

Isotopes as natural recorders of grassland ecosystem functioning and change

Hans Schnyder and Karl Auerswald

Technische Universität München, Lehrstuhl für Grünlandlehre, Am Hochanger 1, D-85350 Freising-Weihenstephan,

E-mail: schnyder@wzw.tum.de

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, C₃:C₄ 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 (¹³C, ¹⁵N, ¹⁸O), biogeochemical cycles, C₃:C₄, 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: ¹²C, ¹³C; oxygen: ¹⁶O, ¹⁷O, ¹⁸O; nitrogen ¹⁴N, ¹⁵N). 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, $\delta^{13}\text{C} = (R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}$, with R the molar abundance ratio, ¹³C/¹²C. In an analogous way the isotope composition of nitrogen is given as δ¹⁵N, and that of oxygen as δ¹⁸O. The standard is Pee Dee Belemnite (PDB) for ¹³C, air for ¹⁵N, and standard mean ocean water (SMOW), or PDB for ¹⁸O.

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 accumulate in the product (or sink), whereas the heavy isotope tends to stay in the substrate (or source). For example, photosynthetic CO₂ fixation prefers ¹²C over ¹³C (¹³C discrimination), so that plants are relatively ¹³C-depleted, whereas atmospheric CO₂ becomes ¹³C-enriched. As another example, isotopically light water evaporates more readily, than heavy water, causing ¹⁸O-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 ¹³CO₂ (relative to ¹²CO₂), which is almost completely suppressed in C₄ plants, because of the localization of Rubisco in the CO₂-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 C₃:C₄ vegetation dynamics, and drought effects on C₃ grassland The carbon isotope composition of all plants (δ¹³C_P) is determined by the δ¹³C of atmospheric CO₂ (δ¹³C_{CO₂}) and carbon isotope discrimination during photosynthesis (¹³Δ), so that $\delta^{13}\text{C}_P = (\delta^{13}\text{C}_{\text{CO}_2} - \Delta) / (1 + \Delta)$ (Farquhar et al., 1989). Δ is variable and, in particular, it differs strongly between C₃ and C₄ plants, allowing a distinction of the photosynthetic types on the basis of their ¹³Δ (e.g. Smith & Epstein 1971, and Figure 1).

C₃ and C₄ plants coexist in many grasslands in the tropics, subtropics and warm temperate regions. Variation of C₃:C₄ abundance has wide biogeochemical and land use implications: it affects the efficiency with which vegetation uses radiation,

water and 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 world's 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.

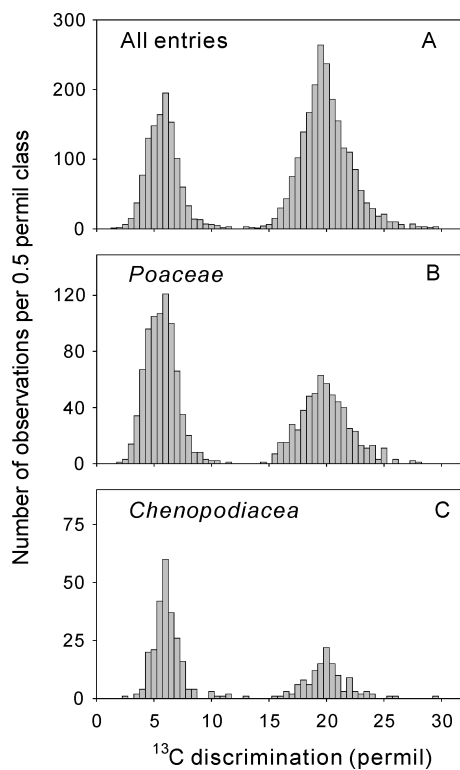


Figure 1 Frequency distribution of ^{13}C discrimination, $^{13}\Delta$, of grassland plants (A). The bimodal distribution of $^{13}\Delta$ is due to the presence of two photosynthetic types with different carbon isotope discrimination: C_4 plants (left hump) and C_3 plants (right hump). Both photosynthetic 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.

