Isotopes as natural recorders of grassland ecosystem functioning and change

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Key points: The use of stable isotope analysis in grassland science has increased enormously in the last two decades. It is providing insight into the functioning of grassland systems, including aspects such as the biogeochemical cycles of carbon, nitrogen and water, $C_3 : C_4$ vegetation dynamics, the nutritional ecology of grassland fauna, and the (agro)ecology of cattle production systems. This paper deals with the natural stable isotope compositions of three major bioelements (carbon, nitrogen and oxygen) and how they behave in organisms and ecosystems, and it describes examples of recent research progress in the ecology of grassland systems, which were made with the help of stable isotope analysis.

Key words: stable isotopes (^{13}C, ^{15}N, ^{18}O), biogeochemical cycles, $C_3 : C_4$, grazer ecology

Introduction Until 1990 stable isotope analysis was used only sporadically in grassland research. Today, more than 6% of all 'grassland' publications appearing in the ISI Web of Science data base employ stable isotope analysis. Several reviews and books have been published in the last years, which covered various aspects of the application of natural stable isotope analysis in ecology and in the study of stable isotope behaviour in the environment and in organisms (e.g. Dawson & Siegwolf, 2007; West et al., 2006; Flanagan et al., 2005; Hobbie & Werner, 2004; Schmidt, 2003; Werner and Schmidt 2002; Dawson et al., 2002; Robinson et al., 2001; Evans, 2001; Handley et al. 1999; Ehleringer et al., 1997), and some of these have also dealt with grassland flora, fauna and ecosystems. The interest in stable isotope analysis in ecology of grassland (and of other biomes) stems from the fact that the natural stable isotope composition of organisms and ecosystems hold many clues about their functioning and physical environment, including the relative availability of resources (or resource limitations).

The major elements of the biosphere (including carbon, oxygen and nitrogen) occur in the form of two or more stable isotopes (carbon: [12]C, ^{13}C; oxygen: [16]O, [17]O, ^{18}O; nitrogen ^{14}N, ^{15}N). The isotopic composition of an element in a sample is conventionally expressed as a δ-value, which is defined as the deviation of the isotope ratio (R) of the sample relative to that of the international standard. Thus, for carbon, $\delta^{13}C = (R_{sample} - R_{standard}) / R_{standard}$, with R the molar abundance ratio, ^{13}C/[12]C. In an analogous way the isotope composition of nitrogen is given as $\delta^{15}N$, and that of oxygen as $\delta^{18}O$. The standard is PeeDee Belemnite (PDB) for ^{13}C, air for ^{15}N, and 'standard mean ocean water' (SMOW), or PDB for [18]O.

The distribution of the isotopes is not homogeneous in the biosphere; but follows characteristic spatial and temporal patterns. These patterns originate from the different behaviour of isotopes in physical and chemical processes, and are controlled by environmental conditions (including anthropogenic factors) and biological properties of organisms and ecosystems. As a rule, the lighter isotope is transferred (e.g. diffuses) faster and is preferred in (bio)chemical reactions, so that the light isotope tends to accumulates in the product (or sink), whereas the heavy isotope tends to stay in the substrate (or source). For example, photosynthetic CO_2 fixation prefers ^{12}C over [13]C (^{13}C discrimination), so that plants are relatively ^{13}C-depleted, whereas atmospheric CO_2 becomes ^{13}C-enriched. As another example, 'isotopically light' water evaporates more readily, than 'heavy' water, causing ^{18}O-enrichment of leaf water during transpiration.

