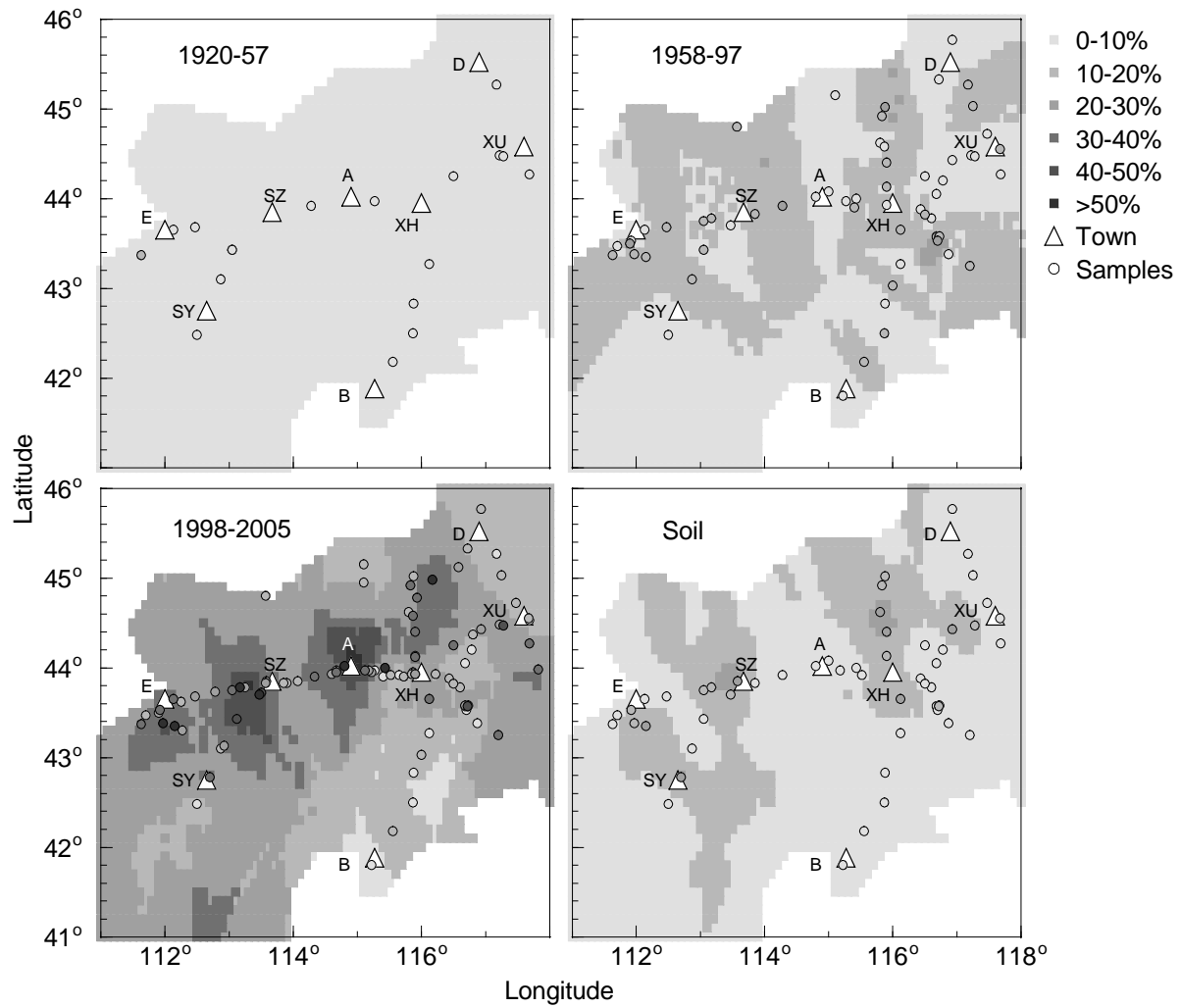


Figure IV.7. Spatial distribution of C_4 abundance within the study area obtained by kriging interpolation of C_4 abundance calculated from isotopic signatures of woolen products for three periods differing in average C_4 abundance and from isotopic signatures of SOC, assuming a mean age of 50 years for soil carbon. Average SD for kriged values at sampled sites was 4% (1920-57 and 1958-97), 14% (1998-2005) and 3% (soil). Circles display sampling sites and their C_4 abundance averaged over all measurements at this site within this period. Towns are A = Abaga Qi, B = Baochang, D = Dong Ujimqin Qi, E = Erenhot, SY = Sonid Youqi, SZ = Sonid Zuoqi, XH = Xilinhot, XU = Xi Ujimqin Qi.

