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Nitrogen and carbon isotope signatures in cattle hair – recorders of agroecosystem processes

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Denen, die mir die Treue gehalten haben

Abstract

This dissertation is about the use of carbon (C) and nitrogen (N) stable isotope composition of cattle hair as a recorder and integrator of C and N cycling components in organisms, agroecosystems, and at the regional scale.

The first aim of this thesis was to develop and assess a method for the extraction of a temporal record of the isotopic history of an animal's diet. Cattle tail switch hair was collected from animals of different breed, sex and age, and in different physiological condition. Hairs were washed, sectioned, and 10-mm-long sections analysed for C and N isotope composition ($\delta^{13}C$ and $\delta^{15}N$). Isotope signatures along paired hairs were similar ($r^2 \approx 0.8$), indicating that a single hair constituted a representative sample and hair growth rate was the same for paired hairs. Plucking hair instead of cutting avoided loss of recently grown hair and information, and allowed to detect hair in the quiescent (telogen) phase from inspection of undamaged hair root. Comparison of isotopic profiles from hair collected at different times identified the segment produced during the respective interval and allowed calculation of average individual hair growth rates (mostly 0.60 to 0.92, mean 0.79 mm d^{-1}).

The method was then used to explore the factors that affect the stable isotope composition on the organism and ecosystem level. Isotope signatures of cows from the same New Zealand dairy farm were similar in mean and pattern, while there were clear differences between farms, indicating that feeding practices and farm system characteristics had strong effects on hair isotope signatures. The $\delta^{13}C$ signal of hair samples collected from a range of cattle operations in Upper Bavaria was primarily determined by the proportion of maize in the diet (r^2=0.96). Thus, for this region, $\delta^{13}C$ of hair provided an indicator of the land use system (arable maize-based forage crop *versus* grassland farming) on which the cattle production system was based. The high precision of the prediction of C_4 proportion in the diet was related to (i) a large difference in the $\delta^{13}C$ of the two main feed components: maize (-12.5‰) and roughage from grassland and clover-grass leys (-28.4‰), and (ii) low variability of the $\delta^{13}C$ within the two components: maize ±0.4 SD; roughage ±0.5‰ SD. The ^{13}C enrichment between diet and hair varied little between animals fed pure C_3 roughage (2.6 to 2.7‰). The $\delta^{15}N$ in the same study was a complex parameter, but the long-term overall signal of adult animals in farms was correlated with stocking rate (r^2=0.55) and N input surplus (farm gate) (r^2=0.78), indicating that farm system $\delta^{15}N$ was

dominated by volatile N losses. Hence, cattle hair ^{15}N signature appears to indicate the 'leakiness' of cattle production systems for N.

The ^{13}C signature of cattle grazing humid temperate grassland (in which C_4 plants are absent) was related to the hydrological conditions of the pastures that they grazed. In a 5-year study, hair analysis revealed that community-scale, season-mean ^{13}C discrimination ($\Delta^{13}C$) only varied between 19.8‰ [on soils with low plant available soil water (PAW) capacity during the drought year of 2003] and 21.4‰ (on soils with high PAW capacity in a wet year). When shifted from pasture to stable, hair ^{13}C signature reached about 85% isotopic saturation of the new dietary signal after seven weeks.

In conclusion, this work demonstrates that hair C and N isotope analysis is a powerful tool to study the nutritional ecology and physiology of cattle, and the ecology of grasslands, agroecosystems, and regions.

Zusammenfassung

Diese Dissertation beschäftigt sich mit der Zusammensetzung der stabilen Isotope des Kohlenstoffs (C) und des Stickstoffs (N) in Rinderhaaren und ihrer Funktion als Archive und Integratoren der C- und N-Kreisläufe in Organismen, Agrarökosystemen und Regionen.

Das erste Ziel dieser Untersuchung war die Entwicklung und Bewertung einer Methode, die es ermöglicht, den zeitlichen Verlauf der isotopischen Zusammensetzung der Nahrung eines Tieres zu erfassen. Zu diesem Zweck wurden Schwanzquastenhaare von männlichen und weiblichen Rindern unterschiedlicher Rassen und Entwicklungsstufen gesammelt. Die Haare wurden gewaschen und in 10 mm lange Stücke unterteilt, welche dann auf ihre C und N Isotopenzusammensetzung ($\delta^{13}C$ und $\delta^{15}N$) hin untersucht wurden. Die Isotopensignaturen von paarweise analysierten Haaren eines Tieres zeigten dabei eine hohe Übereinstimmung ($r^2 \approx 0.8$), was darauf hindeutet, dass bereits ein einzelnes Haar als repräsentative Probe betrachtet werden kann und die Wachstumsraten benachbarter Haare vergleichbar sind. Durch das Auszupfen der Haare ließ sich gegenüber dem Schneiden ein Verlust von Haarmaterial und der darin enthaltenen isotopischen Information vermeiden. Die Untersuchung der intakten Haarwurzel erlaubte es, nicht wachsende Haare, die sich in der Ruhephase (Telogenphase) befanden, zu erkennen. Der Vergleich der Isotopensignaturen entlang von Haaren verschiedener Beprobungszeitpunkte ermöglichte die Berechnung der mittleren, individuellen Haarwachstumsrate (meist 0.60 bis 0.92, im Mittel 0.79 mm d^{-1}).

Diese Methode wurde anschließend zur Erforschung der Einflussgrößen verwendet, welche die Isotopenzusammensetzung auf der Ebene des Organismus bzw. des Ökosystems beeinflussen. Die Mittelwerte und Muster der Isotopensignaturen von Kühen innerhalb einzelner neuseeländischer Milchviehbetriebe zeigten eine hohe Übereinstimmung, während zwischen Betrieben deutliche Unterschiede erkennbar waren. Dies deutete darauf hin, dass Fütterung und Bewirtschaftungssystem einen großen Einfluss auf die Isotopensignaturen in Haaren hatten. Das $\delta^{13}C$ Signal von Haarproben eines breiten Spektrums rinderhaltender Betriebe in Oberbayern wurde in hohem Maße vom Maisanteil in der Futterration bestimmt. Für die untersuchte Region kann die ^{13}C Signatur somit als Indikator des Landnutzungssystems (Mais-basierter Ackerfutterbau gegenüber Grünlandwirtschaft) herangezogen werden, auf dem die Rinderproduktion basiert. Die hohe Präzision der Vorhersage des Anteils der C_4 Pflanzen in der Nahrung beruht auf (i) der

großen Differenz der ^{13}C Signaturen zwischen den Haupt-Futterkomponenten Mais (-12.5‰) und Raufutter von Grünland und Kleegras-Zwischenfrüchten (-28.4‰), und (ii) der geringen Variabilität innerhalb der beiden Komponenten: Mais ±0.4‰ (Standardabweichung), Raufutter ±0.5‰. Die Anreicherung von ^{13}C zwischen Futter und Haar variierte zwischen Tieren, die ausschließlich mit C_3 Raufutter ernährt wurden, nur geringfügig (2.6 bis 2.7‰). In derselben Studie erwies sich die ^{15}N Signatur als komplexer Parameter, wobei das Langzeitsignal ausgewachsener Tiere auf Betriebsebene mit dem Viehbesatz (r^2=0.55) und dem N Import-Überschuss (Hoftor) (r^2=0.78) korrelierte. Dies deutet darauf hin, dass die ^{15}N Signaturen auf Betriebsebene von gasförmigen Verlusten bestimmt wurden. Die ^{15}N Signatur in Rinderhaaren lässt also offenbar Rückschlüsse auf die N-Verluste der Produktionssysteme zu.

Die ^{13}C Signaturen von Weiderindern im feucht-gemäßigten Klima (Fehlen von C_4 Pflanzen) waren von den hydrologischen Verhältnissen der Weiden abhängig. In einer fünf-jährigen Studie konnte anhand von Haaranalysen gezeigt werden, dass auf der Ebene der Pflanzengesellschaft die ^{13}C Diskriminierung ($\Delta^{13}C$) im Mittel der Vegetationsperiode nur zwischen 19.8‰ [auf Böden mit geringer nutzbarer Feldkapazität (nFK) während des besonders trockenen Jahres 2003] und 21.4‰ (auf Böden mit hoher nFK in Jahren mit hohen Niederschlagsmengen) variierte. Erst 7 Wochen nach der Aufstallung wurde im Haar eine etwa 85%ige Sättigung des neuen ^{13}C-Futtersignals erreicht.

Zusammenfassend zeigt diese Arbeit, dass die Analyse der Isotopenverhältnisse von C und N in Haaren ein aussagekräftiges Instrument für das Studium der Ernährungsökologie und Physiologie von Rindern sowie der Ökologie von Grünland, Agrarökosystemen und Regionen darstellt.

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Chapter I: General Introduction

This dissertation is about the use of carbon (C) and nitrogen (N) stable isotope composition of cattle hair as a recorder and integrator of C and N cycling components in organisms, agroecosystems, and at the regional scale. C and N are abundant and essential elements of life. Their reduced forms are main components of food and feed for heterotrophic organisms. Fluxes of C and N are major determinants of ecosystems. They control agronomic yield in crop and animal farming systems and they are in the centre of the most certain man-driven global environmental changes: increasing concentrations of carbon dioxide (CO_2) in the atmosphere, alterations in the biogeochemistry of the global N cycle, and land use/land cover changes (Vitousek 1994).

The stable isotope composition of C and N pools provides information about their origin (source information), and of physical and biochemical reactions that fractionate stable isotopes during transport and transformation processes (process information). Thus, isotope signatures provide information that can be used to quantify nutrient sources, fluxes and pool sizes. They supply an integrated signal of processes over time. Hence, analysis of stable isotope signatures has become an important research tool in earth and atmospheric sciences, and in physiology and ecology to investigate questions ranging from the molecular to the global level (Fritz & Fontes 1980; Schmidt *et al.* 1982; Peterson & Fry 1987; Rundel *et al.* 1988; Lajtha & Michener 1994; Griffiths 1998; Hobson & Wassenaar 1999). Stable isotope signatures are routinely used in food origin assignments and authenticity control (Rossmann 2001).

The isotope composition can be conserved in certain organic materials, which are resistant to decomposition. Such materials are a valuable source of isotopic information for palaeontological and archaeological sciences. With increasing understanding of contemporary environmental influences on fractionation, stable isotope signatures may help to reconstruct palaeoecology and -climatology (Tieszen 1991). Keratinous tissues that are formed continuously, but are metabolically inactive after their formation, like baleen of whales (Schell *et al.* 1989; Best & Schell 1996) or hair of contemporary and ancient animals or man (Jones *et al.* 1981; White & Schwarcz 1994; Hobson *et al.* 1996; Schoeninger *et al.* 1998; O'Connell & Hedges 1999a; O'Connell & Hedges 1999b; Hobson *et al.* 2000; O'Connell *et al.* 2001) provide a particularly useful temporal record of mammalian isotope signatures.

STATE OF KNOWLEDGE

Hair

Similar to hoofs, horn and the outermost layer of the skin, mammalian hair predominantly consists of keratin, a sulphur-rich protein structure, which contains high amounts of cysteine, serine and glutamic acid. Together, these amino acids account for approx. 40% of hair keratin (O'Connell *et al.* 2001). Hair may also contain water (<35%), and lipids (<9%) as well as other proteins, and minerals. Proteins account for more than 90% of the dry mass of hair (Harkey 1993).

Hair is growing from small sac-like organs, called follicles, which are embedded into the epidermal epithelium of the skin. Macroscopically, along the axis of a hair a basal hair bulb and a thin hair shaft can be distinguished. The hair contains three functionally distinct zones: (a) the growth zone around a germination centre in the hair bulb, where hair cells divide and expand, (b) the keratogenous zone directly above the bulb where hair cells gradually die and decompose, long protein chains are formed, bound together to fibres by S-S bonds and cross-linking with other proteins, and combined by a special chemical cement, and finally (c) the metabolically inactive and biochemically inert (Lubec *et al.* 1987; O'Connell & Hedges 1999a) permanent hair, which protrudes from the skin surface. The so formed cylindrical and fibrous structure is called central cortex. It is surrounded by the outer cuticle, a thin layer of overlapping cells (growth zone) or cell residuals (permanent hair) that protects the interior fibre and anchors the hair shaft in the follicle. However, the cuticle can be damaged by chemicals, heat, light and mechanical injury or abrasion and, thus, towards the distal end of the shaft the cuticle becomes less intact. Hair growth is supplied by blood capillaries in the lower third of the follicle. Secretory (sebaceous, apocrine, sweat) glands discharge lipids and other matter into the follicle canal. These substances thus cover the surface and are partly incorporated into the hair (Montagna & van Scott 1958; Harkey 1993).

Hairs do not grow continuously, but in cycles. Periods of growth alternate with periods of quiescence. Three different phases of the hair growth cycle are discerned: (i) the anagen phase, which is the period of hair growth described above, (ii) the catagen, which is a short transition phase during which hair cell division stops and the hair bulb becomes fully keratinised, and finally (iii) the telogen phase (quiescent period) in which hair growth does not occur. During the telogen phase the hair shaft is initially retained in the upper portion of the follicular canal, but can be removed easily by pulling. During the anagen

phase the growth rate of single hairs can vary according to species, type of hair, body region, breed, sex and age of the individual. For human scalp hair growth rates between 0.2 and 1.1 mm/d have been reported (Katz & Chatt 1988; Harkey 1993). The degree of synchronization of growth cycles from hair of an individual or body region determines whether animals have a continuous hair cover or moulting patterns (Harkey 1993).

Isotopes

Nuclides of the same element with identical charge (number of protons) but different mass (different number of neutrons) are called isotopes of a certain element. Unstable isotopes decay radioactively, while stable isotopes are persistent. The abundance of a stable isotope is characterized by R, which is the molar ratio of the rare isotope in relation to the main isotope of the element:

$$R = {}^{15}N/{}^{14}N \text{ or } {}^{13}C/{}^{12}C \qquad \textbf{(Equation I.1)}$$

In nature differences in R are usually very small, generally they occur at the fourth to fifth decimal. To render isotope signatures comparable, they are expressed as relative deviations of isotope ratios from an international standard, the δ-value:

$$\delta^{15}N \text{ or } \delta^{13}C = (R_{sample} - R_{standard})/R_{standard} \qquad \textbf{(Equation I.2)}$$

The international standard for N is AIR (atmospheric N_2, R = 0.0036782), and for C it is V-PDB (fossil carbonate, 'Vienna-Peedee Belemnite', R = 0.01118). A positive δ-value indicates a greater abundance of the rare isotope in the sample as compared to the standard. A negative δ-value indicates a smaller abundance. Increases in δ-values mean increases in the heavy isotope content and vice-versa.

Isotopes have the same chemical properties due to their identical electron sheath. Yet, differences in mass imply different physical properties. These differences are the cause of isotope effects (here denoted as α), which give rise to fractionation of isotopes or isotopomer molecules during the formation and destruction of bonds involving the respective atom or other processes that are affected by mass, such as gaseous diffusion (Farquhar *et al.* 1989).

$$\alpha = R_{substrate}/R_{product} \qquad \textbf{(Equation I.3)}$$

Isotope effects are often classified as being either kinetic or thermodynamic, the distinction really being between nonequilibrium and equilibrium situations (Farquhar *et al.* 1989). Kinetic (nonequilibrium) isotope effects are those in which a substrate is irreversibly converted into a product with fractionation against the heavier isotope. When the substrate

reservoir is large enough to not be appreciably affected by product formation (see below), then the isotope effect, $\alpha_{kinetic}$, is equal to the ratio of isotope ratios of substrate and product (Equation I.3) (Brugnoli & Farquhar 2000). Thermodynamic isotope effects represent the balance of two opposing kinetic effects at chemical equilibrium and are therefore generally smaller than individual kinetic effects (Farquhar *et al.* 1989; Brugnoli & Farquhar 2000).

Isotope composition (δ) is not always a convenient way for expressing results. Thus, isotope discrimination (Δ) is used, which is defined as the deviation of isotope effects from unity:

$$\Delta = \alpha - 1 \qquad \textbf{(Equation I.4)}$$

The Δ-value is calculated from the measured δ-values of the substrate ($\delta_{substrate}$) and product ($\delta_{product}$):

$$\Delta = (\delta_{substrate} - \delta_{product})/(1+\delta_{product}) \qquad \textbf{(Equation I.5)}$$

However, in systems, where the substrate pool is limited and a large fraction of the substrate is eventually converted to product, feed back of the depleted or enriched product on the substrate δ-value occurs. When (a) material is continuously removed from a mixed system, while (b) the fractionation factor α stays constant with product consumption, the isotopic composition in the residual (reactant) material at time t (R_r) is described by the Rayleigh equation:

$$R_r = R_0 * f^{(\alpha-1)}, \qquad \textbf{(Equation I.6)}$$

where R_0 is the initial ratio, and f is the fraction of remaining material. The isotopic composition of the instantaneous product at any time t is enriched or depleted compared to R_r depending on α. The isotope composition in the cumulative product preserves mass balance as substrate is consumed. When the substrate pool is consumed entirely, there is no fractionation (Fritz & Fontes 1980; Mariotti *et al.* 1981; Robinson 2001).

Biogeochemistry

Knowledge of nutrient fluxes and pools, transformation processes and associated isotope fractionation is fundamental for interpretation of isotope signatures. Thus, the following paragraphs give a brief review of relevant processes in terrestrial agroecosystems.

Nitrogen

N accounts for 78 vol.% of air, which is the biggest global N-pool. It is the ultimate source and sink for all N in the upper lithosphere, hydrosphere and biosphere (Letolle 1980). Atmospheric N_2 is well-mixed and has a $\delta^{15}N$ of 0‰ (Mariotti 1983). It can be fixed by free living or symbiotic prokaryotes. This process only shows a small isotope effect and, thus, N from biological fixation has a $\delta^{15}N$ close to 0‰ (e.g. Kohl & Shearer 1980; Shearer & Kohl 1986). In agroecosystems N_2 is predominately fixed by bacteria of the genus *Rhizobium* that live in symbiosis with legumes. Up to 450 kg N ha^{-1} a^{-1} can be fixed by the *Rhizobium*-legume system (Peoples & Craswell 1992). Large amounts of N are also industrially fixed (Haber-Bosch process) to produce fertilizers. Also, high amounts of N are released by fossil fuel burning (Vitousek 1994).

Complex biogeochemical N fluxes and transformation processes like mineralisation, nitrification, ammonia volatilisation or denitrification take place in soils. These transformations are associated with more or less pronounced isotope effects and lead to different ^{15}N signatures in soil N pools (Letolle 1980; Amberger 1987; Handley & Raven 1992; Handley & Scrimgeour 1997; Högberg 1997; Hopkins *et al.* 1998; Robinson 2001). Nitrate (NO_3^-) and ammonium (NH_4^+) are the most important sources of N for plant growth. Where these are in limiting supply, like in most natural ecosystems but also in many agricultural systems, most of the nitrate and ammonia is taken up by the plants and, therefore, fractionation during uptake is negligible (Högberg 1997). The ^{15}N signature may differ between plant parts, but differences were generally smaller than 3‰ in field studies (Shearer & Kohl 1986; Handley & Scrimgeour 1997; Högberg 1997). N can be lost from the soil-plant system to the atmosphere *via* denitrification or ammonia (NH_3) volatilisation, or to the hydrosphere *via* NO_3^- leaching. Especially the gaseous loss components can be heavily depleted in ^{15}N (up to 34‰; Urey 1947; Wellman *et al.* 1968; Delwiche & Steyn 1970; Amberger 1987; Högberg 1997). Higher conservation of N under wet and cool conditions may explain that on the global scale ^{15}N signatures in soil and plants decrease with increasing humidity and decreasing annual temperatures (Amundson *et al.* 2003). In grassland ecosystems grazing animals additionally influence N cycling processes and ^{15}N signatures (Kerley & Jarvis 1996; Frank *et al.* 2000). Grazing and excretion lead to a redistribution of N and, as dung is enriched and urea is depleted in ^{15}N (Steele & Daniel 1978), also to spatial heterogeneity in $\delta^{15}N$. Effects of ammonia volatilisation from urea patches might further enhance the spatial variability of $\delta^{15}N$ (Frank *et al.* 2004).

Generally, the $\delta^{15}N$ of animals depends on their diet (De Niro & Epstein 1981). ^{15}N is enriched along food chains, that is the $\delta^{15}N$ of an animal is higher than that of its diet (Minagawa & Wada 1984). Yet, the magnitude of this enrichment varies between species differing in the chemical form of N excretion (van der Klift & Ponsard 2003), among individuals in different physiological condition and fed on diets of different quality (Ponsard & Averbuch 1999; Roth & Hobson 2000; McCutchan *et al.* 2003; Sponheimer *et al.* 2003b; van der Klift & Ponsard 2003). Also, there are differences in $\delta^{15}N$ between different tissues of an animal (Hobson *et al.* 1996; van der Klift & Ponsard 2003). Actual animal tissue $\delta^{15}N$ depends on the $\delta^{15}N$ of the available source compounds in the body metabolic pool, and tissue-specific ^{15}N fractionation and turnover kinetics (Gannes *et al.* 1997; Gannes *et al.* 1998).

Carbon

Photosynthesis converts atmospheric CO_2 into a (reduced) organic form. In a simple mechanistic model the ^{13}C discrimination of photosynthesis in C_3 land plants ($\Delta^{13}C$) can be described (Farquhar *et al.* 1982b; Farquhar *et al.* 1989):

$$\Delta^{13}C = a + (b-a)\, p_i/p_a\,, \qquad \textbf{(Equation I.7)}$$

where a is the fractionation due to diffusion of atmospheric CO_2 through the stomatal pore (4.4‰),

b the net fractionation caused by carboxylation [27‰, mainly b_3 (see below)] (Farquhar *et al.* 1982a),

p_i the CO_2 partial pressure in the intercellular space, and

p_a the CO_2 partial pressure in the ambient air.

For C_4 plants ^{13}C discrimination in photosynthesis is expressed as (Farquhar 1983; Farquhar *et al.* 1989):

$$\Delta^{13}C = a + (b_4+b_3\phi-a)\, p_i/p_a, \qquad \textbf{(Equation I.8)}$$

where b_3 is the fractionation caused by carboxylation of Rubisco (30‰),

b_4 the fractionation caused by carboxylation of PEP-carboxylase (-5.7‰),

ϕ the proportion of C fixed by PEP carboxylation that subsequently leaks out of the bundle sheath.

Consequently, C_3 and C_4 plants differ strongly in their ^{13}C signatures. Yet, environmental and genetic factors influencing p_i/p_a or ϕ lead to variation in $\delta^{13}C$ within C_3 and C_4 plant types (Deines 1980; O'Leary 1988; Brugnoli & Farquhar 2000). Additionally, the ^{13}C

signature of specific plant tissues and compounds may differ due to fractionation during metabolism (De Niro & Epstein 1977; Deines 1980; Gleixner *et al.* 1993; Hobbie & Werner 2004).

According to variations in photosynthetic and respiration activity the isotope composition of global atmospheric CO_2 varies seasonally, anti-parallel with fluctuations in CO_2 concentration (Keeling *et al.* 1996). In the long term the ^{13}C signature of atmospheric CO_2 declines in association with the increase in CO_2 concentration due to anthropogenic fossil fuel combustion and deforestation (e.g. Keeling *et al.* 1979). Soil organic matter globally contains several times more C than either the atmosphere or living plant biomass and, in general, has a similar $\delta^{13}C$ as the vegetation (Peterson & Fry 1987).

The $\delta^{13}C$ of animals depends on their diet (De Niro & Epstein 1978). Small animals are slightly enriched in ^{13}C compared to their diet (De Niro & Epstein 1978; McCutchan *et al.* 2003). Yet, tissues of different biochemical composition are depleted or enriched in ^{13}C compared to diet (Tieszen *et al.* 1983; Tieszen & Boutton 1988; Tieszen & Fagre 1993). The magnitude of the ^{13}C enrichment between diet and animal might also depend on diet quality and feeding level (Gaye-Siessegger *et al.* 2003). Similar as for $\delta^{15}N$, the actual $\delta^{13}C$ of specific tissues depends on the available source compounds in the body metabolic pool, and tissue-specific ^{13}C fractionation and turnover kinetics (Tieszen *et al.* 1983; Hobson & Clark 1993; Gannes *et al.* 1997; Gannes *et al.* 1998).

AIMS

The following research was based on the hypothesis that (i) cattle hair provides an accurate (C and N) isotopic record over some period of the dietary history of an animal and, (ii) integrates/eliminates small-scale spatial and temporal heterogeneities/gradients in the animal environment. As it would absorb small-scale spatial and temporal variability, it should expose more clearly isotopic gradients on larger scales.

The first aim of this thesis was to develop and assess a method for the extraction of a temporal record of the isotopic history of an animal's diet (Chapter II). This method was then used in a 'proof-of-concept' study to characterize the dietary isotopic history of cattle on different New Zealand dairy farms (Chapter III). Further work explored the potential of cattle hair isotope signatures in revealing dominant agroecological characteristics (N budget and forage production system) of cattle farming systems. This included detailed studies of 13 farms, which represented the full range of cattle farming systems in Upper Bavaria

(Chapter IV). Lastly, the C isotope signature of grazing cattle was related to the hydrological conditions of the pastures that they grazed. This 5-year study included the centennial drought of 2003, and exploited grassland on soils with a vast range of plant available soil water capacity (Chapter V).