In general, the isotopic composition of an element in a given ecosystem, organism or compound is determined by the isotopic composition of its source and isotope effects in transfer or transformation processes. But, isotope effects are fully expressed only if the substrate is infinite (or: only a small fraction is consumed in the reaction). If all substrate is consumed, then the (accumulated) product has the same isotopic composition as the substrate, even if the reaction has a strong intrinsic isotope effect (Robinson, 2001). Isotope effects can be expressed completely in fully open systems, whereas closed systems tend to suppress the expression of isotope effects. One example for the latter is the isotope effect of Rubisco on [13]CO_2 (relative to [12]CO_2), which is almost completely suppressed in C_4 plants, because of the localization of Rubisco in the CO_2-tight bundle sheath cells. Furthermore, a consideration of possible closed-system phenomena is also important for understanding the nitrogen isotope composition of ecosystems. A particular opportunity for the expression of isotope effects is offered at branch points of pathways.

Carbon isotope analysis for studies of $C_3 : C_4$ vegetation dynamics, and drought effects on C_3 grassland The carbon isotope composition of all plants ($\delta^{13}C_P$) is determined by the $\delta^{13}C$ of atmospheric CO_2 ($\delta^{13}C_{CO_2}$) and carbon isotope discrimination during photosynthesis ([13]Δ), so that $\delta^{13}C_P = (\delta^{13}C_{CO_2} - {}^{13}\Delta) / (1 + {}^{13}\Delta)$ (Farquhar et al., 1989). [13]Δ is variable and, in particular, it differs strongly between C_3 and C_4 plants, allowing a distinction of the photosynthetic types on the basis of their [13]Δ (e.g. Smith & Epstein 1971, and Figure 1).

C_3 and C_4 plants coexist in many grasslands in the tropics, subtropics and warm temperate regions. Variation of $C_3 : C_4$ abundance has wide biogeochemical and land use implications: it affects the efficiency with which vegetation uses radiation,

water und nutrients, and it may affect soil carbon storage, water use and nutrient cycling (Connin et al., 1997; Tieszen et al., 1997; Bird and Pousai, 1997; Epstein et al., 1998; Sage and Kubien, 2003; Semmartin et al., 2004). There is abundant evidence that the current distribution of C_4 plants is primarily controlled by growing season temperature, and that this is related to the higher effective quantum yield of CO_2 fixation and higher maximum photosynthetic rate of C_4 plants at high temperature (Ehleringer et al., 1997). But the seasonal distribution of precipitation, aridity, soil fertility, and disturbance (for instance by overgrazing) may exert secondary, modifying effects. Predominance of summer rainfalls benefits the C_4 more than the C_3, whereas predominance of precipitation in the cool season benefits C_3 growth (Murphy et al., 2007). C_4 dicots predominate in hot arid, saline or highly disturbed habitats (Ehleringer et al., 1997). Nitrogen loading can cause a replacement of C_4 grasses by C_3 grasses (Wedin & Tilman, 1996). It may be expected that such secondary controls could have a strong effect on $C_3:C_4$ abundance in those regions which have a growing season mean temperature that is near the $C_3:C_4$ transition-temperature. This is true for a large proportion of the worlds' grasslands (Collatz et al., 1998). For instance, a large part of the steppe of Inner and Outer Mongolia exhibits such a climate, with average temperature near the $C_3:C_4$ transition-temperature during the summer months when most of the annual precipitation falls. In many regions of the world grassland utilization is heavy, with overgrazing leading to degradation and erosion and declines in the carrying capacity of the grassland. Such conditions could promote the spread of ruderal species, including annual C_4 grasses and C_4 dicots (Ehleringer et al., 1997; Wang, 2002), and this could be further promoted by climate warming. However, the effect of warming is offset by the increasing atmospheric CO_2 concentration, which increases the quantum yield in C_3 plants, thus promoting their spread. Collatz et al. (1998) have predicted a general increase in the relative abundance of C_3 plants in mixed $C_3:C_4$ grasslands in the last century.
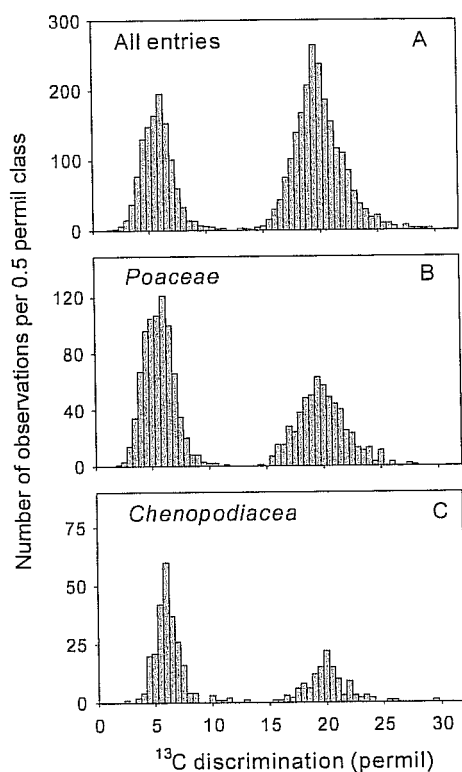
Figure 1 *Frequency distribution of ^{13}C discrimination, $^{13}\Delta$, of grassland plants (A).*
The bimodal distribution of $^{13}\Delta$ is due to the presence of two photosynthetic types with different carbon isotope discrimination: C_4 plants (left hump) and C_3 plants (right hump). Both photosynthetic types occur in the grasses (B), chenopods (C), and other plant families (not shown).
The data were compiled from >40 references with a total of >3000 entries.
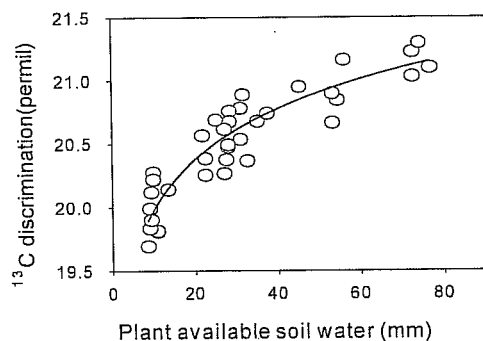
Figure 2 *Influence of soil water availability on ^{13}C discrimination of C_3 grassland (from Schnyder et al., 2006).*