DISCUSSION

Temporal variation of isotopic signatures

The precipitation gradient in Δ_{hair} can be ascribed to two effects. One is the response of C_3 plants to water stress, that lowers the fractionation against ^{13}C (Farquhar *et al.*, 1989). This effect is also visible on sites differing in soil water availability (Chen *et al.*, 2005) and along precipitation gradients (see Chapter III) within the study area. The variation of $\delta^{13}\text{C}$ in C_3 plants with MAP in the study area was in the magnitude of 2‰ (Chapter III). This revealed the need for a correction of the C_3 end-member

according to this parameter in calculations of C_4 abundance by a two-components mixing model. The other effect, responsible for the precipitation gradient in Δ_{hair} is a precipitation-related variation in C_4 abundance. It is founded in the better water use efficiency of C_4 plants, though they can match the CO_2 assimilation of a C_3 plant with about half the stomatal conductance and water loss (Sage, 2002). Moreover C_4 abundance increases with the temperature during the growing period (Ehleringer *et al.*, 1997), which coincides with decreasing precipitation in the study area (Chapter III.). As the effect of variable discrimination of C_3 plants on Δ_{hair} is quantifiable and comparatively low adversely to the effect of variable C_4 abundance, the temporal trend in Δ_{hair} is discussed as effect of variations in C_4 abundance.

The decrease of average Δ_{hair} and the increasing variation in Δ_{hair} from 1920-2005 indicates an increase in C_4 abundance and variability. Geostatistical analysis has shown that the increase in variability is not random but due to the evolution of a spatial pattern. Though a continuous trend of this effect due to temporal trends in environmental parameters could have been expected, moving averages of Δ_{hair} exhibited a decrease within two steps that complied with three periods of constant average C_4 abundance. The increase of C_4 abundance is further combined with a decrease in ecosystem patchiness, portrayed by increasing standard deviation around the average.

The observed $\delta^{15}\text{N}$ gradient with mean annual precipitation is consistent with global trends (Amundson *et al.*, 2003) and can be ascribed to an increase in volatilization losses with decreasing precipitation (Evans & Ehleringer, 1993). The slope and the intercept of the regression remained constant over the three observed periods even when all periods were regressed against the last normal period average precipitation (1961-1990). Hence $\delta^{15}\text{N}$ provides no indication of differences in precipitation between the three periods. The increase in scatter of $\delta^{15}\text{N}$ for each period with higher C_4 abundance is moreover an additional indicator for increased ecosystem instability. Though precipitation data are only available since 1957, the constant $\delta^{15}\text{N}$ signatures since 1928 refers to the absence of a temporal trend in precipitation within the study area.

Temporal variation of calculated C₄ abundance

In contrast to models that predict a decrease in C₄ abundance for wide areas of temperate grasslands due to an increase in atmospheric CO₂ on a global scale (Collatz *et al.*, 1998) C₄ abundance increased in the Inner Mongolian steppe. Significant changes in precipitation are not reported (NNDC, 2006), whereas mean annual temperature in Inner Mongolia increased by 2°C in the period 1951-1999, exceeding by far the global temperature trend (Chase *et al.*, 2000; Schäfer, 2000; Beijing Climate Center, 2005). 75% of this temperature increase can be related to the period from 1979-1997 (Chase *et al.*, 2000). The spatial variation of recent C₄ abundance in the Inner Mongolian steppe indicates an increase of C₄ abundance by 5.5% °C⁻¹ (Chapter III). Applying this spatial trend to the temporal trend observed for mean annual temperature yields a maximum increase of 11% of C₄ abundance due the temperature increase between 1951-99. As this effect should be counteracted by the rise in CO₂ and also should occur continuously with a steeper rise after 1979, it is unlikely that this effect alone is responsible for the stepwise increase in C₄ observed in this study.

As average C₄ abundance and its spatial structure altered within two steps, whereas no significant changes occurred within each period, variations in C₄ abundance can not be ascribed to continuous changes in atmospheric CO₂ concentration or climate. Furthermore, the evolution of a spatial pattern with time is unlikely an effect of a changing temperature or a changing CO₂ concentration. CO₂ and temperature rise should act more or less homogeneously over the entire research area perhaps causing a general trend within the area but certainly not a pattern. Both, the spatial pattern with hot spots around large towns and the discontinuous temporal evolution suggest a connection to effects of human impact like overgrazing and desertification, that increased dramatically during the last 50 years (Xue, 1996). Previous studies showed that overgrazing increases the relative aboveground biomass of C₄ plants or shifts from the dominance of C₃ grasses to the dominance of C₄ plants in the Inner Mongolia steppe ecosystems (Bai *et al.*, 2002). The strengthened human impact is documented in the increasing number of livestock in Inner Mongolia from 1949-98 (7.7 Mio. to 70.3 Mio. sheep units, see Shan, 1996; SBIMAR, 1999) although similar livestock numbers as recently reported have already been observed in former times (70 Mio. sheep units in the 1930s, see Chang, 1933; Sneath, 2000). Case studies, however, found that the highest levels of degradation were reported

in districts with the lowest livestock mobility (Humphrey & Sneath, 1995; Sneath, 2002) because shortage of feed during years of low precipitation can then not be compensated by movement. Mobility indices were hence superior to explain degradation levels than densities of livestock (Humphrey & Sneath, 1995; Sneath, 2002). The collectivization of 1957 with its strong ecological impacts could thus be responsible for the increase in C₄ thereafter.