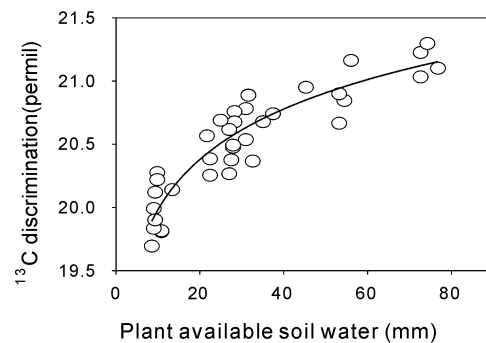


Figure 2 Influence of soil water availability on ^{13}C discrimination of C_3 grassland (from Schnyder et al., 2006).

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 $\delta^{13}\text{C}$ is particularly large in C_3 plants (Figure 1), and much of this may be related to effects of drought (Figure 2). $\delta^{13}\text{C}$ 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: $\delta^{13}\text{C} = a + (b - a) p_i/p_a$, 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 $\delta^{13}\text{C}$ of C_3 plants is mainly controlled by the (stomatal) conductance and photosynthetic activity of leaves. Because of this $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ (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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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).

$\delta^{15}\text{N}$ in the nitrogen cycle As is the case for carbon, the $\delta^{15}\text{N}$ of a plant is dependent on the $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$): 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 $\delta^{15}\text{N}$ (e.g. Dijkstra et al., 2006), opening up opportunities for the assessment of nitrogen partitioning between plants in a community.

Variation of $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ of these plants (Erskine et al., 1998).

Variation in the $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ (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 $\delta^{15}\text{N}$ between farms, and this was unrelated to rainfall. Of all studied parameters, the nitrogen balance surplus of the farms exhibited the strongest relationship with $\delta^{15}\text{N}$ of cattle hair. $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ in some of the farms. Plot-scale variation of $\delta^{15}\text{N}$ in plants and soils was analyzed by Watzka et al. (2006) in long-term experiments on montane grassland. They observed an increase of $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ of these fertilizers. Synthetic fertilizers (and biologically fixed atmospheric nitrogen) have a $\delta^{15}\text{N}$ which is close to that of nitrogen in air ($\delta^{15}\text{N} 0\%$), whereas organic fertilizers are generally (but very variably) enriched in ^{15}N (Bateman et al., 2007).

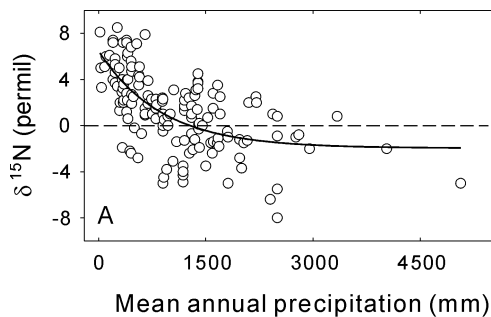


Figure 3 $\delta^{15}\text{N}$ of plants as related to mean annual precipitation. Data compiled from Handley et al. (1999), Jacot et al. (2000), Amundson et al. (2003), and Swap et al. (2004).

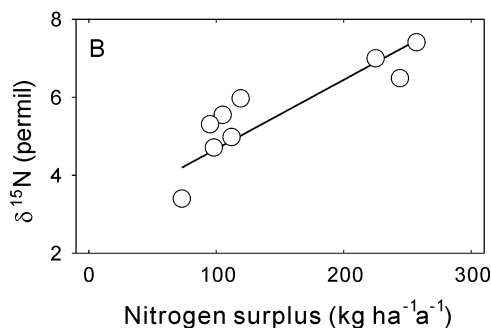


Figure 4 $\delta^{15}\text{N}$ of cattle hair versus nitrogen balance surplus of cattle farms in South Bavaria, Germany (Schwertl et al. 2005).