Whether or not these changes have actually occurred has not been studied in any detail. Answering this question by empirical studies is important from practical and scientific biogeochemical, ecological and economic perspectives. For instance, changes in the $C_3:C_4$ abundance could hold important consequences for the nutritional quality of the herbage, and for the seasonal pattern and abundance of grassland production. A verification of the predictions of Collatz et al. (1998) would enhance our understanding of the interplay of factors controlling $C_3:C_4$ abundance in grassland. Because of the difference in $^{13}\Delta$, the analyses of the $\delta^{13}C$ of grassland and of its changes over time is one promising means by which the relative abundance of C_3 and C_4 plants in grassland can be assessed.

One of the challenges in using ^{13}C for studies of $C_3:C_4$ vegetation dynamics lies in the variability of $^{13}\Delta$ within the photosynthetic types (Figure 1). This variability is not fully understood, and causes uncertainty in the choice of the $^{13}\Delta$ of the C_3 and C_4 end-members in the mixing model used to estimate the relative abundances of C_3 and C_4 plants in grassland

communities.

Potential variation of $^{13}\Delta$ is particularly large in C_3 plants (Figure 1), and much of this may be related to effects of drought (Figure 2). $^{13}\Delta$ in C_3 plants is linearly related to the ratio of leaf intercellular to ambient CO_2 partial pressure (p_i/p_a) according to: $^{13}\Delta = a + (b - a) \ p_i/p_a$, with a the ^{13}C fractionation during diffusion of CO_2 in air (4.4‰), and b the net fractionation caused by carboxylation reactions (mainly Rubisco, approx. 27‰) (Farquhar et al., 1989). This means that $^{13}\Delta$ of C_3 plants is mainly controlled by the (stomatal) conductance and photosynthetic activity of leaves. Because of this $^{13}\Delta$ is also a quantitative indicator of physiological water use efficiency (Farquhar & Richards, 1984), and a measure of the leaf-level coupling of the hydrological and carbon cycles. Drought/aridity often leads to a reduction of stomatal conductance, decreasing p_i/p_a, and $^{13}\Delta$ (e.g. Farquhar & Richards, 1984; Schnyder et al., 2006; Wittmer et al., 2008).