Chapter II: Reconstruction of the isotopic history of animal diets by hair segmental analysis[1]

ABSTRACT

Carbon and nitrogen isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of animal tissues provide information about the diet and, hence, the environment in which the animals are living. Hair is particularly useful as it provides a stable archive of temporal (e.g. seasonal) fluctuations in diet isotope composition. It can be sampled easily and with minimal disturbance from living subjects. However, derivation of the temporal record along the hair length may be subject to errors and uncertainties. This study investigates (and suggests means to minimize) several sources of error, including (a) incomplete sampling, (b) sampling during the quiescent (telogen) phase, (c) non-representative sub-sampling, (d) ignorance of hair growth rate, i.e. time-position relationship of isotope signatures, and (e) non-optimal compromise between analytical/procedural precision and effort/cost. Cattle tail switch hair was collected from animals of different breed, sex and age. Hair was washed, sectioned, and 5- or 10-mm long sections were analysed for C and N isotope composition. Signatures along paired hairs were similar ($r^2 \approx 0.8$) and distances between isotopic minima and maxima nearly identical, indicating that a single hair constituted a representative sample and (except for telogen hair) hair growth rate was the same for paired hairs. However, cutting hair, instead of plucking, caused a variable loss of recently grown hair and information. Telogen hair was identified and data loss due to cutting error reduced when more than one hair from the same animal and sampling region was compared to spot and delimit common and missing regions. Similarly, comparison of isotopic profiles from hair collected at different times identified the segment produced during the respective interval and allowed to calculate average hair growth rate, which varied between animals (0.69 to 1.06 mm d^{-1}). Analysis of alternate 10-mm long sections for two hairs per animal provided a good compromise between precision/resolution and effort. The method should be applicable to other mammalian species including man.

[1] Schwertl M, Auerswald K, Schnyder H (2003) *Rapid Communications in Mass Spectrometry* **17**, 1312-1318.

INTRODUCTION

The analysis of the natural stable isotope composition of animal tissue, products or faeces has become an important tool in studies of the behavioural and nutritional ecology of animals or man (Cobon & Carter 1994; Schoeninger *et al.* 1997; McIlwee & Johnson 1998; O'Connell & Hedges 1999a; Bol & Pflieger 2002). This is because the stable isotope composition of carbon, nitrogen and other elements in tissue depends on the composition of the diet (De Niro & Epstein 1978; De Niro & Epstein 1981; Rundel *et al.* 1988; Tieszen & Boutton 1988), and thus, examination of tissue isotopic composition provides information about dietary components if these exhibit distinct isotopic signatures.

The measurement of whole body isotopic composition is possible only for small animals, e.g. insects. If the diet of larger animals, e.g. mammals is in question, sub-sampling is necessary or certain tissues must be chosen. For dead animals and humans, especially in palaeontological research, bone and bone collagen have been examined (Sealy *et al.* 1987; Vogel *et al.* 1990; Iacumin *et al.* 2000). Because of their low metabolic activity and therefore slow turnover of stable isotopes, these tissues provide a long term dietary information that may integrate decades of their lifetime (White & Schwarcz 1994). Tissues and liquids with high turnover like blood, fat, liver, muscle and brain, however, reflect diet consumed within weeks to months (Tieszen *et al.* 1983; Jenkins *et al.* 2001).

Hair offers perhaps the largest potential for studies of isotope ecology (O'Connell & Hedges 1999a). It is a unique feature of mammalian skin with similar properties for the different species (Leblond 1951). In contrast to most other tissues it can be obtained non-invasively and with minimal disturbance from a living subject. For a certain time, a single hair grows more or less continuously from the base of the hair follicle (Bullough & Laurence 1958; Saitoh *et al.* 1967). It mainly consists of the persistent protein structure keratin, which can preserve the isotopic information for thousands of years (Lubec *et al.* 1987). Therefore, preserved hair, e.g. of human mummies can be used for diet reconstruction (O'Connell & Hedges 1999b; White *et al.* 1999). During growth, keratin is formed in the basal few millimetres underneath the skin (Leblond 1951; Bullough & Laurence 1958). Once the keratin structure is established, hair tissue is metabolically inactive (Montagna & van Scott 1958; O'Connell & Hedges 1999a). Thus, every section of the hair shaft contains isotopic information from the time when it was produced. Therefore, hair can be regarded as an isotopic archive recording dietary changes, which may result

from, e.g., seasonal fluctuations and migration patterns (Nakamura *et al.* 1982; White *et al.* 1999).

We are performing studies of the role of grazers in the flow of energy and nutrients in grassland systems. These include investigations of the isotope signatures along tail switch hair. The switch is the distal part of the tail of cattle and produces especially long hair. Their length mostly varies between 150 and 400 mm. Only recently born calves and older cows have shorter hair. Thus, cattle tail switch hair has a high potential as an isotopic archive, recording spatio-temporal changes in isotopic signals of diet over extended periods of time. However, derivation of the temporal record from isotopic signatures along the length of a sample hair may be subject to several errors, which – to our knowledge – have not been analysed systematically. This study investigates (and suggests means to avoid or minimize) several sources of such errors or uncertainty, including (a) incomplete sampling, (b) sampling hair during the quiescent (telogen) phase, (c) non-representative sub-sampling, (d) ignorance of hair growth rate, i.e. time-position relationship of isotope signatures, and (e) non-optimal compromise between analytical and procedural precision and effort and cost.

EXPERIMENTAL

Animals

The samples were collected from cattle kept and raised on the Grünschwaige Grassland Research Station located at the north end of the Munich Gravel Plain near Freising, Germany (Auerswald 2001). The breeds included Limousin, Aberdeen Angus, Simmental or crossbreeds of the mentioned. Three cows, four suckling calves, six heifers and two steers were examined. During the winter housing period (October to April), the animals were maintained on a diet of grass silage and hay, whereas in summer green pasture was the sole feed source. All feed components were produced on the station.

Sampling and cleaning of hair

From each animal a strand of about 100 switch hairs from about 1 cm^2, was cut close to the skin with scissors at the beginning of the grazing season in April 2001. For six animals, including at least one animal per age group, sampling was repeated in the same way at the end of the grazing season (September/October 2001). All sampling was performed by the same person (M. Schwertl) in the same way and with the same tools. To remove

contaminants like faeces the hairs were soaked and washed by ultra-sonication with deionised water, dried (40°C, 48h), soaked in a 2:1 mixture of methanol and chloroform (ca. 2h), washed with deionised water, soaked in deionised water for another 30 minutes, and rinsed again. Two dried (40°C, 48h) hairs of similar length were then chosen from each hair strand and cut into 5 or 10 mm-long (±1 mm) sections with the help of a stencil. Sections were analysed individually.

Isotopic measurements

To this end a section was enclosed in a tin cup (4x6 mm) and combusted in an elemental analyser (Carlo Erba NA 1110, Milan), interfaced (ConFlo II, Finnigan MAT, Bremen) to an isotope ratio mass spectrometer (Delta Plus, Finnigan MAT, Bremen). The isotope data are presented as $\delta^{13}C$ (‰) relative to V-PDB standard and $\delta^{15}N$ (‰) relative to nitrogen in air and were calculated as follows: $\delta X= [(R_{sample}/R_{standard})-1]*10^3$, where δX is $\delta^{13}C$ or $\delta^{15}N$, and R is the respective $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio. N and C isotope composition of one sample was analysed in the same run using the „Dual Gas Acquisition" feature of the ISODAT7.1 software (Finnigan MAT, Bremen, Germany). In this mode, the peak centring routine is done only for the first peak (N_2) eluting from the GC column because of the small difference in retention time between N_2 and CO_2. The CO_2 peak centre routine was performed before running samples and the jump value thus obtained was used in the following analyses.

As C content in organic samples is usually much higher than N content, the determination of the C isotope composition of samples was done with He dilution. He dilution was adjusted to sample size, so that CO_2 sample peak amplitude ranged between 1 and 3 V. In this range $\delta^{13}C$ was independent of signal intensity. With the same set up of the system, the working gas standard for C isotope determination was calibrated against the laboratory standard, a fine ground wheat flour of known C and N isotope composition ($\delta^{13}C$ –26.54‰ and $\delta^{15}N$ 2.61‰). The same working standard was run regularly after every 10^{th} sample as control. Blank determinations were done routinely before each batch of samples (including working standards) by running empty tin cups. The $\delta^{15}N$ data were blank-corrected. The external precision (standard deviation) was ±0.2‰ for $\delta^{13}C$ and ±0.3‰ for $\delta^{15}N$.

The data were plotted *versus* distance from the hair base (proximal to distal), from the right to the left. This corresponds to a time axis, with the tip of the hair the oldest and the base the youngest part.

Resolution of hair record

Analysis of isotopic signatures along hair always involves trade-offs between analytical precision, spatial (and, hence, temporal) resolution, and effort and cost. Thus, at a minimum linear density of 2 μg C mm^{-1} of hair, and a minimum requirement of 10 μg C per sample for the continuous flow IRMS, the minimum section length for C isotope analysis was 5 mm. Thus, a maximum-resolution analysis of one 400 mm-long hair would require 80 isotope analyses. Although this number may be seen as excessively large, resolution of this type is welcome if two hairs from the same animal are to be tested for the degree of spatial correspondence of their isotope signatures. Options for reducing effort and cost include generation of larger samples, or analysis of a subset (i.e. every second or third) of the total number of samples, and estimation of missing values by interpolation.

For most of the measurements the following compromise was chosen: analysis of alternate 10 mm-sections and linear interpolation of missing values at 5 mm-intervals (Figure II.1).

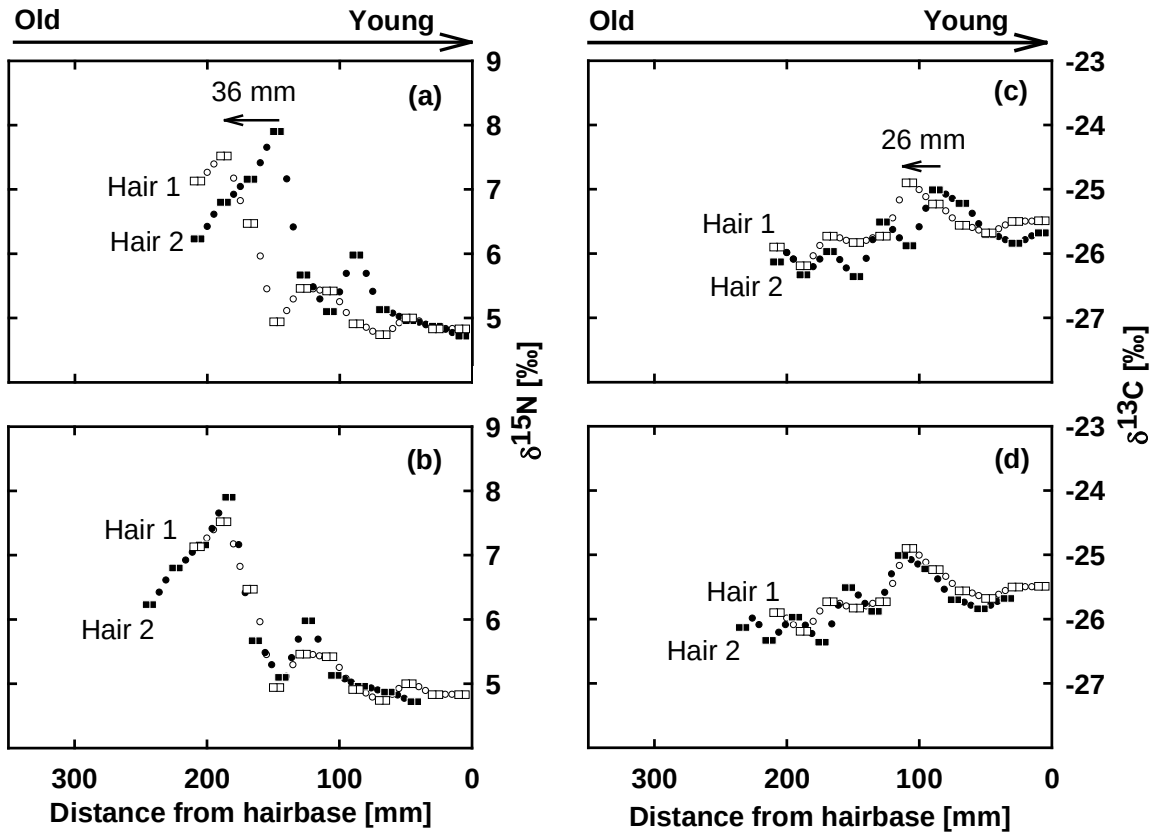

Figure II.1: Nitrogen and carbon isotope signatures ($\delta^{15}N$, $\delta^{13}C$) of two tail switch hairs from Yearling D, plotted *versus* (**a, c**) original distance from (cut) hair base, and (**b, d**) position after correction for cutting or growth cycle error of hair 2, the hair with a missing basal section (for further explanation see text). Measured values are given as double points: hair 1, □□; hair 2, ■■. Circles show values generated by linear interpolation at 5 mm-intervals.

Errors resulting from interpolation, that might obscure the real isotope record along a hair were analysed by comparison of interpolation results with measured values from a single hair that was analysed completely. This was done by sequentially replacing measured values by interpolated values over a variable distance and determination of the δ-difference between measured and interpolated data. This was done in the same way for the $\delta^{13}C$ and $\delta^{15}N$ signature.

Correction for incomplete sampling

If hair is collected by cutting, rather than plucking, a stubble of variable and unknown length is left behind. The stubble is composed of a buried and an exposed part. Since it is missing from the collected hair it involves a loss of isotopic record and since its length is unknown it causes a uncertainty in the assignment of the hair base to a time axis. To

quantify their length, 430 stubbles were plucked from the tail of a slaughtered animal after 4 hair strands had been removed in the same way as with live specimens. Total stubble length was measured and the part below the skin was estimated by visual inspection. For representation of stubble length frequency, kernel density estimation (Victor 1978) was preferred over commonly used histograms because its resolution is not limited by pre-selected class width. This is advantageous where sample size is small, distribution is multi-modal or where differences between distributions are small. For theory of kernel density estimation see Silverman (1986).

The isotope patterns of two hairs from the same sampling area and animal can be displaced relative to each other (Figure II.1). Two different mechanisms may contribute to such a mismatch. A "cutting error" may arise from a difference in stubble length. A "growth cycle error" can originate from sampling hair in different growth phases. A single hair grows for a certain time (anagen phase), remains in the follicle channel for some time afterwards and finally drops (Hopps 1977). After a phase of follicle quiescence a new hair is produced. When cut, an anagen hair cannot be distinguished from a telogen hair, but the latter does not contain recently-formed hair tissue and, hence, misses recent isotopic information. To correct for the mismatch, the isotope signature of one hair had to be shifted relative to the other in mm steps, until best match was obtained (Figure II.1). Correspondence was evaluated by independently regressing the N and C isotope signatures of two hairs while the position of one hair was sequentially shifted relative to the other. The hair with the longer missing basal section was chosen for shifting. After optimum shifting, the most recent information was thus always located near the origin of the x-axis. The optimum shift was defined as that producing the highest r^2. The slope of the regression indicated whether there was any systematic difference in the amplitude of signals on the two hairs.

Hair growth rates

Different signal frequencies (compressed or stretched patterns) along two hairs could be caused by different growth rates. This was evaluated independently of the regression parameters by comparing the distances between corresponding extrema of two hairs from the same animal. Altogether 10 signatures (including 4 pairs of hair for both $\delta^{13}C$ and $\delta^{15}N$) from six animals were analysed.

Hair growth between the spring and autumn sampling was determined by comparing the isotopic signatures of hair collected in spring and autumn. To this end an average isotopic signature for hair collected on one sampling date was first calculated after optimum shifting for each animal. Thereafter, the new growth was identified by matching the two signatures as indicated above.

RESULTS

Comparison of segmental $\delta^{13}C$ and $\delta^{15}N$ values of hair from all animals revealed substantial variability: $\delta^{13}C$ ranged between –27.2‰ and –24.3‰, and $\delta^{15}N$ between 1.7‰ and 9.0‰ (Figure II.2).

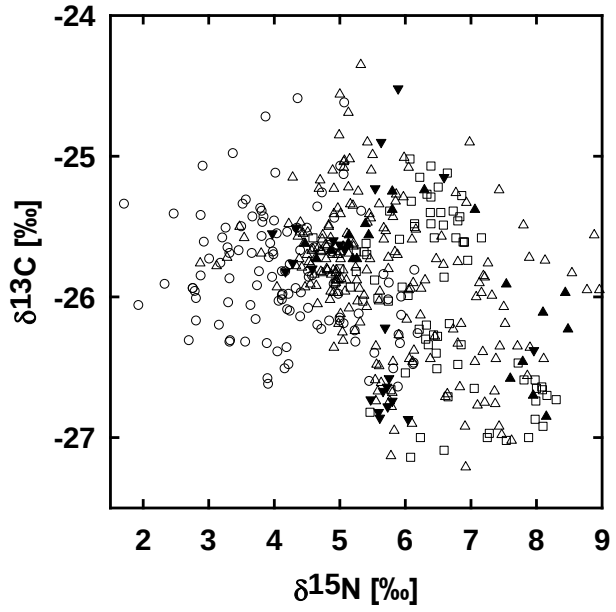

Figure II.2: Nitrogen and carbon isotope signatures ($\delta^{15}N$, $\delta^{13}C$) of all 10 mm-long sections (n = 439) of tail switch hair, sampled from yearlings (△), cows (○), and calves (□). The isotope data for Yearling I obtained in spring (▲) and autumn (▼), are shown separately.

The hairs were 70 to 390 mm long. Along each, the $\delta^{13}C$ and $\delta^{15}N$ signatures varied, with δ-differences between measured maxima and minima in the order of 1‰ to 4‰ for $\delta^{15}N$, and 0.5‰ to 3‰ for $\delta^{13}C$.

The mean absolute difference (MAD) between measured and interpolated values increased with the length of no-data intervals (Figure II.3). The respective MAD was 0.1‰ lower for carbon than for nitrogen isotope signature. In our analysis of cattle hair no-data intervals were never larger than 10 mm. For that case, MAD for interpolated values of carbon as well as of nitrogen was less than the analytical error and 95% of all $\delta^{15}N$ and $\delta^{13}C$ interpolations differed by less than 0.4‰ ($\delta^{15}N$) and 0.7‰ ($\delta^{13}C$) from the measured value.

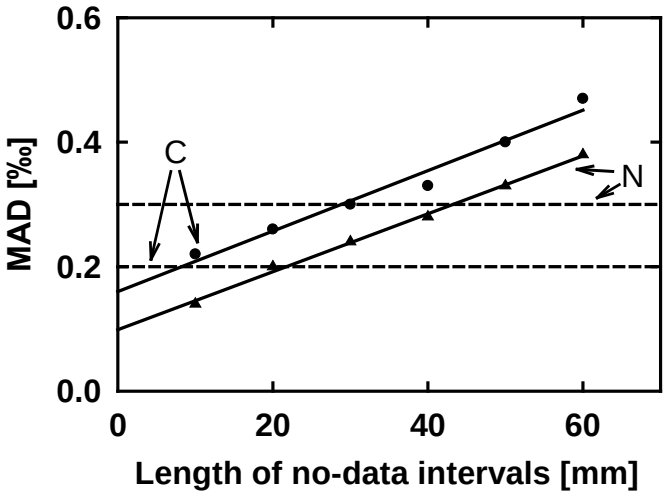

Figure II.3: Impact of spatial resolution and missing values on accuracy of isotopic record. Mean absolute differences (MAD) between measured values and interpolations for different no-data intervals. Data from a (complete) isotopic record of one hair of Yearling J, obtained from measurements of 10 mm-sections at 10 mm-intervals. No-data intervals were generated by sequentially deleting measured values and replacing them by interpolated values. MAD for $\delta^{15}N$ (▲) and $\delta^{13}C$ (●) are shown together with analytical errors for $\delta^{15}N$ and $\delta^{13}C$ (**– – –**), and linear regressions lines (**——**) are given.

The isotopic patterns of two hairs from the same animal at the same sampling time showed remarkable similarity after they were corrected for cutting and/or growth cycle errors. Close correspondence was indicated by high correlation coefficients and slopes of regressions close to 1 (Table II.1). Also, the distances between corresponding isotopic extrema of paired hairs showed high similarity. In most cases differences between corresponding extrema distances were smaller than 10 mm (Figure II.4). Thus, the error was smaller than the size of the sections used for isotopic analysis. The MAD of the 75 extrema distance pairs from the 1:1-line was 7.3 mm.

Table II.1: Correspondence of isotope signatures along two hairs from the same animal. Mean ± standard deviation for parameters (r^2 and slope) of linear regressions between positional isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of paired hairs, sampled simultaneously (in spring or autumn 2001), or at different times (spring and autumn). Regressions are shown for the matching portion of hairs as determined by the optimum shifting procedure explained in Experimental. Twenty-one simultaneously sampled pairs of hair were regressed. Each included 12 to 74 (average 35) data pairs for each isotope. The regressions between spring and autumn signatures were based on average signatures, obtained from combining two spring and two autumn hairs per animal (data from six animals, with 20 to 48 data pairs per animal).

Comparison	r^2	Slope
Regressions between hair 1 and hair 2 of spring or autumn sampling		
$\delta^{13}C$	0.75 ±0.23	0.8 ±0.3
$\delta^{15}N$	0.82 ±0.23	1.0 ±0.2
Regressions between spring and autumn samples		
$\delta^{13}C$	0.65 ±0.16	1.1 ±0.5
$\delta^{15}N$	0.78 ±0.31	0.9 ±0.1

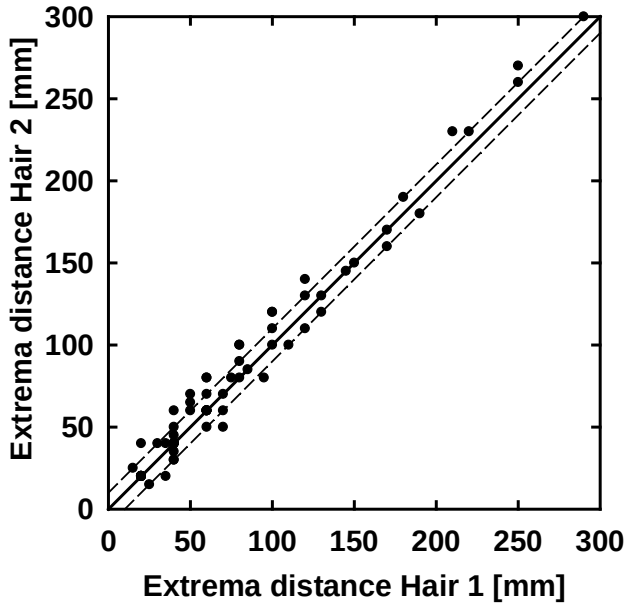

Figure II.4: Corresponding distances between maxima and minima of isotopic signature along hair 1 and 2 from spring sampling; 1:1 line (——) and minimum deviation as caused by the cutting method (- - -) for C and N isotope signatures.

The shift required to obtain best match of paired hairs (optimum shift) can be derived either by comparison of the isotopic signature of the element (carbon or nitrogen) with the largest isotopic variation or the combined information of several elements weighted by their correlation coefficient. We calculated the optimum shift separately for both elements to test the robustness of the method. High correlation coefficients between optimum shifts calculated from $\delta^{13}C$ and $\delta^{15}N$ signatures (Figure II.5) indicated that the applied method to find the optimum shift was appropriate and the original mismatch between the two hairs was indeed due to a cutting or growth cycle error.

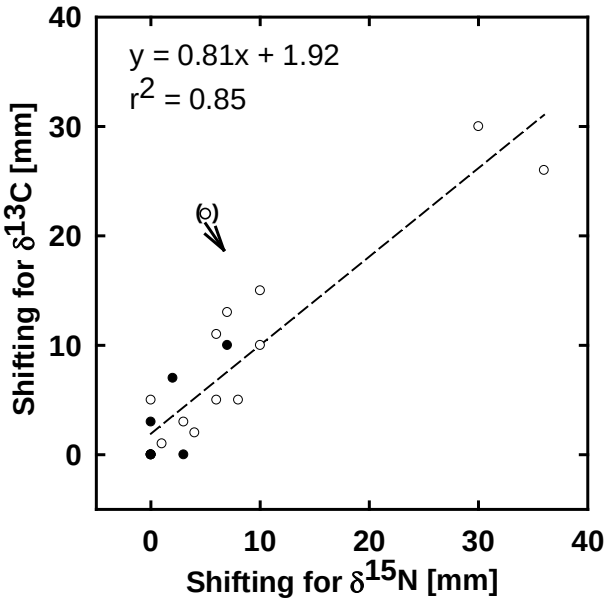

Figure II.5: Optimum shifting distance for best match of simultaneously sampled pairs of hair. Optimum shift was calculated independently for C and N isotope signatures. Data from 15 animals sampled in spring (○) and 6 animals sampled in autumn (●) 2001. The data point in parentheses was excluded from the regression (see text for explanations).

In most cases the optimum shift (i.e. original mismatch between the two) was less than 10 mm (Figure II.5). In two cases the optimum shifting values were larger than 30 mm as indicated by both the $\delta^{13}C$ and $\delta^{15}N$ signatures. In another case the optimum shifts suggested by the $\delta^{13}C$ and $\delta^{15}N$ signatures differed strongly. This data pair was not included in calculating the regression between optimum shifts obtained from $\delta^{13}C$ and $\delta^{15}N$ signatures. Low variation of the isotopic patterns of both carbon and nitrogen along the hair shaft seemed to be the cause for the disagreement in suggested optimum shifts in this

singular case. Other shifting vectors would have yielded similar correlation coefficients but would have brought the data point much closer to the regression line (as shown by the arrow in Figure II.5).