After 1997 the second and even more pronounced increase in C₄ abundance cannot be ascribed to the loss in mobility anymore because the process of settlement was already finished. This increase was mainly found around large towns in the dessert steppe where a large human impact meets a sensible ecosystem. The intensification in land use following the rise of Chinas economic market after 1995 coincides with this second increase in C₄ abundance and patchiness. During that time the possible to increase the income of herdsman occurred (Peng *et al.*, 2005) and encouraged them to increase their number of livestock again, while simultaneously feeding supplement became available to get more animals through the winter. Detailed information on livestock density within our study area is available for the Xilingol catchment steppe around 43° N and 117° E, that is recently grazed by 25 million sheep (Dr. Yong-Fei Bai; pers. comm., Figure IV.8). Here grazing area per sheep decreased from > 5 ha in 1950 to 1.5 ha in 1962 and slightly oscillated around that level until 1994. After that grazing area per sheep decreased again to < 1 ha. These increases in livestock density within two steps match the socioeconomic processes reported above and the increases in C₄ abundance and patchiness.

Though correlations are obvious, the distinct changes in C₄ abundance within sharp steps make human impact as single controlling factor unlikely. However for the East Asian steppe a series of catastrophic climatic events, including sequences of *dzuud* (icy blizzard) and *gang* (drought) are reported (Fernandez-Gimenez, 1999). Especially during 1999-2001 several severe *dzuud* and *gang* events occurred (Blench, 2004). During the same period and probably related, although also connected to commercial activities, plagues of grasshoppers and rodents which compete with livestock for the remaining grass on the pastures occurred. Interviewed herders described the change in grass from nutritious species to less digestible species as well as the spread of toxic grasses (Blench, 2004). The increased C₄ abundance after 1999 seems to be triggered by these events. From the present state of knowledge it is unclear if similar events influenced the increase in C₄ abundance after 1963 and if the

grassland will recover by its own under the recent livestock pressure. Hence the observed increase in C_4 abundance in two significant steps can probably be referred to a combination of extraordinary rising regional temperature, effects of human impact and short-term weather conditions (*dzuud* and *gang* events), which cannot be compensated by movement. Therefore regional changes affect the temporal variation in C_4 abundance of Inner Mongolia by far stronger than effects of global change.

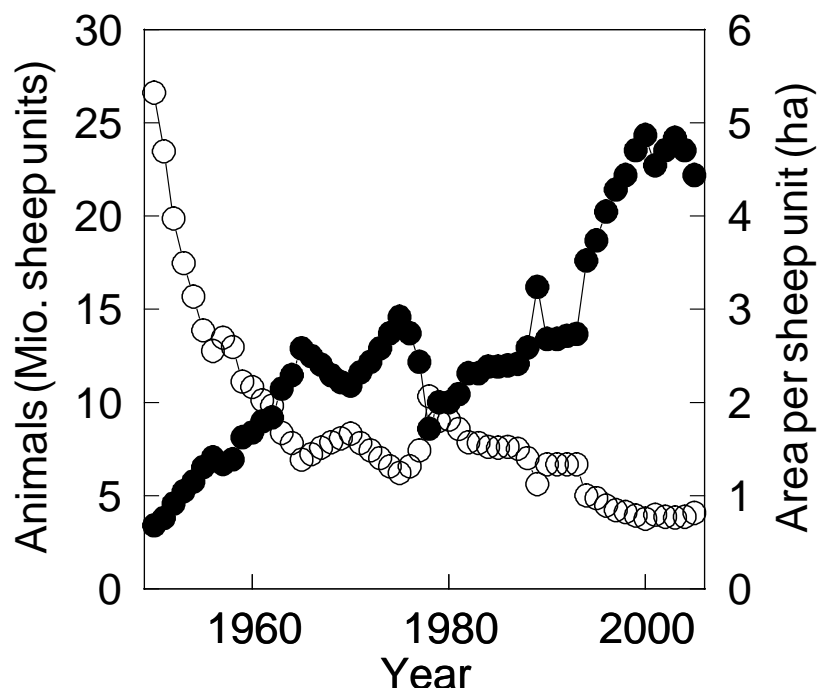


Figure IV.8. Changes in the total number of domestic animals (closed symbols) and the averaged grassland area per sheep unit (open symbols) in Xilingol steppe region from 1950 to 2005.

The calculated C_4 abundance contributing to SOC is similar in average and spatial distribution to the C_4 abundance averaged for the period 1958-1997. This would indicate a rather young SOC age of < 50 yr, which is in contrast to commonly assumed SOC ages (Boutton *et al.*, 1998; Six *et al.*, 1998) but agrees with the SOC ages estimated from ^{14}C near Xilinhot (Dr. Angelika Kölbl; pers. comm.). In some areas SOC indicates even higher abundances, which implies, that very young material (from the period 1998-2005) contributes considerably to SOC in these areas (e.g. near Sonid Zuoqi, north of Xilinhot). This could be expected if old SOC is removed to a greater proportion by soil erosion in these areas (Li *et al.*, 2004) indicating a larger disturbance in these areas than in other areas. These effects are also portrayed by the semivariograms of spatial C_4 abundance, as the semivariances

of soil data are considerably higher compared to the wool samples of the first two periods (Figure IV.6). This strengthens the notion, that the increase and recent spatial pattern of C_4 is caused by disturbances.

