



STABLE ISOTOPES
AS INDICATORS OF
ECOLOGICAL CHANGE

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A VOLUME IN THE TERRESTRIAL ECOLOGY SERIES



The Reaction Progress Variable and Isotope Turnover in Biological Systems

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I. INTRODUCTION

Biological systems under natural field conditions are rarely at steady state, but are frequently responding to changes in the abundance of external resources, environmental conditions, and phenology and development. This poses a challenge for interpreting the stable isotope ratios sequentially recorded in biological materials because those “recorder” tissues may be responding to external resource pools as well as to pools within the organism or ecological reservoir itself. The reaction progress model describes stable isotope turnover in ecological systems; it allows the determination of half-lives, multiple turnover pools, and their relative contribution to the whole, and can identify transit times not controlled by first-order reaction kinetics. In addition, it is useful in determining sampling intervals specific to isotope turnover studies. Thus, the reaction progress model provides a powerful tool for quantitatively reconstructing discrete resource change events (such as a change in dietary food source of an animal) from the effects of other pools that contribute to the isotope ratio of the “recorder.”

Stable isotope analyses have been used as tracers of ecological processes for many different sets of conditions (see chapters in this volume). As a "tracer" or "recorder" of an ecological change, it is the sequential changes in the isotope composition of a material or tissue that serves as a recorder. Examples of a linear ecological recorder include the isotope ratios along the segment of an animal hair, the sequential carbonate deposition in soil caliche, corals, or in mollusk shells, and the xylem cells that form tree rings. In some cases, the stable isotope ratio of that recorder reflects the immediate state of the environment, as would be the case for carbonates in corals laid down in equilibrium with the surrounding ocean water. In other cases, the isotope ratio of a segment of biological tissue reflects a mixing of different pools such as the amino acids in animal hair that can be derived from the most recent food source as well as from internal pools associated with metabolism and decomposition of existing protein pools within the body.

There are various ways in which previous studies have attempted to resolve patterns that involve multiple pools that can contribute to a single ecological recorder. Turnover in biological systems has been described using exponential curves to fit data. This includes animal tissue studies (Fry and Arnold 1982, Hobson and Clark 1992), soil studies (Balesdent and Mariotti 1996), and other studies. A typical turnover experiment involves an instantaneous change and measuring the change in stable isotope ratio over time.

The exponential fit method has the implicit assumption that the system is behaving according to first-order kinetics. In this chapter, we discuss the reaction progress model to describe isotope turnover in biological systems. This concept has been used for isotope exchange reactions in geochemical systems (Criss *et al.* 1987, Criss 1999) and has been applied to isotope turnover in animal tissues (Ayliffe *et al.* 2004, West *et al.* 2004, Cerling *et al.* 2007) and in laboratory scale exchange reactions (Bowen *et al.* 2005). Here we use the reaction progress variable to examine other cases where first-order kinetics is assumed to play the dominant role in isotope turnover.

In this chapter, we describe first-order kinetics and show that it is a more useful way than a simple exponential fit to understanding isotope turnover in biological systems. We then use a number of examples, primarily from existing literature, to demonstrate how rate constants are better understood using this approach. We then discuss implications for changes at the ecosystem scale.

II. METHODS

Stable isotope ratios in natural systems are described by:

$$\delta^k M_A = \left(\frac{R_A}{R_{\text{standard}}} - 1 \right) \times 1000 \quad (11.1)$$

where k is the rare isotope species of interest, M is the element of interest, A is the phase being of interest (e.g., water, diet, and tissue), R_A is the ratio of the heavy to light isotope (e.g., $^{13}\text{C}/^{12}\text{C}$) in the sample, and R_{standard} the isotope standard.

A first-order rate constant is:

$$\frac{dN}{dt} = -\lambda N \quad (11.2)$$

where N is the number of atoms or molecules in the system being described, t is time (sec), and λ is the rate constant (sec^{-1}). Thus, the reaction rate is directly proportional to the amount of material (N) in the system. This is integrated as:

$$N = N_0 e^{-\lambda t} \tag{11.3}$$

This equation is linearized by taking the natural logarithm:

$$\ln\left(\frac{N}{N_0}\right) = -\lambda t \tag{11.4}$$

where N_0 is the initial number of atoms or molecules. The half-life for the system is:

$$t_{1/2} = \frac{\ln(2)}{\lambda} \tag{11.5}$$

and the mean life is:

$$t_{1/2} = \frac{1}{\lambda} \tag{11.6}$$

Many stable isotope turnover experiments are modeled according to an exponential fit model. This assumes first-order rate kinetics, as we show below, and we accept that assumption. The exponential fit is of the form:

$$\delta^t = a e^{-\lambda t} + c \tag{11.7}$$

where δ^t is the stable isotope of interest undergoing isotope exchange with time t , a and c are parameters derived from the "best fit," and λ is a first-order rate constant. Three important parameters result from this equation: λ is the data-derived rate constant from which the half-life is derived as in Eq. (11.5), " c " is the data-derived isotope equilibrium factor δ^{eq} , " a " is the isotope difference between the initial and final equilibrium states (Tieszen *et al.* 1983), so that " $c + a$ " is the data-derived initial isotope value δ^{init} . In many cases, the initial and final isotope equilibrium conditions are known independently and should be compared to those derived from the exponential fit (Cerling *et al.* 2007).