The stubbles left behind after cutting were 3 to 40 mm long with a most frequent length near 8 mm (Figure II.6). The longer stubbles were obtained near the edge of the sampling area, and probably resulted from the round profile of the tail. Sampling error, i.e. loss of isotopic record near the hair base, was reduced when two instead of only one hair was analysed. Monte Carlo simulation predicted that analysis of a second hair reduced the loss of isotopic record by an average 4 mm in 97% of all cases. Including a third hair recovered a further 1 mm-long isotopic record in 28% of all cases.

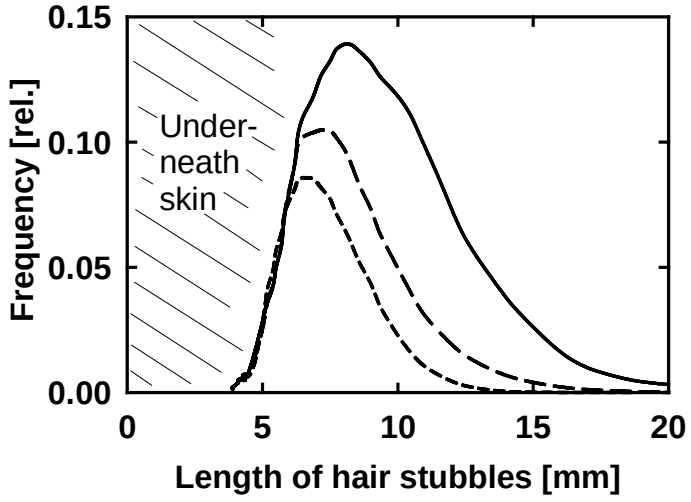

Figure II.6: Kernel density distribution for stubble hair length (——). Monte Carlo simulation was used to assess the length of the shortest stubble when two (– –) or three hairs (- - -) were randomly selected for analysis. The hatched area represents the length of the hair segment underneath the skin.

Comparison of isotopic patterns from autumn and spring sampling of Yearling I exhibited remarkable similarity. The autumn hairs entirely reproduced the spring pattern in their older distal part (Figure II.7). Provided that the cutting error for both sampling dates was the same, the basal portion of the autumn hair which was missing in the spring hair, represents the growth between the two samplings. In the case of Yearling I this was 135 mm grown in 140 days, thus yielding a growth rate of 0.96 mm d^{-1}. The hair growth rate determined in this way for the five other animals sampled twice, varied between 0.69 and 1.06 mm d^{-1}.

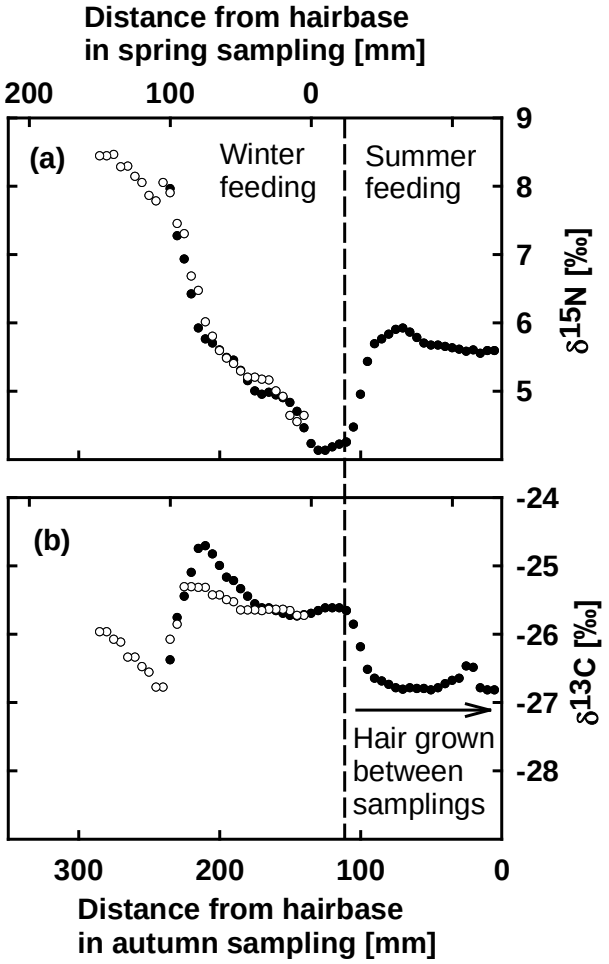

Figure II.7: Mean isotopic signatures for pairs of hair cut in autumn (●) and spring (○) 2001 of Yearling I.

DISCUSSION

Assignment to a time axis

Isotopic signatures along hair provide potentially important insight into the nutritional ecology of animals and the functioning of the ecosystem of which they are a part. However, to become interpretable, the spatial record – as extracted from the hair – must first be transformed into a temporal record. This requires knowledge of the position-time relationship. The present study identified and analysed three sources of uncertainty which can affect the accuracy of position-time transformation: (a) leaving behind/failing to collect a section of recently produced hair (stubble) when collecting hair by cutting, (b) collecting hair from follicles during the quiescent phase, and (c) ignorance of or inaccurate estimates of hair growth rate. These errors and uncertainties can be minimized by combining the

following measures: avoiding sample loss by plucking hair instead of cutting, identification of telogen hair by microscopic examination of the hair root (van Scott *et al.* 1957), and, finally, subject-specific and periodic sampling of hair, and identification of the (subject- and period-specific) hair growth by comparison of the isotopic signatures obtained from hair sampled at the beginning and end of the period of interest. Where collection by plucking is difficult – such as when tail switch hair is firmly held together in strands by dried faeces – and samples must be collected by cutting, at least part of the missing hair base can be recovered by comparison of the isotopic record of several hairs and identification of the hair with the shortest missing section. Also, when cut the hair root is left behind and telogen hair can not be identified by microscopy. To our knowledge, the proportion of telogen hair in the tail switch is unknown. But, it is known that about 15% of human scalp hairs are in telogen phase at any time (Harkey 1993). If the proportion is the same in the tail switch, then the likelihood of obtaining two telogen hairs in a sample of two hairs is about 2% and can be reduced to about 0.3% by choosing a third hair.

One critical condition for this protocol is that all anagen hairs from the same animal and sampling region produce identical isotopic signatures and grow at the same rate. Indeed, comparison of switch hair collected from the same animal demonstrated a high degree of correspondence of the isotope signatures in terms of both the spacing (extrema distance or frequency) and amplitude of the isotopic signals, suggesting identical growth. In general the correlation coefficients between isotopic records of two hairs was higher for nitrogen than for carbon. This was mainly due to larger signals and a higher signal to noise (analytical precision) ratio for $\delta^{15}N$ in our study. However, where larger variations in $\delta^{13}C$ can be expected, for instance following a shift from C_4 to C_3 forage feeding (Jones *et al.* 1981), the correlation coefficient for $\delta^{13}C$ should become much larger.

Hair growth rate (which is a key parameter for position-time conversion of the isotope data) seemed to be nearly identical for anagen hair collected from the same region of the same animal. However, growth rate differed between animals, although a clear relationship between age, sex or breed and hair growth rate was not evident from the present data. Still, age-, sex- and race-specific differences in hair growth rate exist in man (Myers & Hamilton 1951; Hamilton 1958). Also, the rates of hair growth observed in our study were higher than those reported for tail switch hair of Angus and Angus x Charolais cows (0.51-0.63 mm d^{-1}) by Fisher *et al.* (1985). Importantly, hair growth rate can also vary between different types of hair and body regions (Myers & Hamilton 1951; Saitoh *et al.*

1967), with switch hair growing faster than other body hair (Fisher *et al.* 1985). Also, studies by Martin *et al*. (1969) indicate that hair growth in cattle can be impaired by a severe insufficiency in food energy or protein supply. Moreover, environmental factors such as temperature and photoperiod, but also hormonal status may influence hair growth rate (Mohn 1958; Johnson 1977). Yet, Fisher *et al*. (1985) found only small and mostly insignificant ($P>0.05$) monthly variation of switch hair growth rate of four cows grazing a paddock from May to October. Altogether, these results support the notion that periodic, subject- (hair-type and body region-) specific hair growth rate determinations are required for accurate position-time transformation of hair data. Such growth rate determinations may be performed by using the isotopic records collected at different times as shown here (Figure II.7) or by other appropriate means.

Resolution of the isotopic record

Ideally, an isotopic record is able to provide isotopic information with high temporal resolution. However, the resolution that can be achieved or is necessary is limited or determined by technical/analytical, scientific and biological parameters. Thus, there exists a trade-off between precision/resolution and effort/cost. Also, the type of research question asked will determine the required temporal resolution. Lastly, there are biological limitations as to which temporal resolution can be achieved with isotopic records extracted from hair. Thus, a shift in the isotopic composition of the diet will not be instantaneously reflected in the new hair. The full isotopic signature will only be expressed in new hair when the body metabolic amino acid pool feeding the follicles has been turned over completely (O'Connell & Hedges 1999a). Our data offered an opportunity for an assessment of the kinetics of the metabolic pool following transfer from a (winter) silage and hay to a (summer) pasture diet. For this the $\delta^{15}N$ data obtained from Yearling D were position-time transformed and an “exponential rise to maximum” function was fitted to the data (Figure II.8).

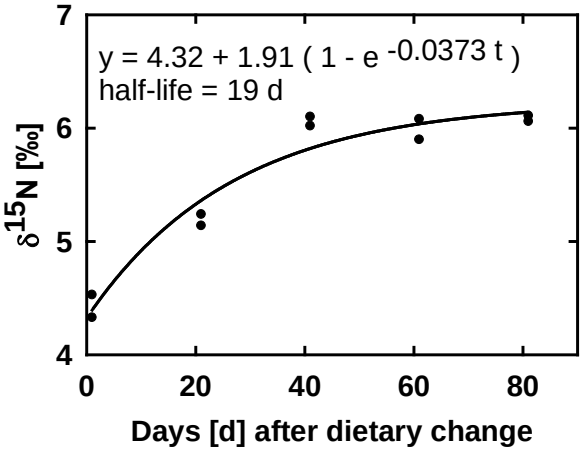

Figure II.8: Kinetics of the body metabolic pool feeding hair growth, as derived from the $\delta^{15}N$ of hair produced following a dietary shift from silage/hay to pasture. $\delta^{15}N$ data were obtained from a segmental analysis of two tail switch hairs of Yearling D, and position-time conversion of data as explained in the text. An "exponential rise to a maximum" function (**——**) was fitted to the data.

This indicated a half-life of the metabolic pool of 19 days. This relationship also demonstrates, that short-lived (< 1 week) isotopic signals ingested with feed are strongly dampened and diluted by the body metabolic pool and will evoke only a relatively weak isotopic signal in the hair. Conversely, sustained dietary isotopic shifts are expressed more prominently. It is evident from the data presented in Figure II.8 that the (temporal) resolution and precision of the pool-kinetic analysis would be improved by an increased spatial resolution of the hair record.

A half-life of the metabolic pool in the order of 20 days also explains why interpolation intervals for no-data sections shorter than 20 mm caused relatively small errors (Figure II.3). At a growth rate of 1 mm d^{-1} 20 mm of new hair growth will be produced until half of a dietary change appears in newly grown hair if the metabolic pool has a half-life of 20 days. The pool kinetics in Figure II.8 was very similar to estimates derived from the kinetics of $\delta^{13}C$ in regrowth hair produced following a change of steers from a C_4 to a C_3 diet (half-life 16 days) (Jones *et al.* 1981). The change in the $\delta^{15}N$ signature observed in our study (Figure II.8) was brought about by transfer from a legume-rich (low $\delta^{15}N$) silage and hay diet to a legume-poor, grass rich pasture with higher $\delta^{15}N$. However, trophic level changes were also a possible cause for changes of the $\delta^{15}N$ along hair. Thus, the decrease of $\delta^{15}N$ between 150 and 100 mm distance from hairbase in the

spring sample in Figure II.7a occurred during weaning of the calf on the pasture. Finally, the $\delta^{13}C$-change between the winter and summer period was related to a difference in $\delta^{13}C$ signature of winter and summer diets: silage and hay was produced on a drought-prone site (producing forage with a less negative $\delta^{13}C$), and pasture grass was grown on peat soil close to the ground-water table, which produced grass with a more negative $\delta^{13}C$ (Figure II.7b).

In conclusion, the present study demonstrates the potential of isotopic analysis of short sections along cattle tail switch hair to provide a reproducible and representative isotopic archive. To our knowledge, this is the first systematic investigation of some common sources of error or uncertainty which may hinder construction of an accurate temporal isotopic record from isotopic analysis along hair. Although procedures were developed and tested with cattle tail switch hair, they should also be applicable to other mammalian species, including man.

ACKNOWLEDGEMENTS

We wish to acknowledge the helpful comments and suggestions of two anonymous viewers. Dr. Rudi Schäufele is thanked for generous assistance with isotope analyses.

Chapter III: Isotopic composition of cow tail switch hair as an information archive of the animal environment[2]

ABSTRACT

Using isotopic signatures from animal tissue, it is possible to recover certain information about the environment of the animal –notably the diet - at the time the hair was laid down. In the case of tail switch hair of cattle, a single hair may often represent an archive of information spanning a year or more in time. Isotopic analysis by mass spectrometry is now becoming cheap enough to be considered accessible for routine diagnostic or scientific investigation. The ratios of ^{13}carbon (C):^{12}carbon and ^{15}nitrogen (N):^{14}nitrogen are ideal for such investigation, since C and N are constituents of all animal proteins. This paper explains the theory of isotopic analysis in layman's terms, and reports an experiment in which tail switch hair of 9 cattle from three Northland dairy farms was analysed in a 'proof of concept' study, to demonstrate the information-retrieval potential offered by isotopic analysis. Changes in isotopic abundance are measured in parts per thousand (δ, ‰). When matching signatures on replicate hairs, the average distance from the 'interpolation' line was ± 0.13‰ for δ^{13}C, and ± 0.11‰ for δ^{15}N. In contrast to this, differences in δ^{13}C between different hair segments analysed exceeded 11‰, while between farm differences in δ^{15}N exceeded 2.0‰. We suggest possible reasons for these differences in isotopic signature.

[2] Schwertl M, Matthew C, Auerswald K, Schnyder H, Crook K (2003) *Proceedings of the New Zealand Grassland Association* **65**, 147-152.

INTRODUCTION

Animal proteins are largely comprised of the elements carbon (C), hydrogen, nitrogen (N) and oxygen. All elements, including these four, have two kinds of particles in their nucleus, protons and neutrons. The number of protons is fixed for a particular element, but the number of neutrons can vary. For example, C has 6 protons in the nucleus, but may have 5 neutrons (^{11}C, radioactive), 6 neutrons (^{12}C, stable), 7 neutrons (^{13}C, stable), or 8 neutrons (^{14}C, radioactive). These different forms of the same element, with different total numbers of protons and neutrons (i.e. different atomic weight), are known as isotopes. For the stable isotopes of C, the natural abundance of the lighter ^{12}C isotope is 98.89% and of the heavier ^{13}C isotope is 1.11%. Similarly, N has 7 protons, and the stable isotopes of N, ^{14}N with 7 neutrons and ^{15}N with 8 neutrons, have a natural abundance of 99.63% and 0.37%, respectively.

While different isotopes of the same element have identical chemical properties, certain environmental and biological processes may operate selectively in favour of either the heavier or lighter isotope. However, such discrimination is typically in the range of the third or fourth decimal place. The abundance of the heavier isotope is thus expressed in parts per thousand (‰), as compared to an international standard, and is referred to as the δ-value, calculated as follows: $\delta^{13}C$ (or $\delta^{15}N$) $=[(R_{sample}/R_{standard})-1] \times 10^3$, where R is the respective $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio. The C isotopic composition ($\delta^{13}C$) is presented relative to 'V-PDB' (fossil carbonate) standard and the $\delta^{15}N$ relative to N in air. Thus, a negative sample δ-value indicates depletion, and a positive δ indicates enrichment, respectively, of the heavy isotope, compared with the standard.

Many grasses of tropical origin such as paspalum and kikuyu (C_4 grasses) have a different photosynthetic system from temperate (C_3) grasses such as perennial ryegrass (e.g. Cambell *et al.* 1996). During photosynthesis in C_3 plants carbon dioxide containing the heavier ^{13}C is less likely to be assimilated [$\delta^{13}C$ –27 ±2‰, (O'Leary 1988)]. This discrimination against ^{13}C is somewhat suppressed under water deficit, and C_4 plants exhibit little or no preference for ^{12}C [$\delta^{13}C$ –13 ±1‰, (O'Leary 1988)]. Reduced discrimination of C_3 plants for ^{13}C when under water deficit has led to a test for water use efficiency (Farquhar *et al.* 1989) based on the ratio of ^{13}C:^{12}C in plant tissue, though the test must be used with caution (Virgona 1993). Similarly, various ecosystem processes can change the natural abundance of the common isotopes of N (Högberg 1997). While N from biological fixation has $\delta^{15}N$ values close to 0‰, values of $\delta^{15}N$ generally increase with

increasing trophic level of the N (Handley & Raven 1992). Herbivores show higher δ^{15}N-values than their diet, carnivores show higher δ^{15}N-values than their prey. In a number of studies the δ^{15}N of animals was about 3‰ higher (enriched in the heavier isotope) than in their diet (e.g. De Niro & Epstein 1981; Hobson *et al.* 1996).

Signatures of animal forages are both incorporated into animal tissues, and modified by metabolic processes of the animals themselves (De Niro & Epstein 1978; De Niro & Epstein 1981; Tieszen *et al.* 1983; Rundel *et al.* 1988). Animal hair, for example, represents an archive of the isotope signatures of food eaten by that animal at the time the hair was laid down. Nowadays, analysis of isotope ratios in animal hair by mass spectrometry, to recover information on isotope signatures is reasonably cheap, and recently some researchers have explored the possibility of using isotopic analysis of animal hair to recover information about the feeding habits and environment of that animal (Hobson *et al.* 1996; Schoeninger *et al.* 1997; McIlwee & Johnson 1998, see also Chapter II). Changes in δ-value along the length of a hair or between hairs therefore allow inference about seasonal ecosystem changes, or may identify differences between production systems. This paper reports the results of isotopic analysis of cow tail switch hair from three dairy farms located near Dargaville, where both seasonal and between-farm difference in pasture botanical composition (i.e. C_4 grass proportion in the diet) would be expected to occur. While it was expected from previous overseas work cited above that animal hair from the respective farms would reflect dietary differences in isotopic abundance, and that mass spectroscopy would detect such differences, we wished to confirm this for data from New Zealand.

METHODS

Hair samples were collected from an organic dairy farm (3 cows sampled as replicates for statistical analysis), a conventional dairy farm with ryegrass dominant pastures (4 cows), and a conventional dairy farm with a significant kikuyu grass component in pastures (2 cows). A tuft of hairs was cut in early December 2002, as close to the skin as possible, from the tail switch of each of these nine cows. To remove faeces and other contaminants, hair was washed by ultra-sound agitation in deionised water, shaken in a 2:1 mixture of methanol/chloroform for 2 hours, and rinsed with deionised water. After drying (40°C, 48 h), duplicate hairs from each animal were selected in order to verify repeatability of analytical procedures. Hairs were stretched on a frame, cut to 10 mm lengths and each alternate length analysed for δ^{13}C and for δ^{15}N value. Each hair segment was enclosed in a

tin cup (4 x 6 mm) and combusted in an elemental analyzer (NA 1108; Carlo Erba, Milan) interfaced (ConFlo II; Finnigan MAT, Bremen, Germany) to an isotope ratio mass spectrometer (Delta Plus; Finnigan MAT) located at the Institute of Grassland Science, Technical University of Munich. The standard deviation was ± 0.2‰ for $\delta^{13}C$ and ± 0.3‰ for $\delta^{15}N$. For further details of the isotopic measurements see Chapter II.

The isotope patterns of two hairs from the same animal can be displaced relative to each other. Two different mechanisms may contribute to such a mismatch. A 'cutting error' may arise from a difference in stubble length. A 'growth cycle error' can originate from sampling hair in different growth phases. A single hair grows for a certain time (anagen phase), remains in the follicle channel for some time afterwards and finally drops. After a phase of follicle quiescence (telogen phase) a new hair is produced. When cut, an anagen hair cannot be distinguished from a telogen hair, but the latter does not contain recently-formed hair tissue and, hence, misses recent isotopic information. To correct for the mismatch, the isotope signature of one hair had to be shifted relative to the other in 1 mm steps, until a best fit to the second hair was obtained, as described in Chapter II. In total, analysis of 18 hairs (2 hairs per animal, 9 animals from three farms) required mass spectrometry measurement of 81 hair samples.

RESULTS

Repeatability of analysis for different hairs of the same animal

The isotopic patterns of two replicate hairs from the same animal typically showed remarkable similarity after optimum shift (Figure III.1). Close agreement between duplicate hairs was indicated by high correlation coefficients for C and N signatures (mean r = 0.82). Similar mean correlation coefficients were found for the two hairs of each of the other animals analysed (0.80 ± 0.15). Over the 9 pairs of hairs the mean distance of individual measurement points from the interpolation line was ± 0.13‰ for $\delta^{13}C$, and ± 0.11‰ for $\delta^{15}N$.

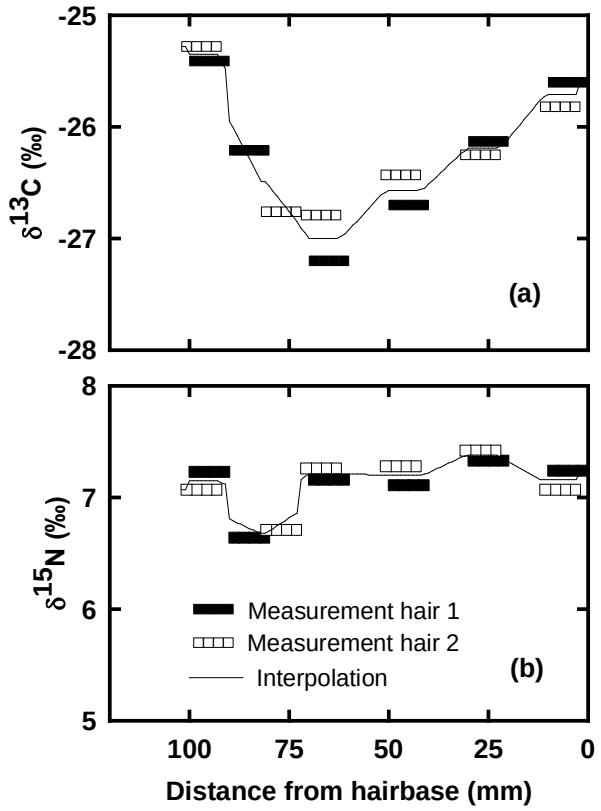

Figure III.1: Isotopic signature for (a) $\delta^{13}C$ and (b) $\delta^{15}N$ of alternate 10-mm sections along two hairs of cow number 13 (Ryegrass farm) shown together with the interpolation line.

Differences between farms

For $\delta^{15}N$, all three farms differed significantly in their isotopic signature, with the organic farm having the lowest $\delta^{15}N$ value, indicating comparative enrichment of the heavier ^{15}N isotope in hair samples from the two conventional farms (Table III.1). For $\delta^{13}C$, each of the farms again exhibited a highly significant difference in mean value (Table III.1).

Table III.1: Mean isotopic signature for $\delta^{13}C$ and $\delta^{15}N$ on the three farms and 95% and 99.9% confidence intervals (CI) for the means. Means different at the 99.9% level are indicated by different letters.

Isotope	Farm	Mean	95% CI	99.9% CI	SD	n
$\delta^{13}C$	Kikuyu	-24.13 A	0.81	1.61	1.35	13
	Ryegrass	-26.29 B	0.16	0.28	0.48	39
	Organic	-20.42 C	1.18	2.12	3.10	29
$\delta^{15}N$	Kikuyu	6.67 a	0.29	0.57	0.48	13
	Ryegrass	7.39 b	0.12	0.21	0.37	39
	Organic	5.28 c	0.28	0.50	0.74	29

However, plotting $\delta^{13}C$ versus the distance of the particular hair segment from the hair base (Figure III.2a), indicated unique temporal patterns in the isotopic signature of each farm, in addition to the difference in farm average $\delta^{13}C$ value (Table III.1). In contrast to the variability over time of $\delta^{13}C$ for isotopic signature, $\delta^{15}N$ signatures for all farms did not change significantly over time (Figure III.2b).

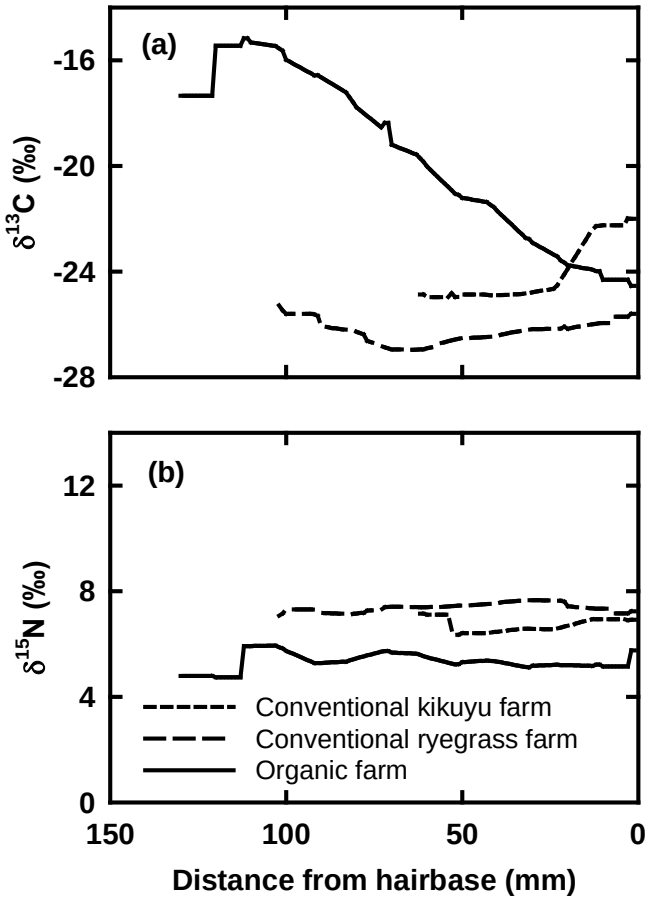

Figure III.2: Average isotopic signatures for (**a**) $\delta^{13}C$, and (**b**) $\delta^{15}N$ of three farms calculated from individual 2-hair averages. Mean deviations of individual lines from average farm line were 0.53 and 0.55‰ (Organic farm, 3 animals), 0.15 and 0.27‰ (Conventional kikuyu farm, 2 animals), and 0.09 and 0.21‰ (Conventional ryegrass farm, 4 animals) for $\delta^{13}C$ and $\delta^{15}N$ respectively. The X-axis reflects a time progression from old hair (left, hair tip) to recently formed hair (right, hair base). The data cover a period of approximately 4 months (early August to 2 December 2002) for the longest hairs.