CONCLUSIONS

The carbon and nitrogen signature recorded in woolen textiles is a valuable source of spatial and temporal changes in vegetation of steppe ecosystems, which otherwise lack persistent archives. Nitrogen signatures gave no evidence for a temporal trend in precipitation. Carbon signatures showed a stepwise increase in C_4 abundance and patchiness in the Inner Mongolian steppe. This is in disagreement with ecological models that predict a general reduction of C_4 plants due to increasing atmospheric CO_2 . The observed temporal and spatial changes in C_4 abundance most likely result from the combination of rising regional temperature, increased land use intensity, loss of mobility and severe short-term weather events. Variations in the C_4 abundance of the ecosystem in the Inner Mongolian steppe could be clearly related to processes at the regional scale, suppressing the effects of processes on a global scale.

Chapter V. GENERAL AND SUMMARIZING DISCUSSION

The Inner Asian steppe is one of the largest biomes of the earth, but little is known about its temporal and spatial variation. In particular, the C_3/C_4 ratio represents an important parameter that needs to be elucidated due to its impact on the magnitude and seasonal distribution of biomass production, the nutritional value of the aboveground biomass, soil C storage, water use, nutrient cycling and biogeochemistry. Due to the large isotopic contrast between C_3 and C_4 plants stable isotopes can serve as appropriate tool to record the C_3/C_4 ratio in arid temperate grasslands. However, several problems had to be solved. First a method to record and integrate the biomass-weighted C_3/C_4 ratio over time and space had to be introduced. By using the isotopic signature of grazer wool as a proxy for the vegetation at the farm-scale the problem of the unknown diet-hair shift and its potential variation due to environmental factors had to be solved. Moreover environmental parameters like altitude or precipitation could affect the $\delta^{13}C$ of C_3 plants, leading to site-specific signatures that deviate from an average $\delta^{13}C$ signature used for the C_3 end-member in the mixing model. Another problem occurred in the reconstruction of the long-term variation in the C_3/C_4 ratio, as the change in $\delta^{13}C$ of atmospheric CO_2 over time influenced the $\delta^{13}C$ of the vegetation.

USE OF STABLE ISOTOPES TO RECORD SPATIAL AND TEMPORAL VARIATIONS IN TEMPERATE GRASSLANDS

This study focused on stable isotopes to estimate spatial and temporal variations in temperate grasslands. Available approaches used the $\delta^{13}C$ and $\delta^{15}N$ signatures at the plant level (Dawson *et al.*, 2002), but transformations of the results to the ecosystem are challenging, as isotope signatures exhibit distinct variations between plant species (Farquhar *et al.*, 1989) and micro-habitats (Ehleringer & Cooper, 1988). Moreover the life-span of the aboveground phytomass in grasslands is short and reflects growing conditions for a period of weeks to a few months. Also, at the plant-level, the spatial integration of soil conditions is small, as it is limited by the expansion of the root system. A consequence is to look for integrative recorders of the plant-level responses to environmental conditions and their naturally occurring or human induced variations.

Other system pools fed by the carbon and nitrogen of the vegetation reflect its isotope signature integrated over larger periods and/or areas. SOC integrates the isotopic signal over decades up to centuries (Boutton *et al.*, 1998; Six *et al.*, 1998). While SOC reflects growing conditions of plants at spatial scales of only a few m², feces of grazing animals integrate growing conditions of months over several hectares, while grazer wool records the signature of grazed vegetation of several km² under the grazing conditions of Inner Mongolia (Chapter III.). Bulk samples of wool give an integrative signal over one up to several vegetation periods, depending on the availability of the respective samples. As hair integrates the isotopic signal of all feed ingested, it also integrates potential seasonal variation, which has been observed, e.g. in C₃/C₄ ecosystems (Ode *et al.*, 1980), but would not be captured in one-time samplings of grassland vegetation. A higher temporal resolution can be achieved by analyzing single wool segments, that can be assigned to certain periods (Chapter II). The scatter around a regression of isotopic signatures against an environmental driver thus decreases significantly with increasing spatial and/or temporal integration of the archive in focus (Figures II.1., II.2, II.4 & III.2). The combination of several carbon or nitrogen pools, differing in their spatial and temporal integration, is therefore useful to explore the spatial and temporal variation of isotope signatures at the ecosystem or regional scale.