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 $\delta^{13}\text{C}$ 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 $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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.,

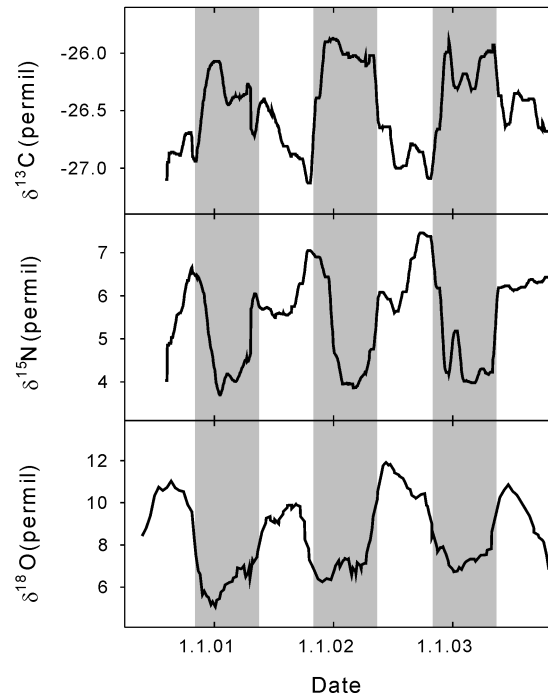


Figure 5 Inter-annual and seasonal variation of carbon ($\delta^{13}\text{C}$), nitrogen ($\delta^{15}\text{N}$) and oxygen isotope composition ($\delta^{18}\text{O}$) in new hair growth of cattle at Grünschwaike Grassland Research Station, Germany.

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 $\delta^{18}\text{O}$ 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 .