DISCUSSION

Repeatability

Given that differences in $\delta^{13}C$ between different hair segments analysed exceeded 11‰ on the organic farm (Figure III.2a), and that between farm differences in $\delta^{15}N$ exceeded 2‰ (Figure III.2b), while mean distance from the interpolation line (Figure III.1) was less than 0.15‰ for both $\delta^{13}C$ and $\delta^{15}N$, the present results underline the sensitivity of mass spectrometry, as a forensic tool to recover historical information on certain aspects of animal environment, as 'sampled' through dietary intake.

Differences between farms

The notable features of the data are time-trends in $\delta^{13}C$ signature, unique to each of the farms sampled (Figure III.2a), and between farm differences in $\delta^{15}N$ (Figure III.2b). Hence, the experimental hypothesis that such differences in isotopic signature would be detected is clearly confirmed. However, since detailed records such as hair growth rate, and animal intake and diet composition on each farm were not collected, interpretation of these results was necessarily intuitive. Each of the farmers was interviewed, and relevant data on cow husbandry and diet obtained.

At the organic farm, the three cows from which hair was collected in early December 2002 had calved in July 2001, December 2001 (both milked over), and July 2002, respectively. Tail switch hairs for cows milked over were trimmed in July 2002, and for the cow calving in July 2002, tail switch hairs were trimmed on completion of colostrum milking (late August 2002), untrimmed tail hair being a sign for the person milking, that milk must be discarded. This husbandry practice on the organic farm allows the start of the record for that farm (120 – 140 mm from the hair base, Figure III.2a) to be placed between late July and late August, depending on the length of stubble left when tail hair was cut. At this time, the $\delta^{13}C$-value of around –16‰ indicates a high presence of C_4 material in the diet. Assuming that: (i) C_4 plant material typically has $\delta^{13}C$-values of about -13‰, (ii) pastures grazed in early spring comprised only C_3-plants with $\delta^{13}C$-values about -27‰ (O'Leary 1988), and (iii) that $\delta^{13}C$ of hair is about 1-2‰ higher than diet (De Niro & Epstein 1978; Jones *et al.* 1981), C_4 material made up about 70% of the animal diet at that time. On our enquiry, the farmer confirmed that in August and part of September, cows were receiving feed supplements of molasses, maize silage, and sorghum, all products derived from C_4 plants. However, the quantity of C_4-derived supplementary feed was estimated at 5 kg DM/cow/day, rather less than 70% of the diet for a lactating cow. Since experience to date is that the assumptions above are fairly accurate for estimating proportion of C_4 material in the diet, this apparent discrepancy, and the variability in signal between cows on this farm (Figure III.2a), are two points for follow up study. We can speculate that between cow differences in $\delta^{13}C$-value at the organic farm (Figure III.2a) could arise from differences in the amount of supplement consumed, more negative values being associated with reduced access to the C_4-derived supplement. However, further measurements with supporting information on cow behaviour are needed to confirm this hypothesis. On the organic farm, the $\delta^{13}C$-value took until late November to reach a value

close to that normally found in C_3 plant material (Figure III.2a), even though C_4-derived supplements were not fed after mid-September. This reflects the gradual elimination of C_4-derived carbon from body tissues by dilution, after feeding of this material stopped. Jones et al. (1981) noted that a period of a little over 70 days was required for isotopic composition of animal tissue to fully adjust after a sudden change of diet. Finally, the proportion of C_4 species in pastures of the organic farm must have been low, since the isotopic signature at the hair base (Figure III.2a), based on our model assumptions (see above), indicated not more than 10% C_4 material in the diet by late-November 2002.

In contrast with the organic farm, no C_4-derived supplements were fed at the conventional farms in early lactation. For the ryegrass farm the $\delta^{13}C$-values are as expected, consistent with there being little or no C_4-derived plant material in the diet. Values for $\delta^{13}C$ at this farm did vary a little with time, being lowest about 60 mm from the hair base, but the reason for this is unknown. For the kikuyu farm, we were at first surprised by the $\delta^{13}C$ values, indicating an average of only 10% of C_4 species in the diet, but with the C_4 component increasing to approximately 30% for hair laid down just prior to sampling, that is in November (Figure III.2a). However, on visiting the farm and inspecting the pastures in May 2003, it was learned that the farmer's practice is to 'mulch' kikuyu pastures in autumn with a heavy mower to break up the stem mat formed over summer and allow C_3 species to provide winter forage. During this visit, C_3-dominant pastures were observed, with annual dicotyledonous species such as chickweed (*Cerastium glomeratum*) also contributing significantly to the animal diet. The farmer estimated C_3 grass content of spring pastures was at least 75%, and November is the month when kikuyu plants in pastures in this region begin to show strong summer growth. Hence, the isotopic record, although unexpected, proved to be explained very well, once the relevant facts were collected.

Although $\delta^{15}N$ differences between diet and animal are reported to deviate from the mean of about 3‰ in some physiological stress situations like fasting (Hobson *et al.* 1993) and water and/or N stress (Ambrose & De Niro 1986; Sealy *et al.* 1987; Ambrose 1991; Cormie & Schwarcz 1996), we would not expect that these factors influenced the present results. The cows were of the same breed and were kept in the same region, in similar physiological condition (lactation), and nutrient and water supply was sufficient. Thus, differences in $\delta^{15}N$ between animals and between farms appear to arise from differences of $\delta^{15}N$ in the diet. We can only speculate on the reason for between-farm (Figure III.2b) differences in $\delta^{15}N$ of herbage, but one possibility that could be investigated is that such

differences reflect differences between farm systems in N loss by volatilisation as this is a process that strongly favours ^{14}N (Gormly & Spalding 1979). The lower $\delta^{15}N$-value for the organic farm is consistent with lower gaseous N losses on this farm.

Potential applications of isotopic analysis

Based on these results, isotopic analysis is a sensitive tool for detection of certain types of variation in dietary composition of animals. In the present study, between-farm differences in the level of C_4-plant-derived material in the animal diet were readily detected, and also a between farm difference in $\delta^{15}N$, of uncertain significance. The methodology could be utilised in animal behaviour studies where percentage of C_4 species in the diet is of interest, potentially can detect practices such as feeding of animal protein to ruminants, or could be used to verify product origin, for example.

ACKNOWLEDGEMENTS

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Chapter IV: Carbon and nitrogen stable isotope composition of cattle hair: ecological fingerprints of production systems?[3]

ABSTRACT

Societal interest in food safety, animal welfare, and environmental quality attributes of food production is increasing, creating a need for reliable indicators of such factors. Here we test the hypothesis that cattle farming systems create unique and meaningful isotopic fingerprints, which can be characterized by analysing cattle tail switch hair. To this end we analysed feeding practices and nutrient fluxes, and sampled hair, feed components and fertilizers from 13 different farms in Upper Bavaria, Germany. The farms represented the range of cattle farming types present in the region and included: conventional confinement dairy, pasture based organic and conventional dairy, suckler cow, and bull and steer and heifer fattening enterprises. Samples were analysed for their carbon (C) and nitrogen (N) stable isotope composition ($\delta^{13}C$ and $\delta^{15}N$). Feed samples could be assigned to one of three groups with characteristic $\delta^{13}C$, which varied very little between and within farms: C_3 forages (including fresh forage, hay or silage from grassland and clover-grass mixtures) with -28.4‰ (±0.5‰ SD), maize *(Zea mays L.)* with –12.5‰ (±0.4‰), and C_3-derived concentrates (including mainly cereal grain and legume seeds) with -26.8 (±1.1‰). The dry matter fraction of maize in the diet explained 96% of the farm average $\delta^{13}C$ of hair. Hair was approx. 2.7‰ enriched in ^{13}C relative to the diet (trophic level shift), and this effect was very similar for growing animals and cows, and seemingly independent of the fraction of maize in the diet. In contrast to $\delta^{13}C$, the $\delta^{15}N$ of individual feed types differed very strongly between – and also within – farms. Only legume seeds had relatively constant $\delta^{15}N$ (1.2 ±0.5‰). $\delta^{15}N$ of cow hair was correlated with stocking rate ($r^2 = 0.55$) and N input surplus (farm gate) ($r^2 = 0.78$), respectively. This correlation was probably caused by increasing losses of ^{15}N-depleted N via ammonia volatilisation, nitrate leaching and denitrification with increasing farm-level N surplus. Heterogeneity of feed ^{15}N signatures indicated within-farm heterogeneity of N fluxes and cycling that was at (least partially) integrated in cattle hair. Thus, cattle hair ^{15}N signature appears to indicate the 'leakiness' of

[3] Schwertl M, Auerswald K, Schäufele R, Schnyder H (2005) *Agriculture, Ecosystems and Environment* **109**, 153-165.

cattle production systems for N. Conversely, the ^{13}C signatures reliably indicates maize feeding and, thus, the type of land use (arable forage cropping *versus* grassland farming) on which cattle production in the region is based.

INTRODUCTION

There is currently an increasing interest in food safety, but also in animal welfare, and environmental and ecological quality attributes of food production (Gregory 2000; Opara & Mazaud 2001). To evaluate agricultural production systems, indicators of resource use and environmental impact are required (Bockstaller *et al.* 1997; Halberg 1999). Health hazards like the mad cow disease (BSE) have shown that, especially in livestock production, traceability systems of agro-processing are necessary to proof authenticity and increase consumers confidence (Latouche *et al.* 1998; Dickinson & Bailey 2002; Hermansen 2003).

The stable isotope composition of different elements in biological products contain information about their geographical and ecological origin (e.g. Rossmann *et al.* 2000). This is because certain biological and environmental processes characteristically discriminate against the light or heavy isotope of an element. That is, they change the isotope composition in the product as compared to the substrate. Thus, isotope signatures can serve as process indicators and tracers of ecosystem sources and sinks. Oxygen isotope composition ($^{18}O/^{16}O$) has provided helpful information for origin assignment and authenticity control of fruit juices, wines, milk and milk products (Bricout 1973; Dunbar 1982; Kornexl *et al.* 1997; Rossmann *et al.* 2000). This is because oxygen but also deuterium/hydrogen (D/H) stable isotope signatures in terrestrial ecosystems largely depend on latitude, distance from sea, altitude, total precipitation and seasonal effects (e.g. Rozanski *et al.* 1992). Carbon isotope composition ($^{13}C/^{12}C$) depends on photosynthetic mechanisms, with C_3 plants depleted in ^{13}C relative to C_4 plants (Smith & Epstein 1971). This signal is passed forward in the food chain (De Niro & Epstein 1978) and, thus, enables examination of the contribution of C_3 or C_4 plants to modern or palaeodiets (Vogel 1978; Teeri & Schoeller 1979) and helps to determine the origin of agricultural products (Kornexl *et al.* 1997; Rossmann *et al.* 2000). Similarly, the nitrogen isotope composition ($^{15}N/^{14}N$) of animal products depends on the ^{15}N abundance of feeds (De Niro & Epstein 1981), which are determined by that of N sources, such as fertilizers or soil N (Högberg 1997). N in plants and soil may be affected by losses from the system, such as leakage of nitrate,

volatilisation of ammonia, and denitrification as is suggested from observations in cropland (Meints *et al.* 1975), forest ecosystems (Högberg 1991), and rangeland (Frank *et al.* 2000).

Still, the practical application of C and N stable isotope composition as an ecological fingerprint is charged with significant uncertainties. Thus, the relationship between the $\delta^{15}N$ of animal products and cropping practices and nutrient fluxes (inputs, outputs, and cycling) and balances of farms has not been analysed systematically. N fluxes in cattle farming systems may be variable, complex, and involve the cycling of large quantities of N (up to 500 kg ha^{-1} a^{-1}). Especially N fertilisation of feed crops *via* manure (including variable rates and types of N losses) may create feed-back mechanisms with uncertain effects on system-scale $\delta^{15}N$. Also, $\delta^{13}C$-based estimates of the fraction of C_3 (or C_4) plants in diets may be highly inaccurate because of variation of the $\delta^{13}C$ within the photosynthetic groups. Reported $\delta^{13}C$ variability is high: C_4 –9 to –16‰, C_3 –20‰ to –35‰; (Deines 1980; O'Leary 1988). Moreover, incorporation of C and N in animal products is associated with isotope effects during digestion and metabolism, leading to a difference between the isotope signatures of feeds and products. Recent work suggests that such trophic level shifts may vary between animals of the same species and depend on feed characteristics (Roth & Hobson 2000; Sponheimer *et al.* 2003b). The overall effect of all these uncertainties on the reliability of isotope signatures as indicators of farming practices can only be assessed by farm-level investigations.

Thus, the present work was conducted to test the hypothesis, that cattle farming systems create unique isotopic fingerprints, which can be interpreted quantitatively in terms of maize *(Zea mays L.)* feeding ($\delta^{13}C$) and N balance components ($\delta^{15}N$). We tested the hypothesis by analysis of feeding and fertilizer practices, and $\delta^{13}C$ and $\delta^{15}N$ of cattle tail switch hair, feed components and fertilizers from 13 farms in Upper Bavaria, Germany. Tail switch hair was chosen since it provides a permanent isotopic record, which – if analysed as a whole – absorbs, dilutes and averages short-term fluctuations of the (isotopic) feeding history of animals. Thus, it should reflect the grand (conservative) characteristics of farming systems.

MATERIAL AND METHODS

Study Area

The study area was located between 47°39' to 48°29' North, and 11°34' to 12°28' East in Upper Bavaria, Germany, and included sites between 430 and 1300 m above sea level. Long term mean annual air temperatures ranged between 6 and 8°C (Enders 1996), except for the alpine farm, F_a (<5°C). Long term mean annual precipitation varied between 750 and 1700 mm (Table IV.1). Soil development in the whole area started in Late Glacial and soils on all farms were Alfisols (Luvisols) and Inceptisols (Cambisols) derived from unconsolidated clastic sediments.

Farms

Farms were chosen to represent the range of cattle production systems present in the area (Table IV.1). Details of land use (plant cultivation, fertilization, yields), livestock husbandry, diet composition, type and amount of imported and exported products for the year before sampling, and information on long term farming practices were obtained by personal interviews with farmers and data of local extension authorities (farms A-I in Table IV.1), or were provided by farmers in cooperation with local extension authorities (farms J-L). On farm F animals were kept on alpine pastures (F_a) from June to August/September, and on the valley home farm (F_v) for the rest of the year. Stocking rates are given in livestock units (1 LU = 500 kg live-weight) per hectare (ha) farm area.

Table IV.1: Details of production systems, number and breed of analysed animals and location

Farm	*Production type*	*Breed*	*Number and type of sampled animals*	*Cattle keeping*	*Production system characteristics*	*Stocking rate (LU/ha)*[4]	*Altitude (m asl)*[5]	*Annual Precipitation (mm)*
A	Dairy	Fleckvieh, Schwarzbunte	5 cows	half-day grazing in summer, slurry	Organic, additional cash crops	1.7	550	1050
B	Dairy	Fleckvieh	4 cows	confinement, slurry	Conventional	2.3	530	1050
C	Cow-calf	Angus	2 cows 2 steers	grazing in summer, deep litter, cattle keeping since 1999	Organic, mainly cash crops	0.6	580	1050
D	Dairy	Fleckvieh	5 cows	grazing in summer, litter	Organic (>40 years), additional cash crops	1.1	550	1200
E	Bull fattening	Fleckvieh	4 bulls	confinement, slurry	Conventional	2.7	470	1300
F_v	Dairy	Fleckvieh	3 heifers	Confinement (September-June), slurry	Organic	1.8	680	1400
F_a	Dairy	Fleckvieh	3 heifers	grazing (June-September)	Organic	0.3	1200	1700
G	Dairy	Fleckvieh	3 cows	confinement, slurry	Conventional	3.5	490	1100
H	Cow-calf	Limousin, Fleckvieh, Angus, crossbred	3 cows 1 steer 1 heifer	grazing in summer, deep litter	Organic (since 1999),	1.3	430	800
I	Steer and heifer fattening	Fleckvieh-Angus crossbred	2 steers 1 heifer	grazing in summer, litter	Organic, additional pig fattening	1.3	500	750
J	Dairy	Fleckvieh	4 cows	confinement, slurry	Conventional	2.4	640	1200
K	Dairy	Fleckvieh	3 cows 1 heifer	half-day grazing in summer, slurry	Conventional	1.6	700	1300
L	Dairy	Fleckvieh	4 cows	confinement, slurry	Conventional	3.0	600	1100

[4] including all livestock, 1 LU = 500 kg live-weight

[5] weighted mean for farm area

Hair sampling

Hair samples were collected from 3-5 animals per farm in September/October 2001 (A-H in Table IV.1), October 2003 (I), or January 2004 (J-L). The breeds included Deutsches Fleckvieh, Limousin, Deutsche Schwarzbunte and Aberdeen Angus (Table IV.1) and crossbreeds of the afore mentioned breeds. All sampled animals were older than 16 months. The bulls of farm E had been kept on the farm for 13 months prior to sampling. All other animals were kept on the same farm for more than 2 years or for their whole life. Growing animals had been weaned at the latest 10 months before sampling. In those cases where cows and growing animals (steers or heifers) were sampled on the same farm (C, H, K) these were fed on similar diets.

From each animal a tuft of tail switch hair was cut with scissors as closely as possible to the skin. The hairs were washed successively with deionised water, and a 2:1 mixture (v/v) of methanol and chloroform, and then dried at 40°C for 48 h. Two hairs of similar length (between 70 and 570 mm, mean for all animals: 230 mm) were chosen from each animal, cut into sections of 10 mm length and alternate sections were placed in tin cups (4x6 mm) for isotope analysis (see below).

Sampling of feeds and mineral fertilizer

Feed components (n = 43), and mineral N fertilizers (n = 6) were sampled from farms A-H, and E and G, respectively. From each component 50-150 g were randomly taken from feeding tables, grassland or stores in June, July, August, or October 2003. Feed samples were dried (1 h at 95°C, then at 70°C for 48 h), and successively ground in an expeller mill and ball mill. Fertilizer samples were ground with a pestle and mortar. Aliquots of homogenized plant samples (0.7 ±0.05 mg) or fertilizers (0.1-0.5 mg to give about 80 µg N) were placed into tin cups (4x6 mm) for analysis.

Isotope measurement

Samples were combusted in an elemental analyser (NA 1110; Carlo Erba, Milan, Italy) interfaced (Conflo II; Finnigan MAT, Bremen, Germany) with an isotope ratio mass spectrometer (Delta Plus; Finnigan MAT). The C and N isotope composition of each sample was measured in the same measurement run. Isotope data are presented in the conventional form, i.e. in parts per thousand (‰) as $\delta^{13}C$ and $\delta^{15}N$, where $\delta^{13}C$ (or $\delta^{15}N$) = $[(R_{sample}/R_{standard})-1]*10^3$, with R the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio in the sample or standard (V-

PDB, "Vienna Pee Dee Belemnite", for ^{13}C ; and AIR, for ^{15}N). Each sample was measured against a secondary laboratory standard, which was previously calibrated against an international standard (IAEA-CH6 for ^{13}C , and IAEA-NO-3 for ^{15}N).

Evaluation of hair isotopic data

The method produced 18 to 149 (mean 90) $\delta^{13}C$ and $\delta^{15}N$ hair measurements per farm, respectively. The $\delta^{13}C$ and $\delta^{15}N$ patterns along two hairs from the same animal showed a high degree of similarity (mean r^2 = 0.68 ±0.18) (for method see Chapter II). Hairs of all growing animals had grown after weaning. Hairs of analysed bulls had grown after the bulls were shifted to this farm. This was ascertained by position-time transformation of the distance values in assuming a minimum hair growth rate of 0.51 mm/d (Fisher *et al.* 1985).

For farm F ^{15}N signatures along each hair clearly changed when the animals were shifted between the valley home farm and the alpine pastures. The corresponding ^{13}C signatures were therefore assigned to separate systems designated F_v and F_a. Hair grown during the first 2 to 4 weeks following the transfer to the alpine pasture was discarded from the data set, because its isotopic composition likely not fully reflected the new forage.

N input and output of farms

For every farm the average N input and output was determined for the year before sampling hair. Only land used for forage production or spreading of manure was included. Thus, set-aside areas and land used exclusively for cash crop production and fertilised minerally was excluded. Generally, the sum of such areas only accounted for a small proportion of the farm land (0 to 2%), except for farm K (20%).

The sum of dry and wet deposition in the study area was rather similar and amounted to17 kg ha^{-1} a^{-1} for the time between 1980 and 1995 (Müller 1997). Weigel et al. (2000) showed that gaseous deposition is of very similar magnitude. Thus, a mean total deposition of 34 kg ha^{-1} a^{-1} was assumed for all land.

N derived from biological fixation of atmospheric N_2 (Ndfa) by legumes was estimated following standard protocols (Hege *et al.* 2003). On grassland estimated Ndfa ranged between 30 and 60 kg N ha^{-1} a^{-1} depending on N fertilisation (sum of mineral and organic fertiliser N). For legume crops and clover-grass estimated Ndfa was between 48 and 254 kg N ha^{-1} a^{-1} depending on legume content and growth period.

Maize in diet

The mean contribution of maize to diet was calculated on a dry matter (DM) basis for the year before sampling by relating average yields of grassland (according to intensity of use), non-maize feed crops and imported feed with yields and import of maize.

RESULTS

Farm gate N balances and balance components

Mean N input varied between 64 (F_a) and 322 kg ha^{-1} a^{-1} (L) (Figure IV.1). Ndfa contributed between 20 (G) and 105 kg ha^{-1} a^{-1} (A) (mean 52 kg ha^{-1} a^{-1}) with low N_2 fixation for conventional farms, or short vegetation periods (F_a).

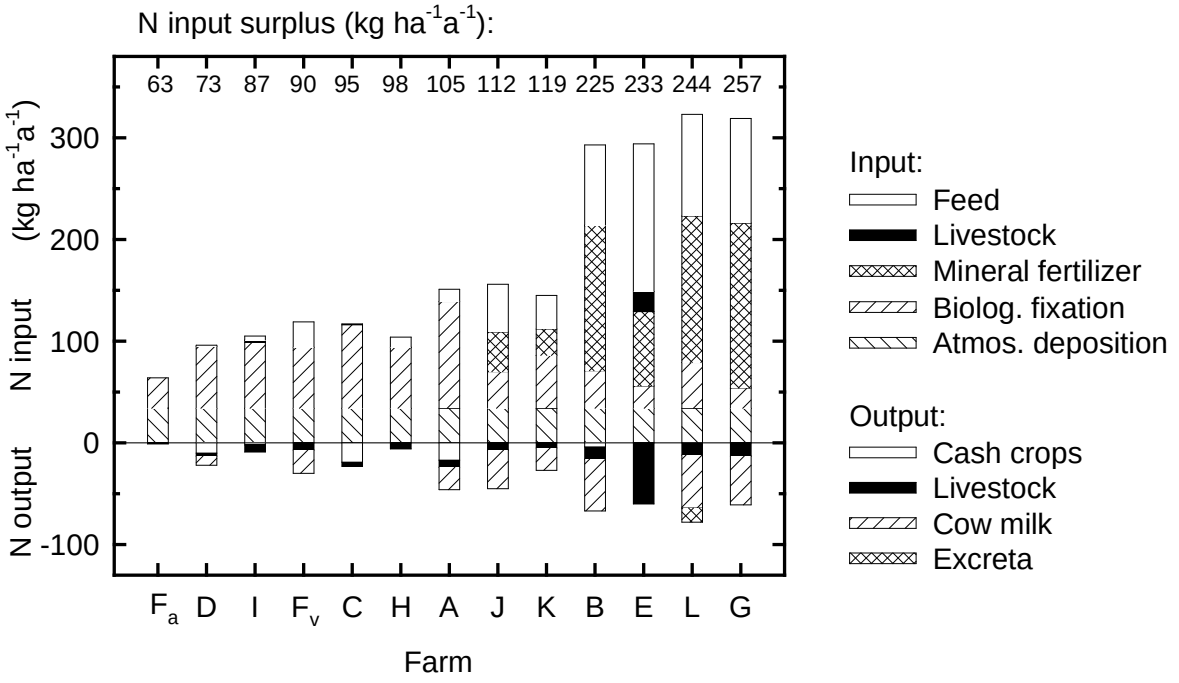

Figure IV.1: Average N input and product output of farms for the year before hair sampling. Farms are ranked according to N input surplus from the left to the right. In steady state condition input surplus must be balanced by equal N loss *via* ammonia volatilisation, nitrate leaching or denitrification. For farm characteristics see Table 1.