Isotope signatures of animal tissues and feces reflect the ingested vegetation, but tissue-specific isotope shifts occur. The diet-hair shift for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in ruminants was independent of altitude, precipitation, temperature and related environmental parameters (Tables II.1 & III.3). This suggests that any metabolic stress or dietary change associated with the respective parameters had no effect on the shifts. Within the scope of biogeochemical studies this finding is potentially useful, because these animals are extensively used to graze grassland and their hair grows near-continuously, integrating the isotopic signal of the grazed vegetation over large areas and long periods. This confirms that hair can provide a spatially and temporally integrated record of the isotopic composition of the grazed vegetation.

The shift for $\delta^{13}\text{C}$ is larger for goats than for sheep in the Eastern Alps (Table II.2), which agrees with Sponheimer *et al.* (2003a) who observed a higher diet-hair shift in goats than in cattle. Though the shift for domestic sheep has not been studied before, this study suggests that it is close to

that of cattle, and less than that of goats (Table II.2). However this effect was not universal, as sheep and cashmere goats in Inner Mongolia did not differ statistically in their diet-hair shift (Chapter III). The reason for this discrepancy is not known. The lacking explanation for this discrepancy is a major drawback of the method because it makes it necessary to determine the diet-hair shift for every application of the method. The diet-hair shift for ^{15}N varied substantially but was unrelated to N concentration in the diet (Chapters II.), which was in contrast to feeding experiments that used diets that differed exceptionally in protein content (Sponheimer *et al.*, 2003b).

While hair is enriched in $\delta^{13}\text{C}$, feces were depleted compared to vegetation (Table III.1). This depletion was in the same magnitude as reported in literature (Jones *et al.*, 1981; Sponheimer *et al.*, 2003a) and is probably due to contamination in the gut by body tissues or fluids with lower ^{13}C signatures. As all these shifts vary distinctly (Table II.2), they have to be determined under the specific conditions of any study to allow a realistic interpretation of isotope data.

Another uncertainty in the interpretation of isotope signatures in grazer hair and feces is selective grazing, as it influences all products of the ingested diet of an animal. Such selection could be expected as e.g. C_4 plants are often less digestible than C_3 plants (Caswell *et al.*, 1973). Even some domestic ruminants select under certain conditions, as the larger variation in $\delta^{15}\text{N}$ of goat hair in the confinement can be ascribed to a more distinct tendency for dietary selection compared to sheep (Figure II.3). Hence, in studies dealing with isotope signatures in animal tissues it is important to exclude or quantify selective feeding of the animals in focus. As the observed diet-hair and diet-feces shifts (Table III.1) for sheep and goats in Inner Mongolia matched shifts detected in pure C_3 or C_4 systems, it could be concluded that animals in the study area show no systematic preference of one photosynthetic type.

The consistency of vegetation-hair and vegetation-feces shifts along gradients of varying C_4 proportions also suggests that the ingested C_3 and C_4 components had a similar digestibility. Thus, for instance, if the C_4 was less digestible than the C_3 , feces should be relatively enriched with C_4 -derived biomass, whereas the hair should be depleted.

As the $\delta^{13}\text{C}$ of C_3 plants depends to some extent on environmental conditions, some variations in the isotopic signature of grazer wool occur independent of changes in the C_3/C_4 ratio. Hence

correction factors for the signature of the C₃ end-member in the mixing model had to be developed. The correlation between $\delta^{13}\text{C}$ of C₃ plants and an environmental parameter could be determined on the ecosystem or regional scale of a pure C₃ environment, whereas a global validity of the respective effect had to be proven. A regional variation in the signature of the C₃ end member could be expected from the influence of altitude (Körner *et al.*, 1988). In the Eastern Alps a linear relationship between $\delta^{13}\text{C}$ of ruminant hair and altitude was detected ($\sim 1.1\text{‰ km}^{-1}$; Figure II.2). The same changes occurred in local grassland vegetation, and in regional to global grassland data sets (Figure II.5).

Moreover, $\delta^{13}\text{C}$ of C₃ plants often decreases with precipitation (Schulze *et al.*, 1998; Liu *et al.*, 2005), though absence of a response to a precipitation gradient in arid regions has also been observed e.g. (Schulze *et al.*, 1996). The gradient of this precipitation effect depends on the vegetation composition, as different species operate at different WUE. Hence, a global validity of this effect was not assumed. Therefore we analyzed $\delta^{13}\text{C}$ of single C₃ plants along a precipitation gradient in Inner Mongolia, created biomass-weighted site-specific C₃ signatures and, thus, quantified that $\delta^{13}\text{C}$ of the C₃ component decreased by 0.008‰/mm MAP (Chapter III). The observed variation in ^{13}C with the longtime parameter MAP is certainly a result of replacement of species with more conservative water use patterns with increasing aridity (Brooks *et al.*, 1997). The variation of $\delta^{13}\text{C}$ in C₃ plants with MAP in the study area was in the magnitude of 2‰. This revealed the need for a correction of the C₃ end-member according to this parameter in calculations of C₄ abundance by a two-components mixing model. By correcting the isotopic signature of the C₃ end-member for the effects of altitude and precipitation, a more accurate determination of the C₃/C₄ ratio becomes possible, especially when both parameters vary across the area in focus.