Another challenge for studies exploring long-term C_3:C_4 vegetation dynamics is presented by the fact that the life span of shoot biomass is very short in grassland (weeks to <1 year) and vegetation composition may change strongly during the season. As did others, Witt et al. (1998) used the wool of sheep and Schnyder et al. (2006) the tail switch hair of cattle to monitor the ^{13}C composition of grassland vegetation. This method takes advantage of the sampling' activity of grazing animals. The wool from a yearly shearing reflects the $\delta^{13}C$ of all feed ingested by the flock during the year. Provided that the sheep graze only grassland, and grazing is non-biased with respect to the isotopic composition of sward components (e.g. sheep do not prefer one photosynthetic type over the other), this method also reflects the $\delta^{13}C$ of herbage on the entire grazing ground of the herd. Other animal tissues such as bones or teeth have also been used. Enamel of teeth is a particularly durable recorder of the isotopic composition of the diet of animals, and has provided evidence for the expansion of C_4 grasslands 6-10 Mio years ago (Morgan et al., 1994; Cerling et al., 1997).

$\delta^{15}N$ in the nitrogen cycle As is the case for carbon, the $\delta^{15}N$ of a plant is dependent on the $\delta^{15}N$ of the external nitrogen sources and isotope fractionation associated with uptake and transformations (Evans, 2001; Robinson, 2001; Werner and Schmidt, 2002). But, identification of the source(s) and estimation (or measurement) of its isotope signature(s) is more difficult for nitrogen than for carbon (which is normally atmospheric CO_2 with a known $\delta^{13}C$): plants can uptake nitrogen from the soil solution or from the air, and uptake can occur in several chemical forms (ammonia, nitrate, amino acids) or by atmospheric nitrogen fixation. The different putative sources of nitrogen in the soil can have widely differing $\delta^{15}N$ (e.g. Dijkstra et al., 2006), opening up opportunities for the assessment of nitrogen partitioning between plants in a community.

Variation of $\delta^{15}N$ among soil nitrogen pools is strongly affected through isotope fractionation in soil nitrogen transformations by soil (micro) organisms. In particular, NH_3 volatilization, and N_2O and NO production during NH_4^+ oxidation have strong isotope effects, which can lead to massive ^{15}N-depletion (up to $\sim$ 60‰) in the respective gasses, if the conversion is incomplete. Such processes lead to ^{15}N-enrichment of soil nitrogen: elevated soil $\delta^{15}N$ is often found in spots of nitrogen accumulation and mineralization such as in wet depressions in landscapes (e.g. Handley et al., 1999) or at urine-or dung-affected microsites in grazed grasslands (e.g. Dijkstra et al., 2006). On the other hand, volatilized (^{15}N-depleted) NH_3 may be absorbed by nitrogen-limited vegetation located downwind of the volatilization source, lowering the $\delta^{15}N$ of these plants (Erskine et al., 1998).

Variation in the $\delta^{15}N$ of plant nitrogen exists at many scales, from the molecule (e.g. amino acids) to the globe (e.g. Werner and Schmidt, 2002; and Figure 3). At the regional or global scale there is a negative relationship between plant or soil $\delta^{15}N$ and rainfall (e.g. Handley et al., 1999; Amundson et al., 2003; see Figure 3). In line with the mechanisms discussed above, the elevated ^{15}N of plants and soils in dry regions has been interpreted in terms of an open nutrient cycle (Swap et al., 2003, Aranibar et al., 2004).