Low amounts of Ndfa in conventional farms corresponded with high import of mineral fertilizer (range: 26 to 162 kg ha^{-1} a^{-1}). Feed N was mainly imported as cereals and cereal by-products (in mean 50% of feed N import), legume seeds (20%), and potato (*Solanum tuberosum L.*) and sugar beet (*Beta vulgaris L. var. altissima Döll*) by-products (20%).

Except for farm C, N exports were mainly in the form of animal products (livestock and milk). About half of the livestock export from farm I was pork, for all other farms only beef livestock was exported. Some animal excreta were sold as slurry by farm L. Notably, each farm could be assigned to one of two groups of production systems differing in N surplus: organic and conventional farms with a surplus <120 kg ha^{-1} a^{-1}, and conventional farms with a surplus >220 kg $ha^{-1}a^{-1}$. When steady state conditions are reached N input surplus must be balanced by N losses *via* ammonia volatilisation, nitrate leaching or denitrification.

Isotope signatures of feeds and fertilizers

^{13}C signatures of the same type of feed were very similar for the different farms (Table IV.2). In particular, a close similarity was found for $\delta^{13}C$ of all roughage components including fresh forage, silage and hay from grassland (mainly *Lolio-Cynosuretum* communities) or clover-grass (mainly dominated by *Lolium perenne L., Trifolium pratense L., Trifolium repens L., Dactylis glomerata L., Festuca pratensis Huds., and Poa pratensis L.*) which averaged –28.4‰ and differed by less than 1.6‰. Similarly, $\delta^{13}C$ of maize components varied very little. Also, except for sugar beet molasses, the (C_3-derived) concentrates differed little in $\delta^{13}C$, but they were consistently enriched in ^{13}C by about 2‰ compared to grassland-derived forage. The ^{13}C signatures of these three groups (i) grassland forage including clover-grass, (ii) maize, and (iii) concentrates were significantly different from each other ($P < 0.01$).

In contrast, there was an extreme scatter in ^{15}N signatures of diet components. Even within the same farm and identical types of feed differences of up to 4‰ occurred. Still, variation in $\delta^{15}N$ between different types of feed and between different farms was higher. Similar ^{15}N signatures within one type of feed was only found for legume seeds (1.2 ±0.5‰). Interestingly, consistently low ^{15}N signatures of all major dietary components were found for farm D (0.4 ±0.6‰).

Table IV.2: Mean isotope signatures ($\delta^{15}N$ and $\delta^{13}C$) of diet components

Farm	Dietary component	$\delta^{15}N$ (‰)			$\delta^{13}C$ (‰)		
		Farm products	Imported legume seeds	Other Imports	Roughage	Maize	Concent-rates
A	Clover-grass, silage	**2.4**			**-29.0**		
	Maize silage	**7.2**				**-13.2**	
	Maize meal	**6.3**				**-12.1**	
	Triticale (*Triticale secale*) seed	**4.2**					**-27.7**
	Field bean (*Vicia faba var. minor*) seed		**1.3**				**-27.6**
	Field pea (*Pisum sativum var. arvense*) seed		**1.8**				**-27.5**
B	Perennial ryegrass (*Lolium perenne*), silage	**3.9**			**-27.9**		
	Grassland, silage	**3.5**			**-27.9**		
	Grassland, hay	**2.8**			**-28.8**		
	Maize silage	**2.0**				**-12.8**	
	Soybean (*Glycine max*) meal		**1.0**				**-26.0**
	Instant dairy concentrate mixture			**1.7**			**-26.0**
	Rape (*Brassica napus*) seed meal			**2.7**			**-26.6**
	Sugar beet molasses			**3.6**			**-28.3**
C	Clover-grass, fresh	**-1.5**			**-27.9**		
	Grassland, hay	**1.8**			**-29.3**		
D	Clover-grass, fresh	**0.3**			**-28.3**		
	Grassland, silage	**-0.2**			**-27.7**		
	Grassland, fresh	**0.5**			**-28.1**		
	Grassland, hay, 2nd growth 2002	**1.3**			**-29.0**		
	Grassland, hay, 1st growth 2003	**0.7**			**-28.0**		
	Grassland, hay, 2nd growth 2003	**-0.3**			**-28.1**		
E	Grassland, hay	**-0.3**			**-28.0**		
	Maize silage	**6.5**				**-12.4**	
	Soybean meal		**0.5**				**-25.1**
F_v	Grassland, fresh	**3.8**			**-28.3**		
	Grassland, hay	**2.0**			**-29.1**		
	Instant dairy concentrate mixture			**4.6**			**-25.0**
G	Grassland, silage	**4.0**			**-28.9**		
	Maize silage	**4.5**				**-12.3**	
	Barley (*Hordeum vulgare*) seed	**0.8**					**-27.8**
	Soybean meal		**1.5**				**-25.5**
	Sugar beet molasses			**5.2**			**-28.0**
	Wheat (*Triticum aestivum*) seed			**2.1**			**-26.7**
H	Grassland, fresh, site 1	**1.8**			**-28.7**		
	Grassland, fresh, site 2	**0.4**			**-28.3**		
	Grassland, fresh, site 3	**1.7**			**-29.1**		
	Grassland, silage, storage a	**1.6**			**-28.4**		
	Grassland, silage, storage b	**1.6**			**-28.4**		
	Grassland, silage, storage c	**1.6**			**-28.9**		
	Grassland, hay, storage d	**4.3**			**-28.1**		
	Grassland, hay, storage e	**1.9**			**-27.9**		
	Grassland, hay, storage f	**3.6**			**-28.7**		
Mean		**2.3**	**1.2**	**3.3**	**-28.4**	**-12.5**	**-26.8**
SD		**± 2.1**	**± 0.5**	**± 1.4**	**± 0.5**	**± 0.4**	**± 1.1**

Fertilizer $\delta^{15}N$ varied between –0.5 and 0.3‰ (Table IV.3) with a mean of –0.1 ±0.3‰, which is close to that of N in air.

Table IV.3: $\delta^{15}N$ of fertilizers used on farms E and G.

Sampled from	Type of fertilizer	N content (%)	$\delta^{15}N$ (‰)[6]
E	Lime ammonium nitrate	27	-0.1
	Urea	46	-0.5
G	N-P-fertilizer (20-20)	20	-0.2
	Lime ammonium nitrate	27	0.2
	N-P-K-S-Mg-fertilizer (20-8-8-5-2)	20	-0.3
	N-P-K-fertilizer (15-10-10)	15	0.3

Isotope signatures of hair

Hair ^{13}C signatures varied between –26.3‰ and –14.1‰ with highest (that is least negative) values for the bull fattening farm, followed by conventional confinement systems and grassland based conventional or organic production systems (Figure IV.2).

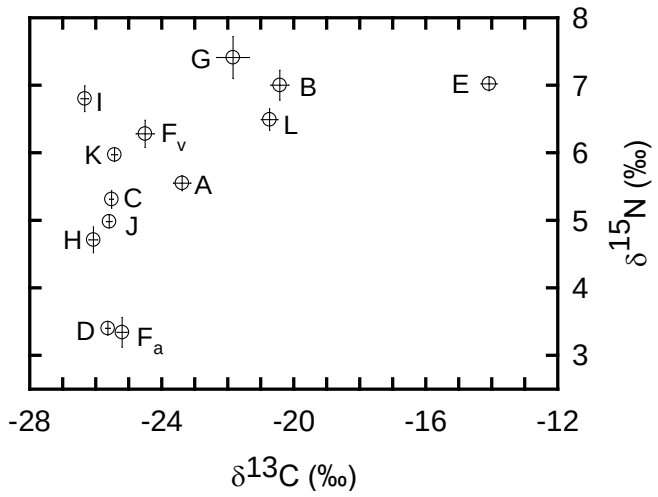

Figure IV.2: Farm mean $\delta^{13}C$ and $\delta^{15}N$ of cattle hair (± 95% CI). For farm characteristics see Table 1.

[6] standard deviations for 2 replicate measurements were below ±0.2‰

Consistently high ^{15}N signatures were found for conventional confinement dairy and bull fattening farms (6.5‰ to 7.4‰), whereas ^{15}N signatures in grassland based systems varied considerably (3.3‰ to 6.8‰). Despite the large variations in feed signatures, the 95% confidence interval was smaller than 1.0‰ in every case, demonstrating the integrative effect of tail switch hair.

When fed similar diets the ^{15}N signatures of growing animals (heifers and steers) were 0.3 to 1.1‰ (mean 0.7‰) higher than those of cows (Figure IV.3a). Differences were negligible for ^{13}C signatures (Figure IV.3b).

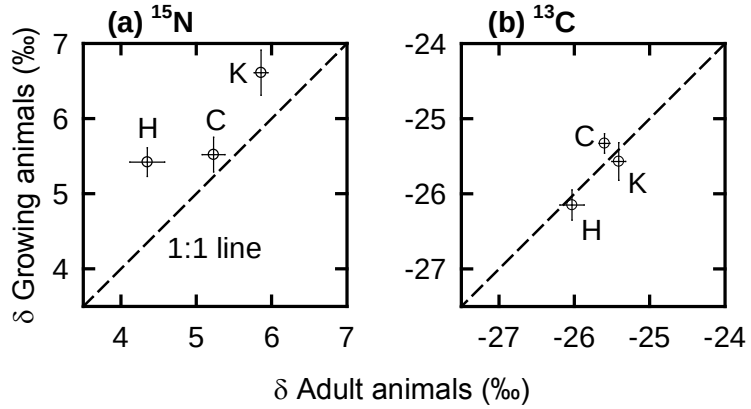

Figure IV.3: Mean hair stable isotope signatures (± 95% CI) of N (**a**) and C (**b**) for adult animals (cows) and growing animals (heifers and steers) from the same farms and fed on similar diets within each farm.

Relationship of $\delta^{13}C$ in hair to maize in diet

The fraction of maize in diet varied between 0 and 75% DM between farms and with hair ^{13}C signatures (Figure IV.4). Hair signatures of farms where no maize was supplied varied between -26.3 (I) and –25.2‰ (F_a) with a mean of –25.7‰ for pure C_3 diets.

The proportion of C_4 in diet was very closely related to $\delta^{13}C$ of hair, C_4 proportion explaining 96% of the variation of $\delta^{13}C$ in hair.

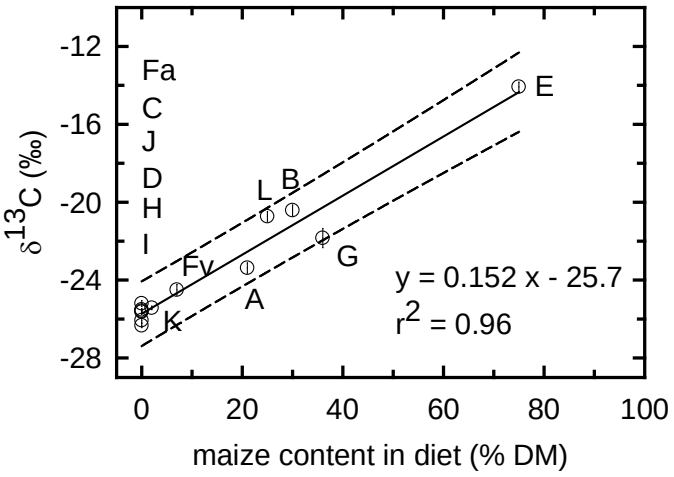

Figure IV.4: Mean ^{13}C hair signatures of farms (± 95% CI) dependent on dietary maize content (% dry matter) for the year before sampling. Regression line (solid) and 95% confidence interval (dashed) for prediction of single values are shown.

Relationship of $\delta^{15}N$ in hair to N balance and stocking rate

Mean hair ^{15}N signatures of cows correlated with stocking rate (r^2= 0.55) (Figure IV.5), but r^2 was reduced slightly (0.52) when growing animals (bulls, steers, heifers) were included. High stocking rates were related with high N inputs, and there was a very close correlation between ^{15}N signatures of cows and N input surplus (r^2= 0.78) (Figure IV.6). Again, the correlation was weaker (r^2= 0.54) when signatures of growing animals were included in the comparison.

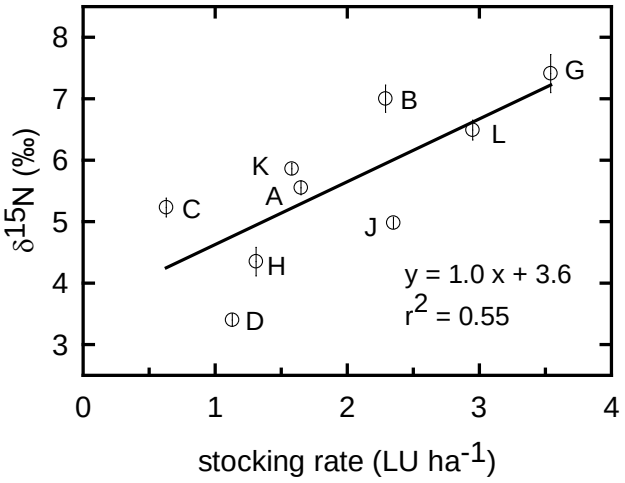

Figure IV.5: Mean ^{15}N cow hair signatures of farms (±95% CI) dependent on stocking rate (1 LU = 500 kg live-weight) with regression (solid line).

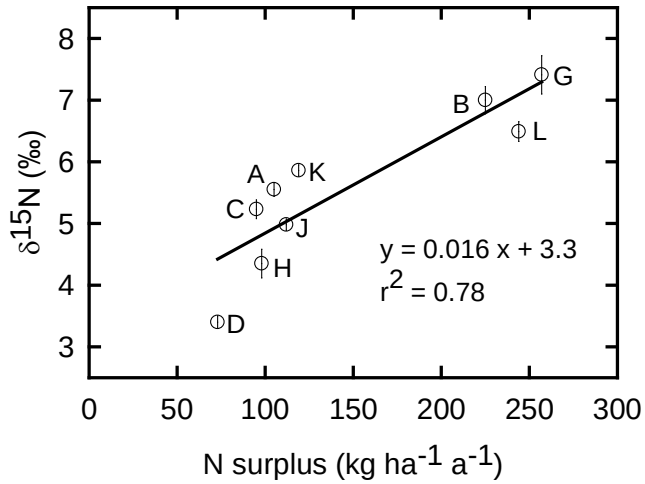

Figure IV.6: Mean ^{15}N cow hair signatures of farms (±95% CI) dependent on yearly N input surplus with regression (solid line).

DISCUSSION

^{13}C signatures

Maize content of the diet explained 96% of the total variation of ^{13}C in tail switch hair of a large variety of cattle production systems, including organic and conventional confinement and pasture based dairying, cow-calf operations, and bull and steer and heifer fattening enterprises. This tight relationship was essentially due to the minimal variation in the δ of the two main components of feeds: (i) forage from grassland, and clover-grass leys, and (ii) maize silage (SD < 0.5‰ for both types of forage). Although the δ of concentrates differed significantly from that of the other components and was more variable, this had only a small effect on hair $\delta^{13}C$, because of the small contribution of concentrates to the diet (average 8% of dietary DM). In those instances where concentrates formed a larger fraction of the diet (12-17% of dietary DM in production systems B, E, G, J and L) it was in the form of instant dairy concentrate mixtures (0-13%), sugar beet molasses (0-7%), soybean meal (0-7%), and wheat and barley seed (0-8%). The δ of these concentrates were enriched in ^{13}C by less than 3‰ compared to that of grassland and clover-grass forage. In consequence, concentrate supplements contributed less than 0.5‰ to ^{13}C enrichment in cattle diet.

In the six farms which fed no maize (in mean 97% DM grassland derived forage), the $\delta^{13}C$ of hair was -25.7‰ (±0.4), 2.7‰ enriched in ^{13}C relative to average $\delta^{13}C$ of grassland and clover-grass forage. This δ-difference between hair and diet is identical to the

trophic level shift found for 8 growing yearlings fed on alfalfa diet (2.7‰) by Sponheimer *et al*. (2003a). Extrapolating the regression in Figure IV.4 for 100% maize leads to a $\delta^{13}C$ hair signature of –10.5‰, giving an estimated 2.0‰ trophic level shift for ^{13}C in the case of a pure C_4 (maize) diet. Thus, the trophic level shift was low in comparison to variability of $\delta^{13}C$ of the C_3 and C_4 feed components, and nearly constant with the different feeds (Figure IV.2). If anything, the trophic level shift was slightly lower for maize-rich diets, which might be related to the larger contribution of C_3 components to protein relative to total dry matter ingestion (maize is poor in protein, but hair is formed of protein). However, if it occurred, the effect was small, perhaps due to the active interconversion of C_3 and C_4-derived C in microbial protein synthesis in the rumen (Bondi 1987; Asplund 1994). Given a relatively constant ^{13}C trophic level shift, a high proportion of mixing of C and N protein compounds in the rumen, and minimal variation in the $\delta^{13}C$ of main feed components, a lower variation from the regression than found in Figure IV.4 could be expected. Especially for production systems A, B, G and L hair signatures deviated somewhat from the general relationship. In these systems, including Fv, the proportion of maize in the diet varied seasonally, leading to pronounced $\delta^{13}C$ variations along single hairs (variation was up to 6.7‰, data not shown). Thus, when periods that were integrated on hairs deviated from the average of the year, mean hair ^{13}C signatures did not accurately represent the year but a shorter or longer period. A more accurate time assignment as suggested in Chapter II would improve the correlation. However, lack of knowledge of the exact seasonal variation of maize proportion in the diet precluded this.

Yet, the close correlation of Figure IV.4, which is also close to the theoretical line of C_3/C_4 mixtures, suggests that analysis of hair signatures could help to characterise (and perhaps quantify) these unknown seasonal variations in feeding regime. A similar conclusion can be drawn from the comparison of individual animals within one farm, which may deviate in mean and pattern. This was especially obvious for farms B and G (data not shown), and might be related to differences in feeding regime, diet selection and intake, digestion or absorption between animals.

C_3 concentrates were on average 2‰ enriched in ^{13}C compared to grassland forage, perhaps due to higher concentration of relatively ^{13}C-rich compounds such as starch and sucrose (Gleixner *et al.* 1993). The relatively high ^{13}C signature of hair produced on alpine pastures (F_a: 25.2‰), was probably due to the altitude effect on $\delta^{13}C$ of vegetation (Körner *et al.* 1991). Differences in $\delta^{13}C$ of grassland vegetation could also be expected due to

different chemical composition of plants (Hobbie & Werner 2004). However, the low variation in $\delta^{13}C$ of silage, hay and fresh forage from grassland and clover-grass, suggest that these factors were of minor importance.

^{15}N signatures

Influence of N surplus

Stocking rate explained 55% of the total variation in cow hair ^{15}N signatures. With increasing stocking rate more animal excreta per area are produced, suggesting that loss of N, e.g. as NH_3, from the system increases. In equilibrium, under standard temperature and pressure, NH_3 emissions are 34‰ depleted in ^{15}N compared to the aqueous NH_4^+ source. In reality fractionation will deviate somewhat from 34‰, e.g. depending on wind speed and temperature (Urey 1947). The NH_4^+ remaining in manure, thus, becomes enriched in ^{15}N depending on isotope fractionation and the fraction of N lost as NH_3. Yet, NH_3 volatilisation also depends on agricultural practices like animal keeping or feeding strategies, as well as storage and spreading of animal excreta. In addition, NH_3 can be emitted from urea or NH_4^+ containing mineral fertilizers (Bussink & Oenema 1998). Agricultural practices, which allow for high losses, can only be maintained on a productive level if these losses are replaced by correspondingly high inputs. A closer correlation was hence found between cow hair $\delta^{15}N$ and N input surplus of farms (farm gate balance). If maintained over long periods, such surpluses will result in equal losses leading to ^{15}N enrichment in the total farm system over time.

Other pathways of N loss may be denitrification and leaching of NO_3^-. These pathways can also lead to an enrichment of the remaining N, but enrichment is less pronounced and highly variable (Wellman *et al.* 1968; Delwiche & Steyn 1970; Ostrom *et al.* 1998). Nitrate leaching is expected to be independent from N input below 120 kg ha^{-1} a^{-1} but increases with higher input under these farming conditions (Jürgens-Gschwind & Owen 1986). This would agree with the steep slope between N surplus and ^{15}N signature below 120 kg ha^{-1} a^{-1} indicating a large contribution of NH_3 volatilisation to total loss, and a decreasing slope at higher input surpluses where the contribution of nitrate leaching would increase. The slope for low N surplus (<120 kg ha^{-1} a^{-1}) farms also remains significantly steeper, when excluding the extraordinary low farm D from regression ($P<0.05$). Losses and, thus, system $\delta^{15}N$ might also depend on site parameters like climate, and – less significant – on soil and topography (Amundson *et al.* 2003). Yet, for the study area

variation between farms was small for most of these factors. A correlation between cow hair $\delta^{15}N$ and precipitation was not evident ($r^2 = 0.01$), but low temperatures on farm F_a probably contributed to low amounts of (gaseous) N loss and, thus, low $\delta^{15}N$ signatures.

The large variation of $\delta^{15}N$ in the different feeds is also consistent with a causal relationship between $\delta^{15}N$ and N input surplus and intensity of animal production system (stocking rate): even for the same types of feed the $\delta^{15}N$ varied greatly between production systems. Again, volatilisation of NH_3 and the contribution of animal excreta (manure) to plant nutrition explains (at least) some of the variation of feed ^{15}N signatures: while maize in farm A was exclusively and on E and G was mainly fertilized with manure, on B mainly mineral fertilizer was applied.

Feed ^{15}N signatures thus can explain the $\delta^{15}N$ variability between hairs of farms. Within farm heterogeneity in feed ^{15}N signatures and temporal and individual differences in feed intake may be the reason for $\delta^{15}N$ variability along hairs and between animals (data not shown).

Influence of non-feed N input

The ^{15}N content of mineral fertilizers was close to that of air (Table IV.3) in accordance with findings of others (Amberger 1987), and, thus, cannot account for the variation of feed $\delta^{15}N$. Similarly, the legume seeds showed a narrow range in $\delta^{15}N$ close to zero, likely due to biological N_2 fixation, which exhibits little isotope fractionation (Högberg 1997). Hence, a $\delta^{15}N$ value close to zero can also be assumed for N_2 fixed within the individual farms. Conversely, $\delta^{15}N$ of atmospheric deposition components have been shown to vary considerably (Hoering 1957; Moore 1974; Freyer 1978b; Freyer 1978a; Heaton 1987). If we assume 50% gaseous, and 50% wet and dry deposition (Weigel *et al.* 2000), and mean isotope signatures of these species as published by Hoering (1957), Moore (1974), Freyer (1978a; 1978b), and Heaton (1987), then the $\delta^{15}N$ of total deposition was about -2‰. Thus, all non-feed N input sources yielded a signature close to zero or less, which cannot explain the $\delta^{15}N$ differences between farms. Accordingly, losses from the systems by NH_3 volatilisation were not isotopically compensated by any input.

Contribution of imported feed

In conventional confinement systems a high proportion of N was imported with feeds (Table IV.1). Compared to input from biological fixation and mineral fertilizer, imported feeds were enriched in ^{15}N and varied considerably. Assuming ^{15}N signatures of 1‰ for legume seeds, and 3‰ for other purchased feeds, signatures of total N input varied between –1 and +1‰ and correlated with hair $\delta^{15}N$ ($y = 1.7 x + 5.6$, $r^2 = 0.48$). This effect clearly contributed to the variation in $\delta^{15}N$ between farms, but did not account for the total variation (4.1‰). However, being directly fed to animals, purchased feed strongly influenced $\delta^{15}N$ in hair, diluting the signal of the farm itself. Thus, hair ^{15}N signatures of farms B, E, and G were probably lowered by feeding high amounts of imported legume seeds.

Yet, while input of purchased feed can alter dietary $\delta^{15}N$ on the short term, this effect is strongly buffered and diluted when routed through the soil N pool. Depending on the turnover time of the soil N pool, ^{15}N signatures of harvested plants integrate processes and fluxes over extended periods of time.

Influence of trophic level shift

Our results demonstrated higher ^{15}N signatures of growing cattle compared to cows when fed on similar diets (Table IV.3a). This suggested a larger trophic level shift for ^{15}N in young growing animals as compared to adults. This is consistent with the data of Roth and Hobson (2000), who found a larger trophic level shift for sub-adult than for adult red foxes. The difference in ^{15}N trophic level shift between cows and growing animals and the variation in ^{15}N trophic level shift within growing animals was probably the main factor reducing the correlation between hair ^{15}N signatures of farms and stocking rate/N input surplus when including growing animals.

In contrast, the close correlation between N surplus of farms and ^{15}N signature of the hair of cows suggests that the trophic level shift within adult animals varies little. Yet, it is known that excretion of ^{15}N depleted urea increases with excess protein in diet (Steele & Daniel 1978; Sutoh *et al.* 1993). Accordingly, Sponheimer *et al*. (2003b) showed that for growing cattle in similar physiological condition the diet-hair enrichment was about 2.5‰ when fed on low protein diet (*Cynodon dactyl*on, 9% crude protein), while it was about 4.0‰ when fed on high protein diets (*Medicago sativa*, 19% crude protein). Absence of differences in trophic level shifts between farms may be due to the fact that farmers tend to

optimise the protein content of diets fed to cows. Still, imbalances may occur when protein content fluctuates, as regularly occurs on pastures, and this may contribute to seasonal variation in ^{15}N signatures of hair.