Due to the increase of fossil CO₂ in the atmosphere, the $\delta^{13}\text{C}$ signature of atmospheric CO₂ decreases over the years, a process called ‘Suess Effect’ (Keeling *et al.*, 1979). This decrease should be also recorded in the vegetation and the animals grazing on it. To account for the Suess Effect in the calculation of C₄ abundance from $\delta^{13}\text{C}$ signatures of wool samples from different years, the discrimination Δ between air and animal hair was used. The signature of air was estimated for every sampled year by empirically obtained functions based on measured values of several gauging stations and ice cores (Figure IV.1). In case of the soil pool, the mean age of SOC and hence the extent of the

Suess effect are difficult to estimate and vary (Boutton *et al.*, 1998; Krull *et al.*, 2003). In addition, higher $\delta^{13}\text{C}$ values in SOC compared to vegetation can also be caused during the decomposition of organic material *via* an enrichment of microbial biomass (Ehleringer *et al.*, 2000), although decomposition experiments without effect on $\delta^{13}\text{C}$ are reported (Boutton *et al.*, 1998). As both effects cannot be quantified, estimates of percent C_4 from $\delta^{13}\text{C}$ of SOC have to be interpreted as minimum estimates.

VARIATIONS IN $\delta^{13}\text{C}$ AND $\delta^{15}\text{N}$ IN INNER MONGOLIA AND ALPINE GRASSLANDS

In Chapter II. the variation of $\delta^{13}\text{C}$ with altitude was investigated at the ecosystem-level. Measurements at the species-level had shown that $\delta^{13}\text{C}$ of C_3 plants increases with altitude even under humid conditions (Körner *et al.*, 1988, 1991) meaning that physiological water use efficiency increases with altitude (Zhang *et al.*, 1993; Hultine & Marshall, 2000). However, it was uncertain if this effect is quantitatively transferred to the community-level, since species may differ in $\delta^{13}\text{C}$ (Körner *et al.*, 1991; Tsialtas *et al.*, 2001) and community composition changes with altitude (Ellenberg, 1978). It could be shown that $\delta^{13}\text{C}$ in grazer hair and vegetation increased with altitude in a similar manner as single plant data from a regional to global data set (Table II.1; Figures II.2 & II.5). The observed difference in community biomass $\delta^{13}\text{C}$ between the lower and upper limit of the altitude gradient (2.3‰) was similar to the effect caused by severe drought (Schnyder *et al.*, 2006) and the range of community- $\delta^{13}\text{C}$ of C_3 dominated grassland ecosystems (Smedley *et al.*, 1991; Schulze *et al.*, 1996a; Schwertl *et al.*, 2005). This implies the imperative to take into account the effect of altitude in ecological studies based on carbon isotope analysis (Chapter III & IV). The similarity of altitude effects on $\delta^{13}\text{C}$ of individual plant species, vegetation and, hair indicates that the effect of altitude on species-level water use efficiency scales up linearly to the community and landscape level. Also it implies no support for a putative modification of the altitude response by species replacement patterns.

The variability of ^{13}C signals is large in environments which support the coexistence of C_3 and C_4 plants, like in arid temperate grasslands. In Chapter III the influence of precipitation, temperature, soil fertility and land use on the variation of $\delta^{13}\text{C}$ within different carbon pools of the Inner Mongolian

grassland was investigated. The regional $\delta^{13}\text{C}$ -signal decreased with mean annual precipitation reflecting an increase in the ^{13}C discrimination of C_3 plants and a decrease in the abundance of C_4 plants (Table III.2 & Figure IV.2). Along the precipitation gradient at a C_3 abundance $> 65\%$ the variation in discrimination of C_3 plants had a higher impact on the variation of the regional ^{13}C signal, while at lower C_3 abundances the impact of the variation in C_4 abundance was more pronounced. $\delta^{13}\text{C}$ also increased with mean July temperature and decreased with soil nitrogen content, reflecting variations in C_4 abundance (Table III.2). Moreover in the dessert steppe $\delta^{13}\text{C}$ decreased with decreasing distance to towns.

The $\delta^{15}\text{N}$ of plants relates to the openness of the nitrogen cycle, with elevated $\delta^{15}\text{N}$ indicating losses of nitrogen from the system (Högberg & Johannisson, 1993; Schwertl *et al.*, 2005). In the Eastern Alps the $\delta^{15}\text{N}$ signature of vegetation and hair decreased with altitude (Figure II.3), mainly reflecting the altitudinal change in precipitation and temperature (Handley *et al.*, 1999; Amundson *et al.*, 2003), while the influence of altitude was identical for a global grassland data set.