References

- Amundson R , Austin AT , Schuur EAG , et al . (2003) Global patterns of the isotopic composition of soil and plant nitrogen . *Global Biogeochemical Cycles* 17 , No 1 , 1031 , doi :10 .1029/2002GB001903 .
- Aranibar JN , Otter L , Macko SA et al .(2004) Nitrogen cycling in the soil-plant system along a precipitation gradient in the Kalahari sands . *Global Change Biology* 10 : 359-373 .
- Ayliffe LK , Cerling TE , Robinson T , et al . (2004) Turnover of carbon isotopes in tail hair and breath CO_2 of horses fed an isotopically varied diet . *Oecologia* 139 : 11-22 .
- Bateman AS , Kelly SD (2007) Fertilizer nitrogen isotope signatures . *Isotopes in Environmental & Health Studies* 43 : 237-247 .
- Bird MI , Pousai P (1997) Variations of $\delta^{13}\text{C}$ in the surface soil organic carbon pool . *Global Biogeochemical Cycles* 11 : 313-322 .
- Bowen GJ , Wilkinson B (2002) Spatial distribution of $\delta^{18}\text{O}$ in meteoric precipitation . *Geology* 30 : 315-318 .
- Cerling TE , Harris JM (1999) Carbon isotope fractionation between diet and bioapatite in ungulate mammals and implications for ecological and paleoecological studies . *Oecologia* 120 : 347-363 .
- Cerling TE , Wittemyer G , Rasmussen HB , et al . (2006) Stable isotopes in elephant hair document migration patterns and diet changes . *Proceedings of the National Academy of Sciences of the United States of America* 103 : 371-373 .
- Cerling TE , Harris JM , MacFadden BJ , Leakey MG , Quade J , Eisenmann V , Ehleringer JR (1997) Global vegetation change through the Miocene/Pliocene boundary . *Nature* 389 : 153-158 .
- Collatz GJ , Berry JA , Clark JS (1998) Effects of climate and atmospheric CO_2 partial pressure on the global distribution of C_4 grasses : present , past , and future . *Oecologia* 114 : 441-454 .
- Connin SL , Virginia RA , Chamberlain CP (1997) Carbon isotopes reveal soil organic matter dynamics following arid land shrub expansion . *Oecologia* 110 : 374-386 .
- Dawson TE , Mambelli S , Plamboeck AH , Templer PH , Tu KP (2002) Stable isotopes in plant ecology . *Annual Review of Ecology and Systematics* 33 : 507-559 .
- Dawson TE , Siegwolf RTW (2007) *Stable Isotopes as Indicators of Ecological Change* Elsevier .
- DeNiro MJ , Epstein S (1978) Influence of diet on distribution of carbon isotopes in animals . *Geochimica et Cosmochimica Acta* 42 : 495-506 .
- DeNiro MJ , Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals . *Geochimica et Cosmochimica Acta* 45 : 341-351 .
- Dijkstra P , Menyailo OV , Doucett RR et al .(2006) C and N availability affects the N-15 natural abundance of the soil microbial biomass across a cattle manure gradient . *European Journal of Soil Science* 57 : 468-475 .
- Ehleringer JR , Cerling TE , Helliker BR(1997) C_4 photosynthesis , atmospheric CO_2 , and climate . *Oecologia* 112 : 285-299 .
- Epstein HE , Burke IC , Mosier AR (1998) Plant effects on spatial and temporal patterns of nitrogen cycling in shortgrass steppe . *Ecosystems* 1 : 374-385 .
- Erskine PD , Bergstrom DM , Schmidt S , et al . (1998) Subantarctic macquarie island—a model ecosystem for studying animal-derived nitrogen sources using ^{15}N natural abundance . *Oecologia* 117 : 187-193 .
- Evans RD 2001 Physiological mechanisms influencing plant nitrogen isotope composition . *Trends in Plant Science* 6 : 121-126 .
- Farquhar GD , Richards RA (1984) Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes . *Australian Journal of Plant Physiology* 11 : 539-552 .
- Farquhar GD , Ehleringer JR , Hubick KT (1989) Carbon isotope discrimination and photosynthesis . *Annual Review of Plant Physiology and Plant Molecular Biology* 40 : 503-537 .
- Flanagan LB et al . (2005) *Stable Isotopes and Biosphere-Atmosphere Interactions : Processes and Biological Controls* , Elsevier .
- Flanagan LB , Comstock JP , Ehleringer JR (1991) Comparison of modelled and observed environmental influences on the stable oxygen and hydrogen isotope composition of leaf water in *Phaseolus vulgaris* L . *Plant Physiology* 96 : 588-596 .
- Handley LL , Austin AT , Robinson D , et al . (1999) The ^{15}N natural abundance ($\delta^{15}\text{N}$) of ecosystem samples reflects measures of water availability . *Australian Journal of Plant Physiology* 26 : 185-199 .
- Hobbie EA , Werner RA (2004) Intramolecular , compound-specific , and bulk carbon isotope patterns in C_3 and C_4 plants : a review and synthesis . *New Phytologist* 161 : 371-385 .
- Kohn MJ (1996) Predicting animal $\delta^{18}\text{O}$: Accounting for diet and physiological adaptation . *Geochimica et Cosmochimica Acta* 60 : 4811-4829 .
- Männel TT , Auerswald K , Schnyder H (2007) Altitudinal gradients of grassland carbon and nitrogen isotope composition are recorded in the hair of grazers . *Global Ecology and Biogeography* 16 : 583-592 .