Influence of system disequilibria and heterogeneities

The $\delta^{15}N$ of hair can only reflect the N balance of the whole farm if the following necessary (but not necessarily sufficient) conditions are met: (i) imported feeds are a small component of total feeds (see above), (ii) nutrient fluxes and cycling involve all farm land to a similar extent ('spatial homogeneity'), (iii) the production system is stable ('steady-state'), and, (iv) seasonal or stochastic variation in fluxes and $\delta^{15}N$ of N sources and feed are accounted for. While condition (iv) can be met by appropriate sampling and analysis (e.g. by analysis of hair which integrates and averages signals over long periods of times), conditions (ii) and (iii) are probably rarely truly fulfilled by real systems. Thus, in farm L the ^{15}N signature of hair was probably lowered by exporting (^{15}N-rich) manure. Spatial heterogeneity may have been a factor in mixed production systems (farms A, C, D, I). In these, grassland and cropland may have represented (partially isolated) subsystems, with differing contributions to nutrient fluxes *via* the animal, recycling of nutrients in manure, and exports of products. Such factors could lead to differing $\delta^{15}N$ in the different subsystems. Violation of the 'steady-state' condition may have affected the $\delta^{15}N$ of hair on farms C and H, which introduced cattle farming and were converted to organic farming, respectively, only 2 years prior to sampling. In these instances, the isotopic signature of the soil N pool did probably not completely reflect present use, but still retained some memory of former agricultural practices and associated ^{15}N signatures. Conversely, the steady-state condition was more likely met by farm D, which had very low $\delta^{15}N$ in feed and hair, probably as a result of organic farming with low inputs (mainly from biological fixation and deposition) and low stocking rates for more than 40 years. Considering the above caveats, we deem the close relationship between $\delta^{15}N$ of hair and N input surplus even more interesting and significant.

CONCLUSION

In conclusion, ^{13}C hair signatures provide a powerful tool to estimate the maize content and, thus, the land use (crop farming in contrast to grassland farming) in cattle production systems. It may assist to identify differences in feed selection over time and between

animals within one farm. ^{15}N signature is a complex parameter, probably dominated by N leakage. Other factors, such as feed import may also contribute to ^{15}N signature in diet and - consequently - $\delta^{15}N$ of hair. Furthermore, differences in ^{15}N diet-hair enrichment (trophic level shift) between growing animals and cows, and within-system heterogeneity of N fluxes and cycling could lead to variation between hair $\delta^{15}N$ and farm gate balances of N. Thus clearly, more research is needed to clarify ^{15}N fractionation in the different processes of N transformation and cycling in agro-ecosystems. Nevertheless, we can see the potential of including isotopic monitoring of animal hair (and of other products and ecosystem pools) in envisaged environmental monitoring programs.

ACKNOWLEDGEMENTS

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Chapter V: The ^{13}C signature of temperate humid grassland as affected by site conditions and interannual variability of weather [7]

ABSTRACT

^{13}C discrimination (Δ) by ecosystems is sensitive to environmental conditions, and provides quantitative information on the biological coupling of water and C cycles. Here we report the effects of plant available soil water (actual PAW) on community-^{13}C signatures of temperate humid grassland. The 5-year study was conducted on two site types (peat and mineral soils) with a large range of PAW capacity, and included the centennial drought year 2003. Community-^{13}C signatures were derived from the $\delta^{13}C$ of grazing cattle hair and vegetation. Hair ^{13}C-signatures provided an assimilation-weighted ^{13}C signal that integrated both small-scale spatial and short-term variability of ^{13}C signatures on a pasture. $\delta^{13}C$ of hair and vegetation increased with decreasing PAW in the same way on both site types. But, at a given PAW, the $\delta^{13}C$ of hair was 2.6‰ less negative than that of vegetation, reflecting the diet-hair isotopic shift. Furthermore, the $\delta^{13}C$ of hair and vegetation on peat soil pastures was 0.5‰ more negative than on pastures situated on mineral soil. This was interpreted in terms of a 10 ppm CO_2 enrichment of canopy air due to ongoing peat mineralisation. Community-scale season-mean Δ varied from 19.8‰ on soils with low PAW capacity during the drought year of 2003, and 21.4‰ on soils with high PAW capacity in a wet year. This suggested relatively small variation in assimilation-weighted p_i/p_a (0.68-0.75) of pastures in a growing season. However, this range is almost the same as that reported from other studies, which encompass the range from subtropical arid to humid temperate grassland.

[7] Schwertl M, Auerswald K, Schäufele R, Schnyder H (submitted to *Global Change Biology*)

INTRODUCTION

The terrestrial hydrological and carbon (C) cycles are coupled via plant stomatal conductance. When water is in short supply, stomatal aperture is decreased to reduce transpiration (*E*). This also causes some reduction in the CO_2 flux into the leaf and a decreased photosynthetic assimilation (*A*). ^{13}C discrimination (Δ) during photosynthesis of C_3 plants is a useful quantitative indicator of this relationship, because it is proportional to the ratio of internal (p_i) to ambient CO_2 partial pressure (p_a), which is controlled by the ratio of stomatal conductance and chloroplast CO_2 demand:

$$\Delta = a + (b - a)\, p_i/p_a \qquad \textbf{(Equation V.1)}$$

[*a* is the ^{13}C fractionation during diffusion of CO_2 in air (4.4‰), and *b* is the net fractionation caused by carboxylation reactions (mainly Rubisco, approx. 27‰)] (Farquhar *et al.* 1982b; Farquhar *et al.* 1989). This effect is conveyed to the ^{13}C signature of whole plant or community biomass (δ_p), since

$$\delta_p = (\delta_a - \Delta)/(1 + \Delta) \qquad \textbf{(Equation V.2)}$$

(δ_a is the $\delta^{13}C$ of atmospheric CO_2). However, this occurs in a dampened fashion, because of the generally lower assimilation under drought conditions. Thus, community-δ provides a lifetime assimilation-weighted measure of p_i/p_a of all community members (provided that δ_a is known and sufficiently constant).

Several studies have confirmed, that community $\delta^{13}C$ is negatively correlated with rainfall, and inversely with aridity over continental (e.g. Stewart *et al.* 1995; Schulze *et al.* 1998; Miller *et al.* 2001) and landscape gradients (Ehleringer & Cooper 1988; Garten & Taylor 1992), and within (Ehleringer *et al.* 1992; Garten & Taylor 1992) and between seasons (Garten & Taylor 1992; Williams & Ehleringer 1996), supporting the notion that leaf level Δ-responses scale up to community, regional and global scale. However, the quantitative relationship between rainfall (or other measures of water availability) and community $\delta^{13}C$ varied greatly among studies. For instance, Stewart *et al*. (1995) observed a strong effect of rainfall on community-scale leaf $\delta^{13}C$ throughout the range of 350 to 1700 mm per year along a transect in Eastern Australia. Conversely, Schulze *et al*. (1998) and Miller *et al*. (2001) studied transects in Northern Australia and observed strong effects on community leaf $\delta^{13}C$ in the range of 200 to 500 mm rainfall, but no or only little effect if precipitation exceeded 500 mm. In transect studies in Namibia (30 to 400 mm rainfall) and Patagonia (125 and 770 mm) Schulze et al. (1991; 1996a) found remarkably small effects

of rainfall on $\delta^{13}C$ of a range of plant life forms and communities. This variability in community-scale Δ-response to rainfall may be explained by many factors, including species composition and replacement patterns, species differences in leaf anatomy, timing of leaf production and shedding, and rooting depths (Schulze *et al.* 1991; 1996b; 1998; Smedley *et al.* 1991; Ehleringer *et al.* 1992; Garten & Taylor 1992; Stewart *et al.* 1995; Williams & Ehleringer 1996; Ehleringer & Cooper 1988; Miller *et al.* 2001). Such relationships may be further modified by nutrient availability (Guehl *et al.* 1995; Livingston *et al.* 1998), plant available soil water (Toft *et al.* 1989; Tsialtas *et al.* 2001) and disturbance regimes (such as fire, herbivory, or human fuel harvesting; Ehleringer *et al.* 1986; Johnson & Matchett 2001; Randerson *et al.* 2002). At present there seems to be no single reliable predictor of community-Δ. In particular, a prediction of community-Δ responses to changing water availability is not possible for ecosystem types in which these responses have not previously been studied. Yet, such understanding is needed for assessment of the effects of changed weather and land use patterns on the terrestrial C and hydrological cycle (Ciais *et al.* 1995; Bowling *et al.* 2001; Randerson *et al.* 2002; Miller *et al.* 2003).

To our knowledge, there are no published systematic studies of community-scale Δ-responses of temperate humid semi-natural grassland (such as that of the Central and Atlantic regions of Europe) to changing water availability as effected by plant available soil water capacity (PAW capacity), rain fall, and intensity of management. Temperate humid (semi-)natural grassland covers a significant fraction of the global land surface. Its effect on global and regional C and hydrological water cycles are even larger, due to the generally high productivity of these biomes. But, although average rainfall is high, periodic droughts are common in these grasslands, and may increase in frequency and severity due to increasing variability in weather patterns (Weltzin *et al.* 2003). Moreover, European grasslands occupy a wide range of soil types, differing in PAW capacities (Klapp 1971), which could cause significant variability in community-Δ. Lastly, variation in land use intensity may also affect community-scale Δ: Smoliak *et al.* (1972) and Greenwood & Hutchinson (1998) found that increasing stocking rates caused consistent decreases of rooting depth, i.e. decreases in the proportion of root mass or root length, surface and volume density per unit soil volume in deeper soil layers. Yet, such responses may not be universal (Bartos & Sims 1974; Pucheta *et al.* 2004).

Thus, here we present results of a 5-year study of the effects of plant available soil water (actual PAW) on community-scale ^{13}C signatures of grassland. These data include the effects of the centennial drought of 2003. Community-^{13}C signatures were derived from cattle hair $\delta^{13}C$ records, and vegetation sampling. Hair ^{13}C-signatures were used because they reflect an assimilation-weighted ^{13}C signal that integrates both the small-scale spatial and short-term variability of ^{13}C signatures on a pasture. The experiment was conducted on two site types (peat and mineral soils) exhibiting a large range of plant available soil water capacity (56 to 186 mm).

MATERIAL AND METHODS

Study location, climate and weather conditions

The study was conducted at Grünschwaige Grassland Research Station which is located 435 m above sea level, latitude 48°23' N, and longitude 11°50' E at the north end of the Munich Gravel Plain near Freising, Germany (Figure V.1).

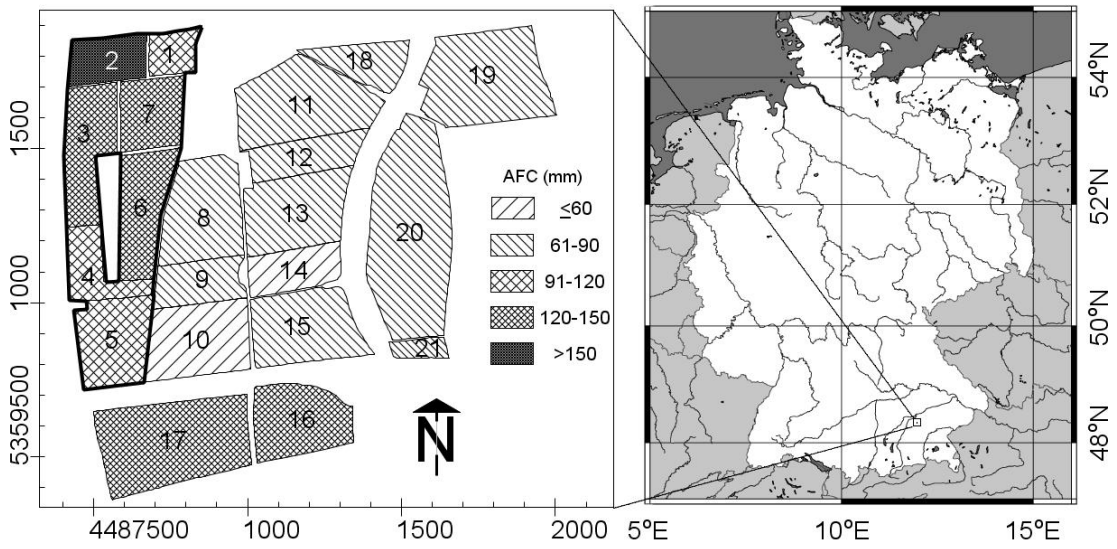

Figure V.1: Situation of Grünschwaige Grassland Research Station in Bavaria, Germany (right panel), and experimental units (left panel, with Gauss-Krüger coordinates). Numbering of experimental units as in Table V.2. Classes of plant available soil water capacity are distinguished by different fill patterns (see inset). Peat soil pastures are framed by a bold line. For details of pasture paddocks see Table V.2.

The climate is temperate humid with a long-term mean annual air temperature of 9.0°C (±0.8 SD), mean annual precipitation of 775 mm (±130), and most rainfall (480 ±80 mm) during the main growing season (mid April to mid October when daily mean temperature >10°C). Meteorological data for the study period (2000-2004) were obtained from a 3 km distant meteorological station (Munich airport) of the Deutscher Wetterdienst. The study period included wet years (e.g. 2002, when annual rainfall was 951 mm) and the centennial drought year 2003, when potential evapotranspiration (PET) during the growing season was more than twice the rainfall (Table V.1).

Table V.1: Long-term average (1996 – 2004) and annual meteorological conditions at Grünschwaige Grassland Research Station during the study period (2000 – 2004): mean annual air temperature and precipitation, and precipitation and potential evapotranspiration (PET) during the main growing season (15 April – 15 October). PET of grassland was calculated according to Penman (1948). All meteorological data were obtained from a 3 km distant meteorological station (Munich Airport) of Deutscher Wetterdienst.

	Long-term average	**Year**				
		2000	**2001**	**2002**	**2003**	**2004**
Mean air temperature (°C)	9.0	9.8	9.0	9.7	9.4	8.9
Precipitation (mm)	775	896	864	951	515	688
Precipitation during growing season (mm)	480	573	442	560	353	412
PET during growing season (mm)	553	572	563	534	753	558

Soils, and the capacity and actual level of plant-available water of experimental units

The site exhibits significant variation in soil type and distance to the groundwater table, and associated differences in land use history. Two site types were distinguished: (a) drained peat soils (Histosols) with average distance of 0.8-1.2 m to the groundwater table that were used as permanent grassland for the previous >45 years, and (b) mineral soils (Luvisols) at 0.9 -1.8 m distance to groundwater that were used as arable land for more than four decades before conversion to grassland between 1997 and 2000 (Table V.2). The groundwater table fluctuated seasonally, with a growing season average that was 30 cm lower than the annual mean.

Table V.2: Characteristics of experimental units (pasture paddocks and meadows) at Grünschwaige Grassland Research Station, for details see Materials and Methods;

Experi-mental unit no.	Main use	Soil material	Area (ha)	Sowing year	Average distance to ground-water table (m)	Average plant avail-able field capacity (mm)	Target sward height (mm)	Measu-red sward height (mm)[8]
1	pasture	peat	2.1	<1960	1.2	111	-	-
2	pasture	peat	3.6	<1960	1.1	186	50	57
3	pasture	peat	6.2	<1960	0.9	139	90	76
4	pasture	peat	3.5	<1960	0.8	111	90	81
5	pasture	peat[9]	6.3	<1960	0.9	100	50	54
6	pasture	peat	5.9	<1960	1.0	130	70	72
7	pasture	peat	4.6	<1960	1.1	135	70	63
8	pasture	mineral	8.0	1999	1.5	66	70	62
9	pasture	mineral	3.9	1999	1.4	70	50	54
10	pasture	mineral	7.6	1999	1.3	56	90	71
11	pasture	mineral	9.8	1998	1.8	68	-	-
12	pasture	mineral	4.1	1998	1.6	62	70	68
13	pasture	mineral	7.7	1998	1.4	66	50	57
14	pasture	mineral	3.9	1998	1.0	59	90	77
15	pasture	mineral	7.8	1999	0.9	65	-	-
16	meadow	peat	6.7	1999	1.0	139	-	-
17	meadow	peat[9]	12.6	1997	1.3	146	-	-
18	meadow	mineral	3.5	1998	1.6	68	-	-
19	meadow	mineral	11.1	2000	1.3	77	-	-
20	mixed use	mineral	16.0	2000	1.2	83	-	-
21	meadow	mineral	1.1	2000	1.1	87	-	-

[8] For details of measurement see Material and Methods

[9] About 30% of the paddock area was situated on mineral soil

The land is flat, and covered a total area of 136 hectares (ha) that was divided in 21 experimental units, including 15 pasture paddocks and six meadows (Figure V.1, Table V.2). Seven pasture paddocks were located on peat soil and nine on mineral soil. Of these, twelve paddocks (six from each soil type) formed part of a grazing experiment (see below). All meadows, but one, were situated on mineral soil.

The plant-available water capacity of soils (PAW capacity) in each experimental unit was estimated from rooting depth, and soil texture and organic matter content according to the tables established by the German Soil Survey Authorities (Ad-hoc Arbeitsgruppe Boden 1994). Rooting depth was given by the sharp discontinuity in texture between the fine-grained soil and the underlying coarse calcareous gravel, which also formed the aquifer. Soil depth and proportion of stones was measured every 20 m on a diagonal transect across each experimental unit. All soils were shallow (25 to 55 cm deep on 80% of the area of both soil types). Soil texture and organic carbon content were determined from composite samples along these transects. A higher resolution was not needed since analyses of several soil pits revealed little horizontal and vertical variation of fine-earth texture. PAW capacity ranged from 56 to 186 mm in the different experimental units, thus covering a large fraction of the range found in grassland in Germany (Klapp 1971; Schmidt *et al.* 1992). The entire range of PAW capacity was present in the paddocks which formed part of the grazing experiment. On average PAW capacity of peat soils (140 mm ±53 SD) was twice that of mineral soils (70 mm ±19 SD), because of much higher organic matter contents (Table V.2).

The actual level of plant available soil water (PAW) in experimental units was modeled with daily resolution for every sampling point (n = 400) between the years 2000 and 2004. Calculations started in late winter (15 February), when soils at each sampling point were assumed to be filled to their specific capacity. The PAW for every day was obtained as the difference between the previous day's PAW, *plus* the current days' rainfall, *minus* its actual evapotranspiration (AET). AET was equated with PET of grassland estimated according to Penman (1948), provided that the current PAW was ≥30% of maximum. If PAW had declined to <30%, then AET was estimated to decrease linearly (in proportion) with the remaining plant available water (Meyer & Green 1981; Serraj *et al.* 1999). Precipitation recharged the soil water reservoir. When precipitation caused PAW to increase beyond PAWC, then the excess was assumed to drain off. Lateral flow was not

considered as the study area is flat (mean slope 0.1%). Also, by-pass flow, capillary rise and hydraulic lift were ignored.

Animal husbandry and grazing experiment

All grassland was used for beef production in cow-calf operations, and steer and heifer ('yearling') fattening. The breeds were Limousin, Aberdeen Angus, Fleckvieh (Simmental) or crossbreeds of the afore mentioned. During the grazing season (approximately end of April to end of October) all animals were kept on pasture. Most animals grazed pasture paddocks that formed part of a grazing experiment, and were kept in groups that were returned to pastures of the same soil type (mineral or peat) every year. During the dormant period animals were housed and fed grass silage and hay, that was harvested from meadows and buffer areas of pasture paddocks (see below). Except for mineral supplements all feed was produced on the station (all grassland, no C_4 plants). Approx. 80% of all forage fed in winter was derived from meadows on mineral soil sites. Pastures received no fertilizers except nutrients returned *via* excreta. Manure was used to fertilize meadows. Mineral fertilizers were not used.

Twelve (out of a total of 15) paddocks formed part of a long-term grazing experiment that was established in 1998/1999 (Table V.2). This experiment tested the effect of grazing pressure (stocking rate) on animal performance on continuously grazed pastures on mineral and peat soils. A constant low, medium and high grazing pressure was maintained by monitoring sward height and adjusting pasture area to maintain swards near target heights of either 50 (high), 70 (medium) or 90 mm (low grazing pressure). To this end each paddock was divided in a continuously grazed 'core' area and a temporarily grazed 'buffer' area. Sward height in core areas was measured at fortnightly intervals. Buffer area was added if actual sward height had fallen below target. All or part of the buffer area was fenced off, when sward height exceeded the target. When nominal sward height fell below target and all buffer area was exhausted some animals were moved to another pasture that was not part of the grazing experiment. Sward height was measured with a rising plate meter (Herbometre®, Agro-Systèmes, La Membrolle sur Choisille, France) at 150 to 250 locations in each pasture on every measurement occasion between 2000 and 2004.

Each sward height treatment was replicated twice on both peat and mineral soils. This was true except for pasture paddock no. 5 (50 mm target sward height), that was

located on the transition from peat to mineral soil (about 30% of paddock area), and was used as permanent grassland for the last >45 years. Paddock no. 5 was assigned to the peat soil site type, but was excluded from data analysis when site types were compared in 2002 and 2003.

The sward height treatments were effective, although the difference between treatments was less than aimed at (Table V.2). On average of the entire experimental period sward height was 55, 66 and 76 mm for the 50, 70 and 90 mm target sward heights. These averages hid considerable small-scale spatial and temporal variation as was indicated by a mean standard deviation of 27, 29 and 34 mm for targets of 50, 70 and 90 mm, respectively.

On every paddock of the grazing experiment the sward dry matter fraction of every species present was assessed on four 1-m^2 permanent quadrates three times per year.

Hair samples

At the beginning and end of grazing seasons 2000-2004 hair was collected from the tail switch of animals. For logistic reasons sampling did not cover all pasture paddocks, although paddocks from both site types (mineral and peat soil) were sampled in all years (see Table V.3 for a list of the paddocks sampled in the different years).

Table V.3: Pasture paddocks (experimental unit no.) from which cattle hair samples were collected in the different years.

Year	Peat soil	Mineral soil
	------- Experimental unit no. --------	
2000	5	13
2001	3	8
2002	3, 4	8, 11, 13, 15, 20
2003	2, 3, 4, 6, 7	8, 10, 11, 12, 14, 20
2004	3	11, 20

The largest sampling effort was made in 2003, when variation of PAW was largest, and hair was collected from two animals per paddock, on five (or six) paddocks per soil type (total 20 animals).

All animals were older than 19 months at the time of first sampling. Hairs were collected by cutting with scissors (2001-spring 2002) or hand-plucking (autumn 2002-2004). Preparation and analysis of hair samples followed the protocols described in Chapter II. Roots of plucked hair were visually inspected (van Scott *et al.*, 1957), and anagen hairs with undamaged root were chosen for further analysis. Two hairs per animal were cut into 10-mm-sections and alternate sections were enclosed in tin cups (4x6 mm) for isotope analysis (see below). On average, a 1-year record was obtained from 23 measurements per animal. On average 17 hair isotope analyses were included in the grazing season mean isotope signature of one paddock. The data set also included a 3 years-long continuous hair record from one suckler cow (Fleckvieh x Angus cross, born on 11 July 1997), that was obtained by sampling hair between 2001 and 2003. This animal grazed peat soil paddocks in all years (paddock no. 5 in 2000, and no. 3 in the following years).

Herbage samples

In 2003 herbage was collected in paddocks no. 6 and 8, two adjacent paddocks with same target sward height (70 mm) but located on different soil types. Sampling occurred, at fortnightly intervals between mid June and the end of October. Each sample consisted of bulked herbage from 80-190 randomly distributed hand-pluckings taken from the whole grazed area simulating cattle grazing (De Vries & Daleboudt 1994). The vegetation on both pastures was dominated by *Poa pratensis* L. and *Lolium perenne* L. On average over the entire grazing season the two species accounted for about 60% of the standing dry matter biomass on both pastures. Other abundant species (> 5% of total standing herbage dry mass) were *Agrostis stolonifera* L., *Potentilla reptans* L. and *Poa trivialis* L. on paddock no. 6, and *Dactylis glomerata* L., *Phleum pratense* L., *Taraxacum officinale* Web. and *Trifolium repens* L. on paddock no. 8.

To relate the C isotope composition of herbage to a measure of PAW during growth of that herbage, the $\delta^{13}C$ of a sample was related to the average PAW of the respective paddock during the 450 degree days (dd, 0 ^{o}C base temperature) prior to sampling. The rationale for this was that the dominant species (*L. perenne* and *P. pratensis*) were in the vegetative stage, in which shoots are composed almost entirely of leaves. *L. perenne* is a

well studied species having a leaf life span of about 330 dd (Lemaire 1988) while the leaf life span for *P. pratensis* seems to be almost 50% higher (Ryser & Urbas 2000). Thus, a leaf life span of 450 dd for the whole standing herbage was assumed because *P. pratensis* accounted for a higher proportion of the standing biomass. However, shorter periods were also possible. Yet, sensitivity analysis showed, that average PAW was similar for periods between 400 and 450 dd prior to respective sampling dates (r^2>0.98). We assumed that older leaves were either dead and had passed over to the litter fraction, or that they had been grazed by the cattle.

Herbage samples were dried (1h at 95°C, then at 70°C for 48h), and subsequently ground in an expeller mill and ball mill. Aliquots (n = 3) of homogenized samples (0.7 ±0.05 mg) were placed in tin cups (4x6 mm) for elemental and isotope analysis.

Isotope and elemental analysis

Hair-sections or vegetation samples were combusted in an elemental analyzer (NA 1110; Carlo Erba, Milan, Italy) interfaced (Conflo III; Finnigan MAT, Bremen, Germany) with an isotope ratio mass spectrometer (Delta Plus; Finnigan MAT). Carbon and nitrogen elemental contents and isotope composition were measured in the same measurement sequence. Carbon isotope data are presented in the conventional form, i.e. as $\delta^{13}C$, where $\delta^{13}C = [(R_{sample}/R_{standard})-1]$, with R the $^{13}C/^{12}C$ ratio in the sample or standard (V-PDB). Nitrogen isotope data are not presented here, but were used (in addition to ^{13}C data) for matching common regions of hair that was sampled from the same animal at different times (see below). Each sample was measured against a laboratory working standard gas, which was previously calibrated against a secondary isotope standard (IAEA-CH6 for ^{13}C, accuracy of calibration ± 0.06‰ SD). After every tenth sample a solid internal lab standard (SILS) with similar C/N ratio as the respective sample material (fine ground wheat flour for herbage samples, fine ground protein powder for hair) was run as a control. The SILS was previously calibrated against an international standard (IAEA-CH6). The long-term precision (SD) for hair and herbage ^{13}C analyses was better than 0.2‰.