The same magnitude of scatter was present in vegetation and hair samples of this study and in the global data set, although all three data sets reflect different spatial and temporal scales associated with different heterogeneities (Figure II.5). Altitude may thus serve as a proxy in regional studies, where spatially resolved long-term measurements of precipitation and temperature are lacking. Increasing losses of depleted nitrogen with increasing temperature and decreasing precipitation also appear in the Inner Mongolian steppe. This effect is documented in the signature of the remaining plant available nitrogen in the system that is recorded in animal wool *via* the grazed vegetation (Chapter IV). $\delta^{15}\text{N}$ increased by $0.5\text{‰}/^{\circ}\text{C}$ mean July temperature, and decreased by 1.0‰ for each 100 mm increment in mean annual precipitation (Table V.1). The scatter around the regression of the Alpine wool data set (Figure II.3) is lower compared to the Inner Mongolian data set as temperature and precipitation strongly co-vary with altitude in the Alps, while their correlation is lower within the Inner Mongolian steppe.

Parameter	Range	$\delta^{15}\text{N}$ -response	SE (‰)	r^2	P
MJulT	18 – 23 °C	0.5‰/°C	± 0.1	0.29	0.001
MAP	120 – 370 mm	-0.010‰/mm	± 0.002	0.29	0.001

Table V.1: Slope of mean July temperature (MJulT) and mean annual precipitation (MAP) on $\delta^{15}\text{N}$ of wool of sheep and goats.

C₄ abundance in the Inner Mongolian steppe

The mechanisms of competition between C₃ and C₄ plants are relatively well-known in theory and are referred mainly to the day-time temperature of the vegetation period (Ehleringer *et al.*, 1997). However under temperature regimes where both photosynthetic types operate with a similar efficiency, controls and patterns of the C₃/C₄ distribution under field conditions remain poorly understood. A better knowledge on the interaction and controls of environmental parameters and the impact of land use on the C₃/C₄ distribution will contribute to the understanding of the global carbon cycle. This is particularly valid for arid temperate grasslands like the huge steppe of Inner Mongolia, where both plant functional types are abundant and compete with each other. There C₄ abundance increased with mean July temperature (Figure III.3) but decreased with mean annual precipitation and soil nitrogen content. A geostatistical analysis confirmed to some extent a spatial variation of C₄ abundance along environmental gradients, but a major control of spatial C₄ abundance was the distance to towns within the dry steppe was (Figures III.5 & III.6). Increased C₄ abundance around towns seemed to result from enhanced disturbance/overgrazing and coincided with a decreased soil nitrogen contents. The observed soil nitrogen gradient may also be supported by desertification effects due to overgrazing. N-contents below 0.2% appear in more arid parts of the study area as well as in some individual sites in the wetter part. These sites exhibit a low vegetation cover combined with a layer of residual stones above the top soil. They are suspected to underlie heavy wind erosion causing a depletion of organic matter from the soil (Li *et al.*, 2004) and accelerating the desiccation of the top soil. As these degradations are the consequences of overgrazing and destruction of the vegetation cover, a higher C₄ abundance at these sites could also be caused by invading annual and dry tolerant ruderal C₄ plants (Wang, 2002c).

C₄ plants contribute a significant (average 26%), but highly variable share to primary production of Inner Mongolia grassland. This contribution is higher than has been suggested based on species abundances (Wang, 2002b; Liu *et al.*, 2004), but as this study is based on integrative recorders covering a large area (approx. 225 000 km²) over several years, they better reflect C₄ abundance in the area than plant inventories. C₄ contribution to annual phytomass production at spatial scales of 1 ha obtained from $\delta^{13}\text{C}$ of vegetation ranged from 0-100% while wool-data induce a range from 4-64% at scales > 1 km². SOC reflects an integrated long-term signal ranging from 0-43% C₄ abundance at spatial scales of 1 m². In contrast to other C₃/C₄ ecosystems exhibiting seasonal variations in C₄ abundance (Ode *et al.*, 1980; Schwertl *et al.*, 2003b), no systematic difference in average C₄ abundance along hair segments grown between two shearings occurred. Absence of strong seasonal variation of C₄ abundance may be related to the fact that the main period of grassland production is short and concentrated in the warm summer months (June to September), during highest precipitation.

The ^{13}C of SOC did not exhibit the town effect to the same extent as the recent vegetation and wool samples (Figure III.5). This indicates that the C₃/C₄ pattern experienced a large-scale change. The question arises, when and how this change occurred. As soil carbon does not allow a temporal resolution, the only surviving and datable product of pure grassland areas is wool. Hence in Chapter IV, the temporal variation of $\delta^{13}\text{C}$ in the Inner Mongolian steppe was quantified by an analysis of woolen textiles. The discrimination Δ_{hair} between air C and wool C for different decades decreased twice, after 1957 and 1998 (Table IV.1 & Figure IV.3). Average C₄ abundance calculated from Δ increased from 2% (1928-57) to 9% (1958-97) and 25% (1998-2005) (Table IV.4 & Figure IV.5). Simultaneous increasing variation in Δ_{hair} and mean C₄ abundance of periods indicates increasing system instability (Figures IV.3 & IV.5). While the three periods highly significantly differed in mean abundance and in the standard deviation, no significant trends in C₄ abundance and scatter occurred within any period. Furthermore, the increase in C₄ in time is in contradiction to models predicting decreases in C₄ abundance due to rising CO₂ concentrations. The stepwise increase and its order of magnitude makes a causal connection to rising temperature (Beijing Climate Center, 2005) unlikely. Moreover the question arises, why the observed scatter increases with time. A geostatistical analysis revealed, that spatial patchiness increased for the period 1958-97, while period 1998-2005 exhibited a