Handley et al. (1999) and others have pointed out that the negative correlation between water availability and soil or plant $\delta^{15}N$ (evident at the regional or global scale) often fails at the landscape scale, where wet spots can be ^{15}N-enriched relative to their drier surroundings. Schwertl et al. (2005) analyzed hair of cattle from farms representing the whole spectrum of cattle production systems in South Bavaria, Germany. There was large variability in $\delta^{15}N$ between farms, and this was unrelated to rainfall. Of all studied parameters, the nitrogen balance surplus of the farms exhibited the strongest relationship with $\delta^{15}N$ of cattle hair. $\delta^{15}N$ increased with nitrogen surplus ($r^2 = 0.78$), again indicating increasing volatile losses of nitrogen with nitrogen balance surplus. The same study also yielded evidence for substantial plot-scale variation of $\delta^{15}N$ in some of the farms. Plot-scale variation of $\delta^{15}N$ in plants and soils was analyzed by Watzka et al. (2006) in long-term experiments on montane grassland. They observed an increase of $\delta^{15}N$ of top soil and plants with increasing amounts of applied fertilizer and nitrogen balance surplus. Again, this result was interpreted in terms of a stimulation of soil processes, which discriminate against ^{15}N and enhance the loss of ^{15}N-depleted compounds from the system. In the same experiment soil $\delta^{15}N$ depended also on the type of fertilizer, with organic fertilizers causing a stronger ^{15}N-enrichment of soils and vegetation than synthetic fertilizers. This was related to the different $\delta^{15}N$ of these fertilizers. Synthetic fertilizers (and biologically fixed atmospheric nitrogen) have a $\delta^{15}N$ which is close to that of nitrogen in air ($\delta^{15}N$ 0‰), whereas organic fertilizers are generally (but very variably) enriched in ^{15}N (Bateman et al., 2007).
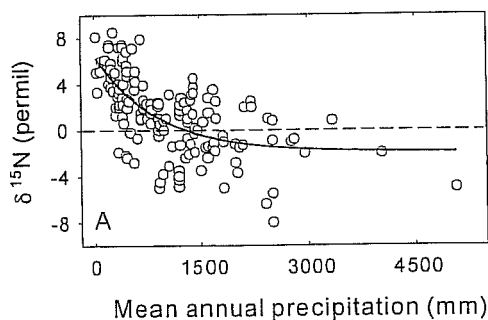
Figure 3 $\delta^{15}N$ *of plants as related to mean annual precipitation. Data compiled from Handley et al. (1999), Jacot et al. (2000), Amundson et al. (2003), and Swap et al. (2004).*
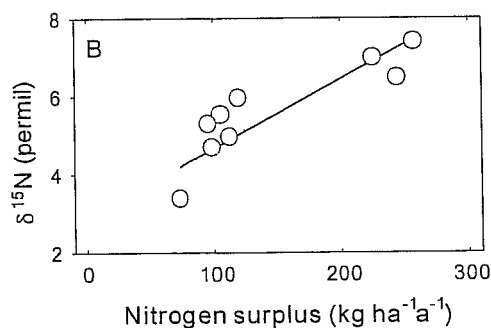

Figure 4 $\delta^{15}N$ *of cattle hair* <u>versus</u> *nitrogen balance surplus of cattle farms in South Bavaria, Germany (Schwertl et al. 2005).*

Figure 5 *Inter-annual and seasonal variation of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and oxygen isotope composition ($\delta^{18}O$) in new hair growth of cattle at Grünschwaige Grassland Research Station, Germany.*

Animals grazed grassland with high soil water capacity in summer. During winter (shaded period) animals were held in confinement and were fed with hay and silage harvested from (sown) grassland with low soil water capacity (see Schnyder et al. 2006). The time course was retrieved by segmental analysis of cattle tail switch hair sampled at different times. Time-assignment of the segmental data was performed as described by Schwertl et al. (2003).