Evaluation of hair ^{13}C signatures

Hair analysis produced positional isotope data: each 10 mm-long segment was specified by the distance of its proximal and distal limit relative to the base of the hair. It is intuitively clear, that these positions reflect points in time relative to the time when the hair was

eradicated. To be useful for comparison with the PAW data, the spatial record had to be converted to a temporal framework. Position-time conversion was accomplished as detailed in Chapter II. Briefly, this involved matching the common region (overlapping portion) of hair collected at different times. This was achieved by statistical comparison of the spatial patterns of ^{15}N and ^{13}C signatures of two hairs. The procedure thus also revealed that region of the younger hair that was not present in the older one, because it was produced after the sampling of the old one. The latter also provided a measure of the mean rate of hair growth during the respective period: hair growth rate (mm d^{-1}) = length of new hair portion / time interval between samplings. For position-time assignment within a given sampling interval we assumed that the respective hair was growing at a constant rate. Indeed, hair growth rate appeared to be reasonably constant for a given animal. For instance, for the cow that was followed over a period of 3 years (see Figure V.3), the hair growth rate ranged between 0.72 and 0.82 mm d^{-1} (mean 0.79) in the different periods. Yet, for every position-time conversion, we used each animal's period-specific hair growth rate. Overall, hair growth rates ranged between 0.60 and 0.92 (mean: 0.79) mm d^{-1}, suggesting some variation of hair growth rate between animals.

Comparison of the isotope signatures of common regions of hair provided a measure of the overall precision of the method: the mean absolute deviation of the $\delta^{13}C$ of two hairs after positional alignment was 0.20‰ (±0.16 SD) for hair collected on the same sampling date, and 0.27‰ (±0.27 SD) for hair collected on different sampling dates.

Hair ^{13}C signatures were used to calculate season-mean animal- and pasture-specific average ^{13}C signatures. Isotope analyses demonstrated that about seven weeks were needed for a complete isotopic equilibration of the body metabolic C following a change of diet (see Results section, and Figure V.4). Thus, the hair produced during the first seven weeks following turnout in spring was omitted when calculating an animal- or pasture-specific season-mean $\delta^{13}C$.

RESULTS

Plant available soil water

The actual level of plant available soil water (PAW) varied strongly between years and soil types (Table V.4).

Table V.4: Modeled mean plant available soil water availability (PAW) of peat soils and mineral soils on pasture paddocks at Grünschwaige Grassland Research Station, and associated mean hair $\delta^{13}C$ for different years. For details see Material and Methods.

	Year				
	2000	2001	2002	2003	2004
Mean PAW on peat soil (mm)[10]	85	76	73	29	73
Mean PAW on mineral soil (mm)[11]	38	35	28	11	27
Mean hair $\delta^{13}C$ for peat soil (‰)[12]	-26.9	-26.8	-26.8	-26.3	-27.0
Mean hair $\delta^{13}C$ for mineral soil (‰)[12]	-26.0	-25.7	-25.9	-25.3	-25.9
Mean difference between mineral and peat soil hair $\delta^{13}C$ (‰)	0.9	1.1	0.9	1.0	1.1

Overall, season mean PAW varied between 10 and 80 mm between different pastures and years. On average of the whole growing season PAW was 2-3 times less in 2003 than in the other years. In all experimental units PAW was less than 30% of total capacity for most of the growing season of 2003 (see Figure V.2 for comparison of an average peat soil and an average mineral soil site).

[10] for mean plant available field capacity of peat soil (140 mm)

[11] for mean plant available field capacity of mineral soil (70 mm)

[12] 95% C.I. was <0.2‰ in every case

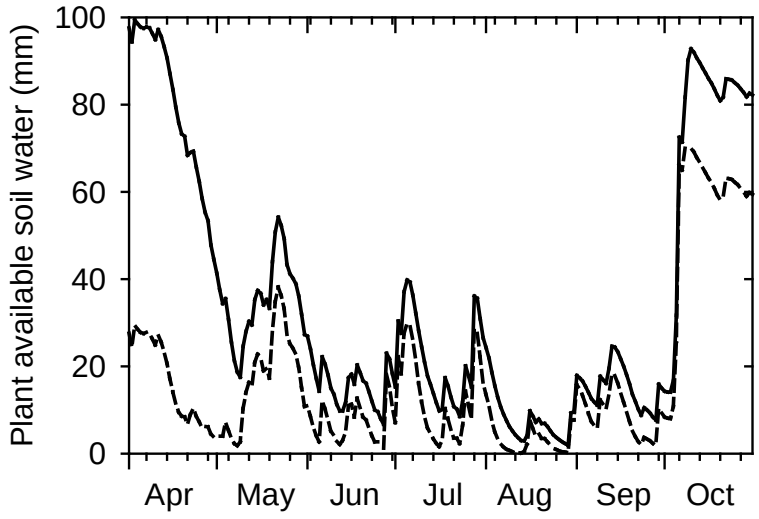

Figure V.2: Modeled plant available soil water (PAW) during the growing season of 2003 for average peat soil (140 mm PAW capacity, dashed line) and mineral soil sites (70 mm PAW capacity, solid line).

In each year the growing season mean PAW of peat soils was more than twice that of the mineral soil (Table V.4, Figure V.2). PAW also varied among different experimental units within a site type, which was mainly due to differences in PAW capacity (Table V.2, Figure V.1). Similarly, the spatial variation of PAW within an experimental unit was substantial, as was indicated by the SD of PAW which varied between 5 and 80 mm, and 1 and 13 mm within experimental units of peat and mineral soil, respectively (data not shown) .

Long-term hair ^{13}C record

Figure V.3 shows a 3-year-long continuous record of hair-δ^{13}C of one cow that grazed peat soil pasture in every grazing period.

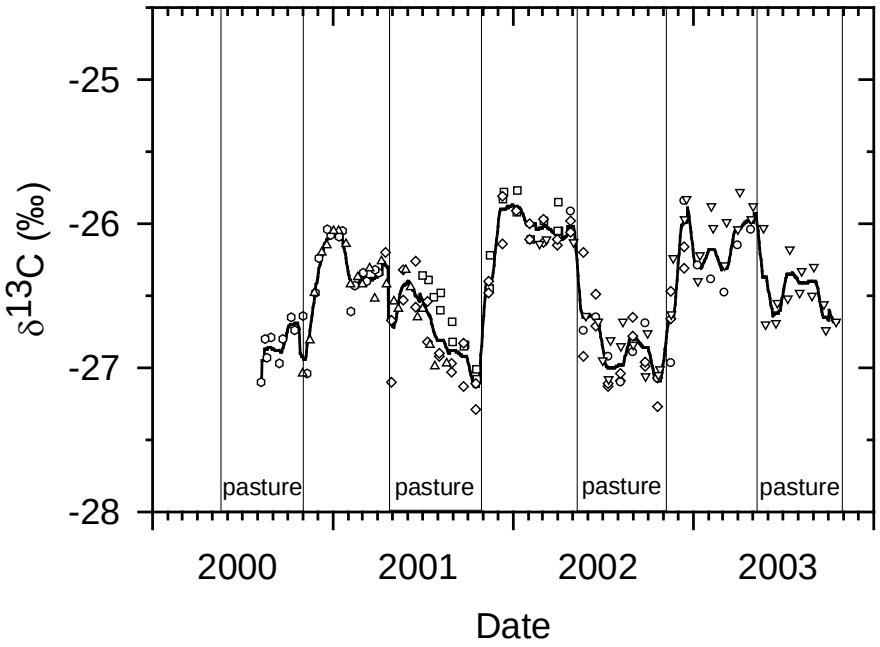

Figure V.3: Continuous δ^{13}C record constructed from 10 mm-long sections of tail switch hair collected from a Fleckvieh x Angus cow between spring 2001 and autumn 2003. Different symbols indicate ^{13}C signatures from hair sampled at different dates. Two hairs were analyzed on each sampling occasion, except for the spring 2003 sampling (one hair). The solid line gives the interpolation line. Periods on pasture are indicated. Position-time conversion of ^{13}C data was performed as explained in Materials and Methods.

The temporal fluctuations of δ^{13}C presented a regular saw-tooth pattern. During the dormant period, when animals were housed, δ^{13}C varied between –25.4 and –26.4‰. Following turnout in spring δ^{13}C decreased gradually by 0.5 to 1.2‰. After pasture closure the δ^{13}C returned rapidly to approx. -26‰. Yet, the annual patterns revealed specific differences in the temporal evolution and rate of change of δ^{13}C following turnout to pasture in spring. These temporal patterns were highly reproducible, as was evident from the close similarity of δ^{13}C values at given times obtained from different hairs collected on one occasion or of hairs collected on different occasions.

Site ^{13}C signatures

Animals kept on different site types exhibited different temporal patterns of hair-δ^{13}C. This was demonstrated by a comparison of 10 animals in two grazing seasons (2002 and 2003) and the interim winter housing period (Figure V.4).

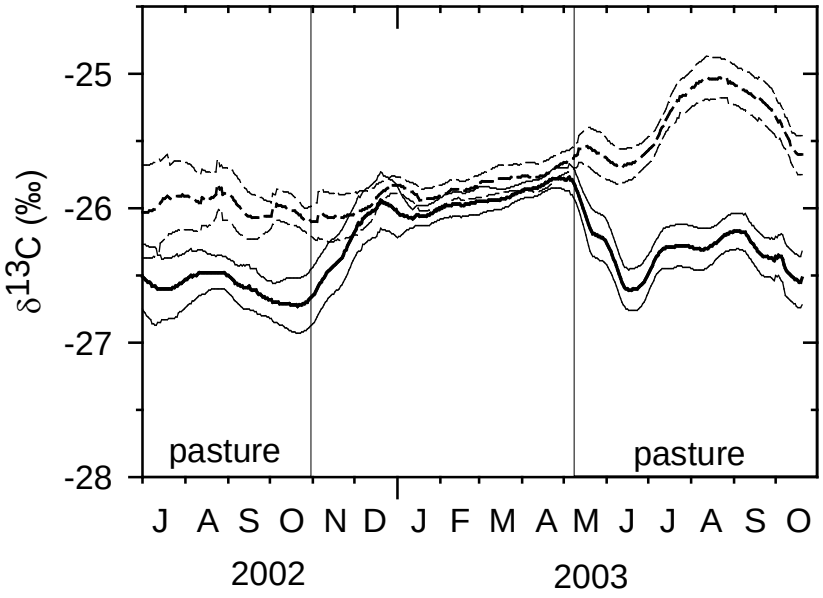

Figure V.4: Hair- δ^{13}C record for cattle grazing peat soil pastures (solid thick line) or mineral soil pastures (dashed thick line) in 2002 and 2003. All animals were fed the same diet during the interim winter housing period. Ten animals per site type (peat or mineral soil), and five paddocks per site type (two animals per pasture) were analysed. The 95% CI are given by thin lines.

Again, all animals grazing peat-soil pastures showed the temporal pattern that was already seen in a single animal (Figure V.3). Conversely, animals grazing pastures on mineral soils produced different patterns during the grazing period: in 2002 hair-δ^{13}C fluctuated little and was similar to that during the following period of winter feeding. During the 2003 grazing period the δ^{13}C of hair changed little following turnout, but started to increase in mid-June. Thereafter it remained relatively high (near -25‰) and stable until the end of September, when it decreased again. The increase in June 2003 was also evident in the hair of animals grazing peat soil pastures (Figure V.3, Figure V.4). Also, hair-δ^{13}C in these animals remained relatively high until the end of September, when it started to decrease again. The

changes observed during the grazing period 2003 are consistent with effects of drought on the $\delta^{13}C$ of pasture grass (and are discussed in more detail below).

When all animals were housed at the end of October 2002 and fed the same silage and hay diet, the ^{13}C signature of hair rapidly converged (Figure V.4). After 7 weeks the hair-^{13}C signatures of the two groups of animals were the same. Yet, during the remainder of the winter housing period the $\delta^{13}C$ of animals from peat soil pastures was 0.1‰ more negative than that of animals from mineral soil pastures, although this effect was not statistically significant. However, supposing the effect was real, then it meant that some of the C used for hair growth was mobilized from a slowly turning over body pool. A simple estimate of the contribution of this pool to total hair growth was obtained by relating the 0.1‰ $\delta^{13}C$-difference to the 0.7‰ $\delta^{13}C$-difference that was observed between the two groups prior to housing. Accordingly, a slowly turning over body pool would have contributed about 14% of the C used in hair growth. This demonstrates that the totality of body pools feeding hair growth was almost completely turned over after 7 weeks on a new diet. In consequence, we have omitted the first 7 weeks of hair growth when calculating pasture-specific ^{13}C signatures form hair data.

Notably, the hair-^{13}C signatures of different animals from one site type showed a very high degree of similarity in their hair ^{13}C record (Figure V.4). Thus, the 95% confidence interval for the $\delta^{13}C$ of a group hardly ever exceeded ±0.2‰.

The relationship between hair-$\delta^{13}C$ and plant available soil water

Comparison of grazing season-mean hair-$\delta^{13}C$ of individual animals and the season-mean PAW of the respective pastures revealed a strong relationship. Hair-$\delta^{13}C$ became less negative as PAW decreased (Figure V.5).

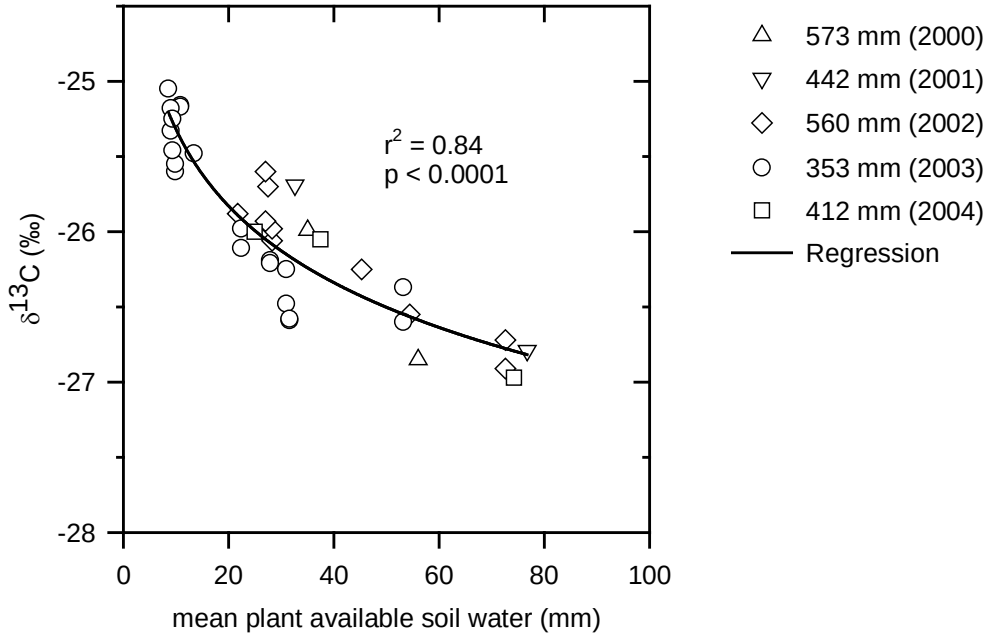

Figure V.5: Season mean hair $\delta^{13}C$ as related to the season mean plant available soil water of respective pastures. Symbols indicate years with different precipitation during growing season (see inset).

This data set contained all data collected in 5 years (Table V.3). A logarithmic model gave the best fit, indicating that the effect of PAW on hair-$\delta^{13}C$ increased with decreasing PAW.

Overall, the mean hair-$\delta^{13}C$ of individuals varied between –27.0 and –25.0‰ among different grazing periods and pastures. Concomitantly, the season-mean PAW of the different pastures varied between 10 and 80 mm (Figure V.5). Within years season-mean PAW differed by up to 20 to 50 mm, with the largest range occurring in 2003 (Fig. 5). PAW capacity alone explained 64% of the $\delta^{13}C$ variation in hair ($y = -1.17 * \ln(x) - 20.76$, $P<0.0001$), while year effects (including effects of precipitation and evapotranspiration; determined by fixing the season mean PAW at 150 mm for all pastures) explained only 17% of the scatter in $\delta^{13}C$ ($y = -0.46 * \ln(x) - 24.21$, $P=0.01$). Yet, the interaction of all

weather factors (evaporation demand, precipitation, temporal distribution) with soil, as determined by mean PAW explained 84% of the variation in $\delta^{13}C$ in a logarithmic model (y = -0.73 * ln (x) – 23.63; P<0.0001, see Figure V.5). Sward height did not contribute to explaining the remaining variation (r^2 = 0.12; P> 0.05).

However, when the hair-$\delta^{13}C$ *versus* PAW relationship was analysed separately for the two site types, it was noted that there was a significant offset between the two site types (P<0.05), although the slope of the relationship was the same for both (P<0.05) (Figure V.6).

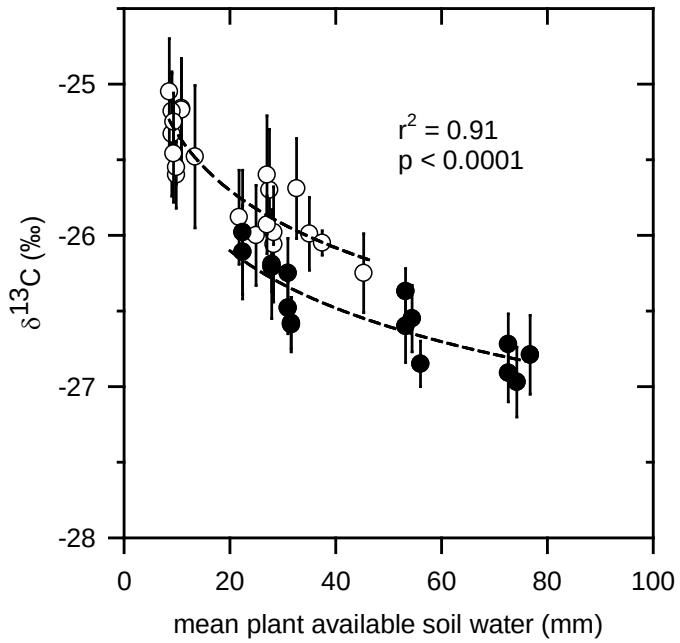

Figure V.6: Season mean hair $\delta^{13}C$ as related to the season mean plant available soil water of peat soil pastures (open circles) and mineral soil pastures (closed circles). Error bars indicate seasonal variation (SD)

This meant, that for any PAW, the hair-$\delta^{13}C$ on peat soil pastures was 0.5‰ more negative than on mineral soil pastures. Overall, the regression model including the site type offset explained 91% of the hair-$\delta^{13}C$ variation on pastures. Again, sward height did not contribute to explaining the remaining variation (r^2 = 0.004; P>0.05).

Relationship with herbage-$\delta^{13}C$

If it was causal, then the relationship between $\delta^{13}C$ and PAW, that was observed in hair, should also exist in vegetation. This relationship was analysed in one peat soil paddock and one (adjacent) mineral soil paddock during the 2003 season, when variations in PAW were largest. The data confirmed a relationship between $\delta^{13}C$ of vegetation (herbage) and PAW (Figure V.7) that was similar to that of hair-$\delta^{13}C$ and PAW (Figure V.6), although the time-scale of the comparison was different for the two data sets.

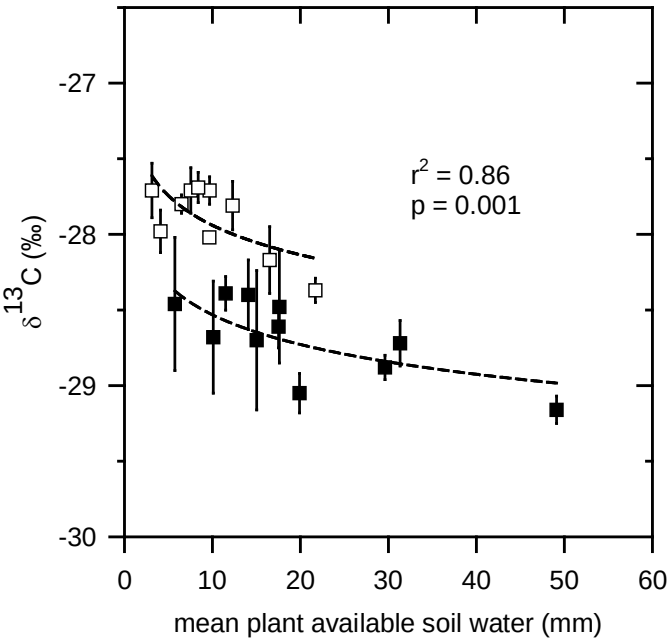

Figure V.7: $\delta^{13}C$ of herbage (± SD) sampled from a peat soil pasture (open squares) and a mineral soil pasture (closed squares) as related to the mean plant available soil water during the 450 dd prior to sampling. Samples were collected every 2 weeks between June and October 2003. Three replicates were obtained from each pasture on each sampling occasion.

Again, a logarithmic regression that included an offset between mineral and peat soil pastures explained the variation best (r^2 = 0. 86), demonstrating that the offset seen in hair was related to a different $\delta^{13}C$ of herbage produced on the two types of sites. In addition, the slope of the relationship between $\delta^{13}C$ and PAW was the same for the peat and the mineral soil pasture, and the slopes of the herbage-^{13}C *versus* PAW, and hair-^{13}C *versus* PAW relationship did not differ significantly (P>0.05).

Notably, however, at a given PAW there was a systematic $\delta^{13}C$-difference of 2.6‰ (±0.1 SD) between hair and herbage samples. This meant that hair was systematically enriched in ^{13}C by 2.6‰ relative to herbage ('diet-hair shift').

In summary, the entire data set, including the herbage and hair data, was well described by a regression model which included the effects of (i) PAW, (ii) the offset between mineral and peat soil, and (iii) the diet-hair shift. This model explained 98% of the total variation ($P<<0.0001$).

DISCUSSION

Grazer hair integrates pasture $\delta^{13}C$

The $\delta^{13}C$ of tail switch hair of grazing cattle provided a temporally and spatially integrated, assimilation-weighted measure of grazed vegetation $\delta^{13}C$. This derived from (1) a fixed relationship between the $\delta^{13}C$ of herbage and hair (constant 'diet-hair shift'), (2) sufficiently fast turnover of the body metabolic pool feeding hair growth, (3) cattle roaming large areas during grazing, and (4) relatively non-selective grazing.

The diet-hair shift found in this study (+2.6‰) was near-identical to that found in Chapter IV and other studies with cattle (Sponheimer *et al.* 2003a). Overall, the studies of Sponheimer *et al.* (2003a), Chapter IV and the present data cover a very large range of environments and diets, and included animals of different breeds, sex, physiological condition, and age. Considering the systematic differences in $\delta^{13}C$ which occur between different chemical components in plants (Deines 1980; Gleixner *et al.* 1993; Hobbie & Werner 2004), and the large variability in chemical composition of forage plants (van Soest 1994) the apparent constancy of the diet-hair shift in these data sets is quite remarkable. Probably, the constancy derives (at least in part) from extensive ^{13}C scrambling during ruminal, and post-ruminal metabolism (leading to isotopic equilibration among metabolites), and constant isotope effect(s) during biosynthesis of hair keratin. Whatever the cause, the constancy of the diet-hair ^{13}C-shift is important for biogeochemical investigations employing hair ^{13}C data.

The turnover of the body metabolic pool was near-complete after seven weeks. This was somewhat faster than the 74 days reported by Jones *et al.* (1981) for studies with two steers. But, it still implied that short-term isotopic signals ingested with feed were strongly attenuated in the hair ^{13}C-record (West *et al.* 2004), and that hair produced during the first seven weeks of a grazing season contained some isotopic signal derived from winter (hay

and silage) feeding. Elimination of the first 7 weeks of hair grown following turnout in spring allowed us to obtain a 'clean' pasture signal, although some isotopic signal from the beginning of the grazing season was also lost in this procedure.

There was a very close agreement in the $\delta^{13}C$ of hair of different animals (in 12 pairs of animals grazing the same pasture the hair $\delta^{13}C$ differed by less than 0.13‰, see also Figure V.4), and of different hairs of one animal (Figure V.3), showing that – in principle – one hair provided an accurate and representative isotopic record of a pasture The agreement between animals is related to the fact that cattle graze in herds, in which grazing is spatially and temporally synchronized for all animals. Also, cattle graze relatively non-selectively (e.g. Agnusdei & Mazzanti 2001), particularly in conditions of controlled grazing, where stocking rates are adjusted to the carrying capacity of the land. Adjustment of grazing pressure aims at efficient herbage use and is a common pasture management practice in most temperate humid grasslands.

Assimilation-weighting of hair-$\delta^{13}C$ resulted from the fact that herbage growth is a function of assimilation rate, which must have varied in time (as a function of weather conditions etc.) and space (due to variation in PAW capacity etc.). In consequence, sites and periods with sub-average forage production contributed less to total feed intake, and therefore had a lesser effect on $\delta^{13}C$ of hair.