- Morgan ME , Kingston JD , Marino BD (1994) Carbon isotopic evidence for the emergence of C₄ plants in the Neogene from Pakistan and Kenya . *Nature* 367 : 162-165 .
- Murphy BP , Bowman DMJS (2007) Seasonal water availability predicts the relative abundance of C₃ and C₄ grasses in Australia . *Global Ecology and Biogeography* 16 : 160-169 .
- Phillips DL , Newsome SD , Gregg JW (2005) Combining sources in stable isotope mixing models : alternative methods . *Oecologia* 144 : 520-527 .
- Post DM (2002) Using stable isotopes to estimate trophic position : Models , methods , and assumptions . *Ecology* 83 : 703-718 .
- Robinson D (2001) $\delta^{15}\text{N}$ as an integrator of the nitrogen cycle . *Trends in Ecology and Evolution* 16 : 153-162 .
- Rossmann A , Haberhauer G , Hölzl S , et al . (2000) The potential of multielement stable isotope analysis for regional origin assignment of butter . *European Food Research and Technology* 211 : 32-40 .
- Sage RF , Kubien DS (2003) Quo vadis C₄ ? An ecophysiological perspective on global change and the future of C₄ plants . *Photosynthesis Research* , 77 , 209-225 .
- Schnyder H , Schwertl M , Auerswald K , Schäufele R (2006) Hair of grazing cattle provides an integrated measure of the effects of site conditions and interannual weather variability on $\delta^{13}\text{C}$ of temperate humid grassland . *Global Change Biology* 12 : 1315-1329 .
- Schwertl M , Auerswald K , Schnyder H (2003) Reconstruction of the isotopic history of animal diets by hair segmental analysis . *Rapid Communications in Mass Spectrometry* 17 : 1312-1318 .
- Schwertl M , Auerswald K , Schäufele R , Schnyder H (2005) Carbon and nitrogen stable isotope composition of cattle hair : ecological fingerprints of production systems ? *Agriculture Ecosystems & Environment* 109 : 153-165 .
- Semmartin M , Aguiar MR , Distel RA , Moretto AS , Ghersa CM (2004) Litter quality and nutrient cycling affected by grazing-induced species replacements along a precipitation gradient . *Oikos* 107 : 148-160 .
- Smith BN , Epstein S (1971) Two categories of $^{13}\text{C}/^{12}\text{C}$ ratios for higher plants . *Plant Physiology* 47 : 380-384 .
- Swap RJ , Aranibar JN , Dowty PR , Gilhooly WP , Macko SA (2004) Natural abundance of C-13 and N-15 in C-3 and C-4 vegetation of southern Africa : patterns and implications . *Global Change Biology* 10 : 350-358 .
- Tieszen LL , Reed BC , Bliss NB , Wylie BK , Dejong DD (1997) NDVI , C₃ and C₄ production , and distributions in great plains grassland land cover classes . *Ecological Applications* 7 : 59-78 .
- Wang RZ (2002) Photosynthetic pathways , life forms , and reproductive types for forage species along the desertification gradient on Hunshandake desert , North China . *Photosynthetica* 40 : 321-329 .
- Watzka M , Buchgraber K , Wanek W (2006) Natural ^{15}N abundance of plants and soils under different management practices in a montane grassland . *Soil Biology & Biochemistry* 38 : 1564-1576 .
- Wedin DA , Tilman D (1996) Influence of nitrogen loading and species composition on the carbon balance of grasslands . *Science* 274 : 1720-1723 .
- Werner RA , Schmidt HL (2002) The in vivo nitrogen isotope discrimination among organic plant compounds . *Phytochemistry* 61 : 465-484 .
- West JB , Bowen GJ , Cerling TE , Ehleringer JR (2006) Stable isotopes as one of nature's ecological recorders *Trends in Ecology & Evolution* 21 : 408-414 .
- Witt GB , Moll EJ , Beeton RJS , Murray PJ (1998) Isotopes , wool , and rangeland monitoring : let the sheep do the sampling . *Environmental Management* 22 : 145-152 .
- Wittmer M , Auerswald K , Radnaakhand T et al . (2008) Influence of aridity on carbon isotope discrimination in leaves of *Stipa* and other C₃ species in Central Asian Grassland . *Proceedings of the 21st International Grassland Congress and the 8th International Rangeland Congress* , 29 June-5 July 2008 , Hohhot , China .