Isotopes in the ecology of animals and production systems The isotopic composition of animals reflects that of their diet (DeNiro and Epstein, 1978, 1981; Kohn et al., 1996), so that (single-or multi-element) isotope analysis of animal bodies, tissues or products can be used to study the production ecology of cattle farming systems (Schwertl et al. 2003), the contribution of different diet sources (Phillips et al., 2005) or the behavioural ecology of animals, including migration patterns and dietary preferences (Cerling and Harris, 1999; Cerling et al., 2006; West et al., 2006). Different types of animal tissues have been used for such analysis, including bones (apatite or collagen), teeth and hair. Animal products have been studied with the aim of characterizing/authenticating production systems and to trace their origin (e. g. Rossmann et al., 2000). Hair is a particularly interesting object for studies of animal ecology, because it allows reconstruction of the dietary history with high temporal resolution (Schwertl et al., 2003; Ayliffe et al., 2004; and Figure 5).

The $\delta^{13}C$ of animal tissues or products gives an estimate of the proportion of C_3 and C_4 plants in the diet, allowing distinction of grazers, browsers and mixed feeders in savanna systems (Cerling and Harris, 1999), or the proportion of maize in the rations of dairy and beef cattle (Schwertl et al., 2005). The use of ^{15}N in studies of food webs/trophic networks is probably one of the most popular examples of the use of stable isotopes in ecology (e. g. Post 2002). It takes advantage of the fact that the ^{15}N of consumers is generally enriched by about 3‰ relative to their diet, allowing estimation of the trophic position of organism in food chains, based on the $\delta^{15}N$ of food chain components(Diet and hair also exhibit a systematic isotopic shift for ^{13}C (e. g. Männel et al., 2007)). Cattle also track environmental effects on the isotopic composition of plants/feed, such as the effect of altitude on the $\delta^{13}C$ and $\delta^{15}N$ of grassland vegetation in the European Alps (Männel et al., 2007).

The oxygen isotope composition of animals is greatly influenced by that of drinking water and free water in food (Kohn et al.,
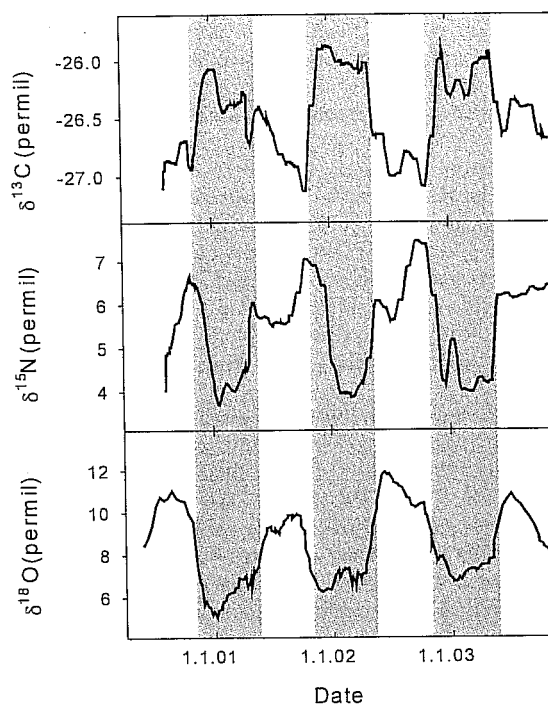
1996). In cattle grazing fresh pasture a large proportion of the ingested water is in the form of leaf water. Leaf water is enriched in ^{18}O due to fractionation during transpiration (Flanagan et al., 1991), distinguishing it from drinking (well) water which has a δ^{18}O close to that of meteoric water. Accordingly, rations with different proportions of fresh herbage should produce different ^{18}O signals in animal bodies and products, such as meat and milk. However, the ^{18}O of water also exhibits strong geographic variation, which is related to effects of altitude, latitude, distance from the coast, amount of precipitation, and season on the ^{18}O of meteoric water (e. g. Bowen and Wilkinson, 2002). Thus, drinking water contains geographic information, which is imprinted in animal tissues and products.

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Multifunctional Grasslands in a Changing World

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