distinct increase in C_4 abundance around towns in the dessert steppe that seemed to result from enhanced disturbance/overgrazing (Figure IV.7). This study substantiates that C_4 abundance in the steppe of Inner Mongolia depends on human impact in combination with severe snow and drought events inducing a huge modification and changing spatial C_4 abundance over time, independent of changes in CO_2 concentration, though influences of temperature could not be excluded. During the observed period long-term changes in precipitation are lacking, while mean annual temperature increased by $2^\circ C$ between 1951-1999 (Schäfer, 2000; Beijing Climate Center, 2005), exceeding by far the global temperature rise. Moreover each increase in C_4 abundance correlates with an increase in stocking rate, whereas the second increase after 1994 was of low magnitude, but had an extreme impact, as the carrying capacity of the pastures seems to be already reached before (Figure IV.8). The increase of C_4 abundance most likely results from a combination of increasing temperature, increasing human impact and severe weather events, as climatic changes alone are not in the magnitude to explain the increase of C_4 plants, particularly as the rise in atmospheric CO_2 should have damped the influence of climatic changes. Hence temporal changes in the C_4 abundance of the Inner Mongolian steppe are related to processes at the regional scale, suppressing global effects.

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APPENDIX

Abbreviations & Symbols	Description	Units
C	Carbon	
C ₃	Plants that exhibit the C ₃ photosynthetic pathway	
C ₄	Plants that exhibit the C ₄ photosynthetic pathway	
CH6	IAEA secondary standard for the calibration of CO ₂ as laboratory working standard gas	
CI	Confidence interval	
CO ₂	Carbon Dioxide	
FWLS	Final weighted least square regression	
HCl	Hydrochloric Acid	
H ₂ O	Dihydrogen monoxide (water)	
IAEA	International Atomic Energy Agency	
LTS	Least trimmed square regression	
N	Nitrogen	
n	Amount	
NH ₃	Ammonia	
N ₂ O	Dinitrogen Oxide	
NO3	IAEA secondary standard for the calibration of N ₂ as laboratory working standard gas	
P	Probability value of a significance test	
r ²	Stability index	
R _{standard}	Ratio of ¹³ C/ ¹² C or ¹⁵ N/ ¹⁴ N of an international standard (Vienne Pee Dee Belemnite or air nitrogen respectively)	
R _{sample}	Ratio of ¹³ C/ ¹² C or ¹⁵ N/ ¹⁴ N of a sample	
SD	Standard deviation	
SE	Standard error	
SILS	Solid internal laboratory standard	
SOC	Soil organic carbon	
X	Any element	
A	Net assimilation	g m ⁻² d ⁻¹
a.s.l.	Altitude above sea level	m
c _a	Atmospheric CO ₂ concentration	ppm
c _i	Intercellular CO ₂ concentration	ppm
g	Stomatal conductance	mmol m ⁻² s ⁻¹

MAP	Mean annual precipitation	mm
MJulT	Mean July temperature	°C
P _{C4}	Percent contribution of C ₄ plants to aboveground biomass	%
SNC	Soil nitrogen content	%
WUE	Water use efficiency	μmol CO ₂ / mmol H ₂ O
Δ	Isotope discrimination	‰
Δ _{C3}	Carbon isotope discrimination between air and C ₃ plants	‰
Δ _{C4}	Carbon isotope discrimination between air and C ₄ plants	‰
Δ _{hair}	Carbon isotope discrimination between air and animal hair	‰
δ ¹³ C	The deviation of the ¹³ C to ¹² C ratio of a specimen from that of the international standard Vienna Pee Dee Belemnite	‰
δ ¹³ C _{product}	δ ¹³ C of the product of discrimination	‰
δ ¹³ C _{source}	δ ¹³ C of the source for discrimination	‰
δ ¹⁵ N	The deviation of the ¹⁵ N to ¹⁴ N ratio of a specimen from that of the international standard air nitrogen	‰
δ _{Base}	δ ¹³ C of the C ₃ component of a plant community at a MAP of 250 mm	‰
δ _{C3}	δ ¹³ C of C ₃ vegetation	‰
δ _{C4}	δ ¹³ C of C ₄ vegetation	‰
δ _d	Offset between δ ¹³ C of diet and hair (diet-hair shift) or feces (diet-feces shift)	‰
δ _{diet}	δ ¹³ C of an animal's diet	‰
δ _v	δ ¹³ C of bulk vegetation	‰
δ _x	δ ¹³ C of hair or feces	‰

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