The effect of site type on $\delta^{13}C$

Our investigations revealed a significant offset between site types in the relationship between $\delta^{13}C$ and PAW, and this offset was evident in both hair and vegetation samples (Figure V.6 and Figure V.7). Theoretically, the offset between site types could have resulted from any site type related factor that can affect community level-Δ or the $\delta^{13}C$ of canopy air (see Equation V.2). Importantly, however, it could *not* derive from weather conditions, which were the same on all sites. Also, it seems unlikely that the effect was related to differences in water relations not accounted for in our assessment of PAW, such as hydraulic lift or capillary rise (Caldwell *et al.* 1998), because the $\delta^{13}C$-difference between site types was practically the same in contrasting years, and also occurred when PAW was high on both site types (Table V.4, Figure V.6 and Figure V.7). Still, there remain several factors which could bring about a 1‰ difference in $\delta^{13}C$, including differences in species composition (Smedley *et al.* 1991; Tsialtas *et al.* 2001) and nitrogen status of plants (Guehl *et al.* 1995; Livingston *et al.* 1998). Yet, we found no relationship

between N content of vegetation and $\delta^{13}C$ ($P>0.05$), and both site types were dominated by the same two grasses: *L. perenne* and *P. pratensis.*

Could a difference in $\delta^{13}C$ of canopy CO_2 between the two site types explain the effect? The drained peat soil pastures at the Grünschwaige had a much higher organic carbon content than the mineral soil pastures (18.5% ±2.9 SD versus 6.0% ±2.1 SD). Drained peat soils have a large potential for CO_2 release to the atmosphere (Armentano 1980; Maljanen *et al.* 2001; Nieveen *et al.* 2005). This might cause some CO_2 enrichment inside the canopy, although this effect would be counteracted by turbulent conditions during daylight. We used the $\delta^{13}C$-offset between peat and mineral soil pastures to estimate the CO_2 enrichment and associated shift in $\delta^{13}C$ that was required to explain the offset. When assuming a $\delta^{13}C$ of -8.3‰ and a concentration of 359 ppm for background CO_2 at Grünschwaige (Gamnitzer & Schnyder, unpublished data), and the same $\delta^{13}C$ for peat-derived CO_2 as for organic C in peat soils (-26.8‰), then an enrichment of canopy CO_2 of only 10 ppm derived from peat mineralization was sufficient to explain the 0.5‰ offset between site types. Although definitive proof is lacking, we feel that this is the most likely explanation for the $\delta^{13}C$ offset between site types.

^{13}C discrimination and plant available soil water

Thus, community-level Δ could be estimated from the hair and vegetation ^{13}C data (considering the diet-hair shift when inferring Δ from $\delta^{13}C$ of hair, and accounting for the different δ_a on mineral and peat soil pastures), and the relationship with PAW explored (Figure V.8). This demonstrated a saturating response of Δ with increasing PAW, that was consistent with the effect of drought on Δ in C_3 plants (Farquhar *et al.* 1982b; Farquhar *et al.* 1989).

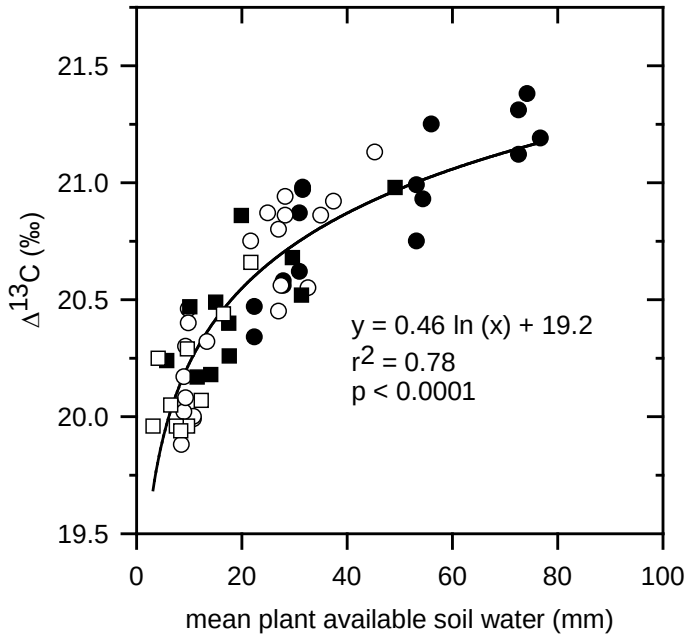

Figure V.8: Community-level ^{13}C discrimination (Δ) as related to plant available soil water of pastures. Δ was calculated from the hair (circles) and vegetation (squares) ^{13}C data shown in Figure V.6 and Figure V.7. A diet-hair shift of 2.6‰ was considered when inferring Δ from $\delta^{13}C$ of hair. It was assumed that the $\delta^{13}C$ of canopy CO_2 (δ_a) was –8.3‰ on the mineral soil site (open symbols, Gamnitzer & Schnyder, unpublished), and –8.8‰ on the peat soil site (closed symbols). For further explanations, see text.

The effect of PAW on Δ was relatively small: in the centennial drought year 2003 community-level season-mean Δ of the most drought prone pastures was 19.8‰, only 1.6‰ less than that of the least drought prone pastures in a wet year. However, this range of Δ corresponds closely to the range of community-average or species-mean Δ of the C_3 component in several grassland ecosystems, which together span the range from subtropical arid to temperate humid conditions [19.0‰, Red Butte Canyon Grassland, Utah, (Smedley *et al.* 1991); 19.3‰, grass flora of Namibia, (Schulze *et al.* 1996a); 19.5‰, Greek semi-arid upland grassland (Tsialtas *et al.* 2001); 20.2‰ tallgrass prairie in Kansas, (Lai *et al.* 2003); 20.5‰ tallgrass prairie pasture in Oklahoma, (Still *et al.* 2003); 20.7‰ temperate humid grassland in Upper Bavaria, (Chapter IV)]. Thus, the Δ-responses seen at Grünschwaige reflected almost the entire variation in community-level Δ of grassland ecosystems at the global scale. Much larger variation at the species-level, and on shorter time-scales has been reported [up to 5-7‰ in studies in arid and semi-arid grassland by Smedley *et al*. (1991) and Tsialtas et al. (2001)]. Yet, on average of all species Δ varied by

only about 1 to 2.5‰ during the growing seasons in these studies (Smedley *et al.* 1991; Tsialtas *et al.* 2001), a variation that is not much larger than that seen at the Grünschwaige during the growing season of 2003. Evidently, the smaller variation at the level of seasonal-average community-level Δ is due to the averaging of the interspecific variation (whereby the community-Δ is skewed to the Δ of the species which dominate in the ecosystem), and the assimilation-weighting of the different periods of the year. The fact, that drought had only little effect on community-level seasonal Δ, was likely due to low assimilation during drought periods. In grassland low assimilation during drought may be partially related to side-effects of grazing/herbivory, which are a lesser factor in most other ecosystem types. The restitution of photosynthetic activity through refoliation is very sensitive to drought. Recovery of nutrients lost by defoliation is hampered by the low availability of soil nutrients under drought. Therefore, periods with high PAW should have a particularly large effect on the community-level season-mean Δ in grassland. Notably, grazing pressure had no apparent effect on Δ. This, may be related to the fact that all soils were rather shallow, so that their PAW capacity could be exploited efficiently even with rather shallow root systems.

As the range of Δ was relatively small, the season-mean assimilation-weighted p_i/p_a varied in a narrow range ($0.68<pi/pa<0.75$). This indicates that – on a whole-season basis - these grasslands exhibited little plasticity in the relationship between transpiration flux and CO_2 uptake. This is in agreement with several studies with herbaceous and woody plant communities (Smedley *et al.* 1991; Garten & Taylor 1992; Schulze *et al.* 1998; Miller *et al.* 2001; Tsialtas *et al.* 2001) and, again, is likely due to the small contribution of assimilation in drought conditions to whole season assimilation.

In conclusion, this study has shown that the $\delta^{13}C$ of cattle tail switch hair can provide accurate information on Δ of grassland communities on seasonal time-scales. As cattle graze large areas, and feed more on productive than non-productive sites, the hair $\delta^{13}C$ is a assimilation-weighted signal of vegetation covering a large area. This work also reveals that variable site conditions (PAW capacity of soils) and interannual variation of weather can bear the same range of whole-season community-mean Δ in temperate humid grassland as is observed in grassland covering the range from subtropical arid to humid grassland.

Chapter VI: General and summarizing discussion

GENERAL FINDINGS

The present study focussed on N and C fluxes and processes that affect stable isotope signatures in cattle hair and agricultural ecosystems. In Chapter II a method that allows position-time transformation of isotope signatures (or other properties) along hair of living animals was developed. Several sources of error and uncertainty when trying to get an accurate and representative temporal record from hair were analysed and means to avoid them were suggested. It was shown in Chapter III that feeding practices and farm system characteristics of New Zealand dairy farms have strong effects on hair isotope signatures. Isotope signatures of cows from the same farm were similar in mean and pattern, while there were clear differences between farms, even within a region of high climatic and geographic similarity. Chapter IV demonstrated, that the hair ^{13}C signature of cattle in Upper Bavaria was primarily determined by the proportion of maize in the diet, providing an indicator of the land use system (arable maize-based forage crop *versus* grassland farming) on which individual cattle production systems were based. The $\delta^{15}N$ in the same study was a complex parameter, but the long-term overall signal of adult animals in farms was correlated with farming intensity (stocking rate) and farm N balance components (input surplus), indicating that farm system $\delta^{15}N$ was dominated by volatile N losses. Again, on the basis of hair analysis, Chapter V revealed that ^{13}C discrimination of humid temperate grassland (in which C_4 plants are absent) is affected by plant available soil water.

RELEVANCE OF THE STUDY

The results of Chapter II were already helpful in other studies on the organism level, such as investigations of human physiology and body N-balance (Fuller *et al.* 2004) and - with increased temporal resolution (1-mm hair sections) - short-term C pool kinetics in horses (West *et al.* 2004). Also, studies on dietary habits of contemporary bears (Mizukami *et al.* 2005) and on the seasonal isotopic history of a mammoth, that was living about 42000 years BP (Iacumin *et al.* 2005) have referred to the methodology described in Chapter II of this thesis. Thus, the results of this dissertation have already had an impact on research at the organism, ecosystem and regional level.

Studies on the organism level

The isotopic composition of hair is determined by (a) the diet and (b) physiological processes within the organism. Thus, interpretation of hair isotope signatures, e.g. for

dietary reconstruction, requires knowledge about fractionation processes and pool kinetics within an animal. This dissertation contributes to a better understanding of these processes in cattle: Hair growth must have been partly supplied from the body C pool, since hair growth reached about 85% of ^{13}C saturation only seven weeks after a dietary shift (Figure VI.1a). These kinetics appeared to be similar in animals of different age, sex and breed and in different physiological condition (Figure VI.1a). Evidently, the ^{13}C signal deposited in hair reflected that of diet consumed over an extended period of time. Conversely, the potential of hair isotope signatures to record short-term dietary changes is clearly limited.

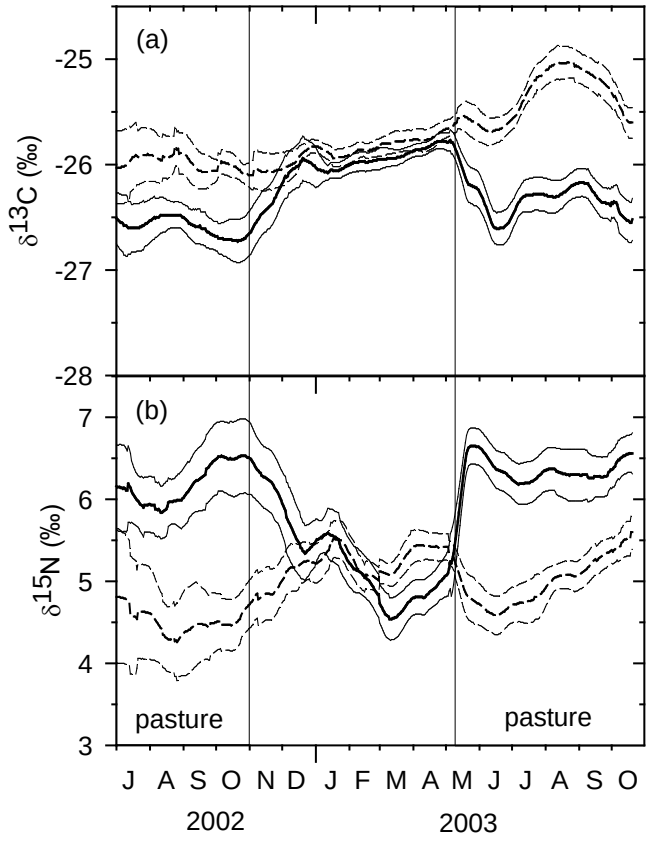

Figure VI.1: Hair $\delta^{13}C$ (**a,** see Figure V.4) and $\delta^{15}N$ (**b,** (Schwertl *et al.* 2005)) record for cattle grazing peat soil pastures (solid thick line) or mineral soil pastures (dashed thick line) of the Grünschwaige in 2002 and 2003. All animals were fed the same diet during the interim winter housing period. Ten animals per site type (peat or mineral soil), and five paddocks per site type (two animals per pasture) were analysed. The 95% CI are given by thin lines.

The $\delta^{15}N$ which was simultaneously analysed for the same animals (Schwertl *et al.* 2005) revealed that the mean turnover time for the corresponding N pool was in the same range (7

weeks to reach about 90% of ^{15}N saturation, Figure VI.1b). This was slightly shorter than was expected from preliminary results on the N pool kinetics of one steer from Figure II.8 (time to 90% saturation: 8 weeks), and it was in the same range as the ^{13}C saturation in regrowth hair of two steers after a C_4-C_3 dietary change and vice-versa (time to 90% saturation: 7 weeks; Jones *et al.* 1981). The turnover time determined in this study was the mean for a larger number of animals (n=20) of different breed, age, sex and physiological condition during grazing and stable keeping. This makes it less sensitive to individual differences in diet selection and intake, digestion, absorption and metabolism. The so determined turnover time is independent from unknown variations in the new diet itself and from estimation of the ^{13}C diet-hair enrichment. The close similarity in the turnover time of the C and N metabolic pool may be taken to mean that the respective pools were composed of amino-acids, and that there was little exchange of amino-C and -N after absorption from the digestive tract.

The isotope signatures in hair are affected by fractionating processes in the animal. Interestingly, the mean ^{13}C enrichment between diet (bulk C) and cattle hair was almost constant (2.6 to 2.7‰) for different C_3 plant feeding systems and individuals of different age, sex and breed in different physiological conditions (Chapters IV and V). An identical mean ^{13}C diet-hair shift (+2.7‰) was observed for 8 steers fed on alfalfa (*Medicago sativa*) hay (Sponheimer *et al.* 2003a), and for one cow grazing a ryegrass (*Lolium perenne*) dominated pasture (Minson *et al.* 1975). Considering the systematic differences in $\delta^{13}C$ which occur between different chemical components in plants (Deines 1980; Gleixner *et al.* 1993; Hobbie & Werner 2004), and the large variability in chemical composition of forage plants (van Soest 1994) the apparent constancy of the diet-hair enrichment in these data sets is quite remarkable. Probably, the constancy derives from extensive isotopic scrambling during ruminal metabolism (leading to isotopic equilibration among metabolites), and constant isotope effect(s) during biosynthesis of hair keratin (see Chapter V). Thus, dietary differences in $\delta^{13}C$, which were smaller than 1‰ were clearly detected (Chapter V). Still, for unknown reasons a lower diet-hair shift (+1.8) was detected for two steers fed on cowpea (*Vigna sinensis*) hay (Jones *et al.* 1981), and a higher shift (+3.1‰) was found for one cow fed on ryegrass dominated pastures (Minson *et al.* 1975). Also, the ^{13}C diet-hair shift seemed to be slightly lower for pure C_4 plant diets (+2.0‰, Chapter V). This was confirmed by other studies on pure C_4 plant diets, where the ^{13}C diet-hair shift was 1.8‰ for 2 steers grazing *Digitaria decumbens* pastures (Jones *et al.* 1981), and 1.9‰ for 3 cows

grazing *Heteropogon contortus* pastures (Minson *et al.* 1975). Still, the close correlation between dietary maize content and hair $\delta^{13}C$ (Figure IV.4) indicated that the diet-hair enrichment was similar for mixed C_3-C_4 diets, which also suggested intensive scrambling of C_3 and C_4 compounds in the rumen (see above).

No similar results as for ^{13}C are available for the ^{15}N diet–hair shift. Preliminary results for two cows (grazing Grünschwaige pasture no. 6 in 2003) suggested that hair was 3.4 to 4.1‰ enriched in ^{15}N compared to diet. The close similarity in hair $\delta^{15}N$ for animals grazing the same pasture (Figure VI.1) but differing in age, breed, sex and physiological condition indicates that the ^{15}N diet-hair enrichment may be fairly independent of these factors. However, the ^{15}N signature of growing animals was higher than that of adult animals when they fed on a similar diet (Figure IV.3a) suggesting a larger diet-hair shift for growing animals. For human hair Fuller *et al*. (2004) showed that the diet-hair shift decreased (up to 1.2‰) with increasing weight gain, i.e. increasing N demand, during pregnancy. Thus, the ^{15}N diet-hair enrichment might also reflect the N balance of an organism. This is consistent with a study of Sponheimer et al. (2003b), who found that the ^{15}N diet-hair enrichment for steers was higher (4.0‰) when the dietary N content strongly exceeded the demand (*Medicago sativa*, 19% crude protein) than when the dietary N content was low (*Cynodon dactylon*, 9% crude protein). Still, the biochemical fractionation processes that control the ^{15}N diet-hair shift are not well understood (Gannes *et al.* 1997). Controlled feeding studies are required to test the quantitative influence of the organism N balance on the ^{15}N diet-hair shift.

Studies on the ecosystem, regional and global level

The animals fed on forage harvested or grazed from larger areas. Hence, the hair isotope signatures provided information about the environment in which they were living. Depending on hair length and growth rate a single hair contained environmental information of 0.3-2 years (Figure III.2 and Figure V.3), and enabled tracing temporal trends over this time.

$\delta^{13}C$

The ^{13}C signal is essentially determined by the photosynthetic pathway ($\delta^{13}C$ difference between C_3 and C_4 plants in Upper Bavaria: ~16‰, Table IV.2). Hair $\delta^{13}C$ thus clearly reflected the proportion of C_4 plants in the diet (Figure IV.4). In temperate humid agroecosystems, where C_4 plants do not occur in natural vegetation and, maize is the only important C_4 plant in cattle diet, the hair $\delta^{13}C$ is an indicator of land-use, i.e. grassland farming as opposed to arable crop farming (Chapter IV). In grassland ecosystems with mixed C_3-C_4 vegetation, e.g. in the tropical and subtropical zones, the $\delta^{13}C$ hair signal of grazing cattle will provide information about the content of either photosynthetic type in the diet and seasonal variations therein.

Within C_3 roughage from a temperate-humid region the maximum $\delta^{13}C$ difference was less than 2‰ (Table IV.2). Accordingly, effects of (i) different biochemical composition, (ii) plant discrimination against ^{13}C (Δ, see Equation I.7), and (iii) the $\delta^{13}C$ of the CO_2 source (=substrate, Equation V.2) on whole plant $\delta^{13}C$ were relatively small. Yet, cereal and legume seeds were systematically enriched in ^{13}C by about 2‰ compared to whole plant roughage (Table IV.2), probably due to the high concentration of ^{13}C rich compounds like starch or glucose (Gleixner *et al.* 1993), and low concentrations of (relatively ^{13}C-depleted) cell wall material. Cattle graze relatively non-selectively (e.g. Agnusdei & Mazzanti 2001) on the whole above-ground biomass of pasture paddocks, provided that stocking rate is adjusted to the productivity of the pasture. Hence, the $\delta^{13}C$ in hair of grazing cattle enabled detection of environmental factors affecting $\delta^{13}C$ *via* plant discrimination (Δ) or the $\delta^{13}C$ of the CO_2 source, given the relative constancy in ^{13}C enrichment between dietary C_3 roughage and hair (see above).

Plant available soil water influences stomatal conductance (Schulze 1986) and, hence, also *pi*/*pa* and Δ (Equation I.7). The Grünschwaige Grassland Research Station offered near-ideal conditions to study the relationship between water supply and Δ on the ecosystem level because (a) the grassland included sites with contrasting plant available soil water capacity, (b) the area was split into experimental pastures that provided a clear gradient in water supply, (c) each experimental pasture was grazed by cattle over the whole vegetation period, (d) a large range of parameters like soil texture and organic matter content, vegetation composition, and weather conditions, but also isotope signatures of different ecosystem compartments were controlled and measured (Auerswald 2001;

Schnyder *et al.* 2004). Thus, it could be shown from cattle hair $\delta^{13}C$ that seasonal mean Δ of temperate humid grassland is correlated with plant available soil water (Figure V.8). However, a large variability in weather and site conditions was followed by a $\delta^{13}C$-difference in hair of only 1.6‰, which is equivalent to a range of *p*i/*p*a from 0.75 to 0.68 (Chapter V). This was likely due to low assimilation during drought periods and, hence, a low effect of drought on assimilation-weighted season-mean Δ.

Plant $\delta^{13}C$ also depends on the $\delta^{13}C$ of the CO_2 source (Equation V.2). Regionally, the CO_2 content in canopy air might be enriched, e.g. by a high release of CO_2 (with a $\delta^{13}C$ close to the plant source) from drained peat soil. Increased release and assimilation of respired CO_2 on peat soil was the most likely explanation for the lower $\delta^{13}C$ on peat soil compared to mineral soil (Chapter V). If this is true, then ^{13}C signatures may indicate the function of a grassland site as a source for atmospheric CO_2. This may be an important information to model the global C cycle and its response to climatic change (Ciais *et al.* 1995) as soil is an important C pool on the global scale and grassland covers a large fraction of the land area (24%, Shantz 1954). Information about the source CO_2 can also be obtained from old hair, which is stored in textiles (Auerswald *et al.* 2005) or found at excavation sites (Iacumin *et al.* 2005). Hence hair $\delta^{13}C$ may contribute to the reconstruction of former ecological and global conditions.

$\delta^{15}N$

In contrast to ^{13}C, ^{15}N signatures did not allow to assign dietary components to different groups of plants or plant components. Only legume seeds and roughage with high legume content showed consistently low ^{15}N signatures (Table IV.2), probably due to high contributions of biological N fixation to N supply of these plants and plant communities. However, clear similarities in mean and temporal pattern of cattle hair $\delta^{15}N$ within farms, and differences between farms (Figure II.2, Table III.1 and Figure III.2) indicated, that farm-system characteristics controlled ^{15}N signatures.

N fluxes in modern cattle farming systems are complex and heterogeneous, in that they may produce both forage and cash crops, import large amounts of forage and mineral fertilizer, and possibly even export large quantities of manure. Additionally, the long turnover time of the soil N pool may complicate the interpretation of ^{15}N signatures (Chapter IV). However, mean hair ^{15}N signatures of adult animals from different farm systems correlated strongly with farming intensity (stocking rate, Figure IV.5) and farm N

input surplus (Figure IV.6). Hence, the $\delta^{15}N$ at the farm level was probably dominated by N losses (Chapter IV). This is consistent with observations in cropland (Meints *et al.* 1975), forests (Högberg 1991) and rangeland ecosystems (Frank *et al.* 2000), and also with the global trend of decreasing $\delta^{15}N$ values in soils and plants in cooler and wetter conditions where conservation and recycling of mineral N is more efficient (Amundson *et al.* 2003).

In cattle farming systems NH_3 volatilisation is considered to be responsible for most of the increase of ^{15}N signatures given the high NH_3 loss potential of cattle farms (Bussink & Oenema 1998), and the strong isotope effect associated with NH_3 volatilisation (34‰ under standard conditions). In reality, fractionation will deviate somewhat from 34‰ depending on environmental conditions (Urey 1947). Also, feed-back of NH_3 volatilisation on system scale $\delta^{15}N$ depends on the fraction of NH_3, which is lost from a pool or sub-pool: with increasing fraction of NH_3 loss the $\delta^{15}N$ in the remaining substrate pool increases following the Rayleigh equation (Equation I.6), but the effect on system scale $\delta^{15}N$ decreases due to a lower contribution of the remaining substrate to total N in the system. Thus, when all the NH_3, which is stored in a certain pool (e.g. in a drop of slurry) is lost, there is no effect on system scale $\delta^{15}N$. This makes the correlation between NH_3 loss and farm system $\delta^{15}N$ complex. More knowledge about NH_3 fluxes and connected isotope effects under farming conditions for the most important NH_3 loss pathways in agroecosystems is needed to improve the understanding of farm scale ^{15}N signatures, and their relationship with different types and rates of N losses.

Fertilisation with manure caused increasing $\delta^{15}N$ in maize crops (Chapter IV). In animal farm systems N recycling via manure (including storage and application to plants) is the most important source for NH_3 volatilisation (Bussink & Oenema 1998), and thus probably also for ^{15}N enrichment. Hence, soils which are mainly fertilized with manure, e.g. non-legume crops in organic farms, should produce plants with high ^{15}N signatures (e.g. see maize silage and maize meal of Farm A in Table IV.2). Similarly, in grazed grassland, plants that were growing on urine patches were enriched in ^{15}N compared to controls. Yet, leaves of plants were depleted in ^{15}N, indicating that they may have been a sink for volatilised (^{15}N depleted) NH_3 (Frank *et al.* 2004). However, volatilised NH_3 may also be transported over longer distances and deposited in other ecosystems. Hence low ^{15}N signatures in (non-fertilized) natural ecosystems, like in bogs (Bragazza *et al.* 2005), may indicate NH_3 input from adjacent agroecosystems.

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