Isotope turnover or exchange reactions begin under some initial isotope ratio conditions and proceed to the final state. The progress along this path can be normalized to the nominal fraction 1.0 at $t = 0$ by (Criss 1999):

$$\frac{\delta_A^t - \delta_A^{eq}}{\delta_A^{init} - \delta_A^{eq}} = 1 - F \tag{11.8}$$

where δ_A^{init} , δ_A^t , and δ_A^{eq} are the isotopic δ -values of "A" under the initial conditions, at time " t " after exchange begins, and for the final equilibrium conditions, respectively and where $F = 0$ at the beginning of the exchange reaction and $F = 1$ in the final state (*i.e.*, $t \gg 1/\lambda$). This term $(1 - F)$ describes the reaction progress. Criss (1999) pointed out that for trace isotope changes in an open system that:

$$\frac{R_A^t - R_A^{eq}}{R_A^{init} - R_A^{eq}} = e^{-\lambda t} \tag{11.9}$$

This equation is to be converted to the common " δ " notation as:

$$\frac{\delta_A^t - \delta_A^{eq}}{\delta_A^{init} - \delta_A^{eq}} = e^{-\lambda t} \tag{11.10}$$

Equations (4), (8), and (10) are combined to give:

$$\ln(1 - F) = -\lambda t \tag{11.11}$$

This expression describes various biological and geological “turnover”, exchange, and reaction progress experiments that are commonly treated as exponential functions. Cerling *et al.* (2007) show that tissue turnover experiments, when treated in this fashion, can yield far more information about the system than an exponential description; for example, multiple rate constants can be derived, delayed release of red blood cells can be determined along with a more accurate determination of the rate constants for turnover, and this leads to a forward model to calculate dietary input from sequential measurements of animal tissues such as hair.

III. RESULTS

In this section, we provide examples of biological systems where rate constants can readily be determined using the reaction progress variable. The first example is of a “dry-down” experiment of hair. The second example concerns turnover of soil carbon during an agricultural change from C_3 to C_4 plants. The third example involves elimination of feces by an animal that changed from a C_3 -based to a C_4 -based diet. The fourth example discussed is in the case for multiple turnover pools in ecological systems. Cerling *et al.* (2007) have already discussed examples of tissue turnover during diet-switch experiments and the determination of multiple rate constants. These examples illustrate the variety of applications of considering biological turnover experiments in the reaction progress model context.

1. Bowen *et al.* (2005) carried out an isotope exchange experiment where hair samples were equilibrated with water vapor-enriched D and ^{18}O (δD and $\delta^{18}\text{O}$ of the liquid water was +192‰ and +4.9‰, respectively) and then freeze dried for up to 10 days. This represents a “classical” reaction progress experiment as described by Criss (1999) for geochemical systems. Figure 11.1 shows that the rate constant for isotope exchange for both D and ^{18}O is similar, with both having half-lives of 1.4 days for isotope exchange.
2. The amount of soil carbon is approximately twice the amount of carbon in the terrestrial biosphere and therefore has a significant role in the global carbon budget. In this section, we use the data of Balesdent and Mariotti (1996) to illustrate turnover of carbon in a modified ecosystem where a C_4 crop was maintained on a field for 13 years; previously this field had a C_3 vegetation. Figure 11.2 shows the original data of Balesdent and Mariotti, along with data plotted as the reaction progress variable. Although the data is sparse, the reaction progress variable gives half-lives of 62, 9.9, and 5.6 years for the <10- μm , 20- to 200- μm , and 200- to 2000- μm size fractions. In addition, the nonzero intercept of the 200- to 2000- μm size fraction plot indicates that a very short-lived turnover pool may be present (<1 year).
3. The third example illustrates the delayed release of a substance and the simultaneous determination of a first-order rate constant. Sponheimer *et al.* (2003) conducted a controlled diet experiment where alpacas (*Llama pacos*) and horses (*Equus caballus*) were switched from a C_3 to a C_4 diet. Feces were collected and analyzed during this experiment. Figure 11.3 shows the original data along with the data plotted as the reaction progress variable for two different alpaca. After about 3 days, the $\ln(1 - F)$ versus time plot is linear for two different individuals, consistent with a first-order rate constant for fecal carbon turnover. However, this analysis also gives a “transit” time of about 35 h from ingestion to expulsion. The turnover rate of fecal carbon for these two individuals had an average value of 46.3 h (Figure 11.3B). Application of this model to the equids of this study shows that the two animals had slightly different physiological behaviors, with “transit” times of 17 and 20 h with half-lives of 4 and 9 h, respectively. The traditional method for describing such an experiment would include the transit time where such a time represents the first appearance of a marker; in our case, this is an equivalent definition. Mean retention time is the time to which half the material has been passed; in this case the mean retention time does not

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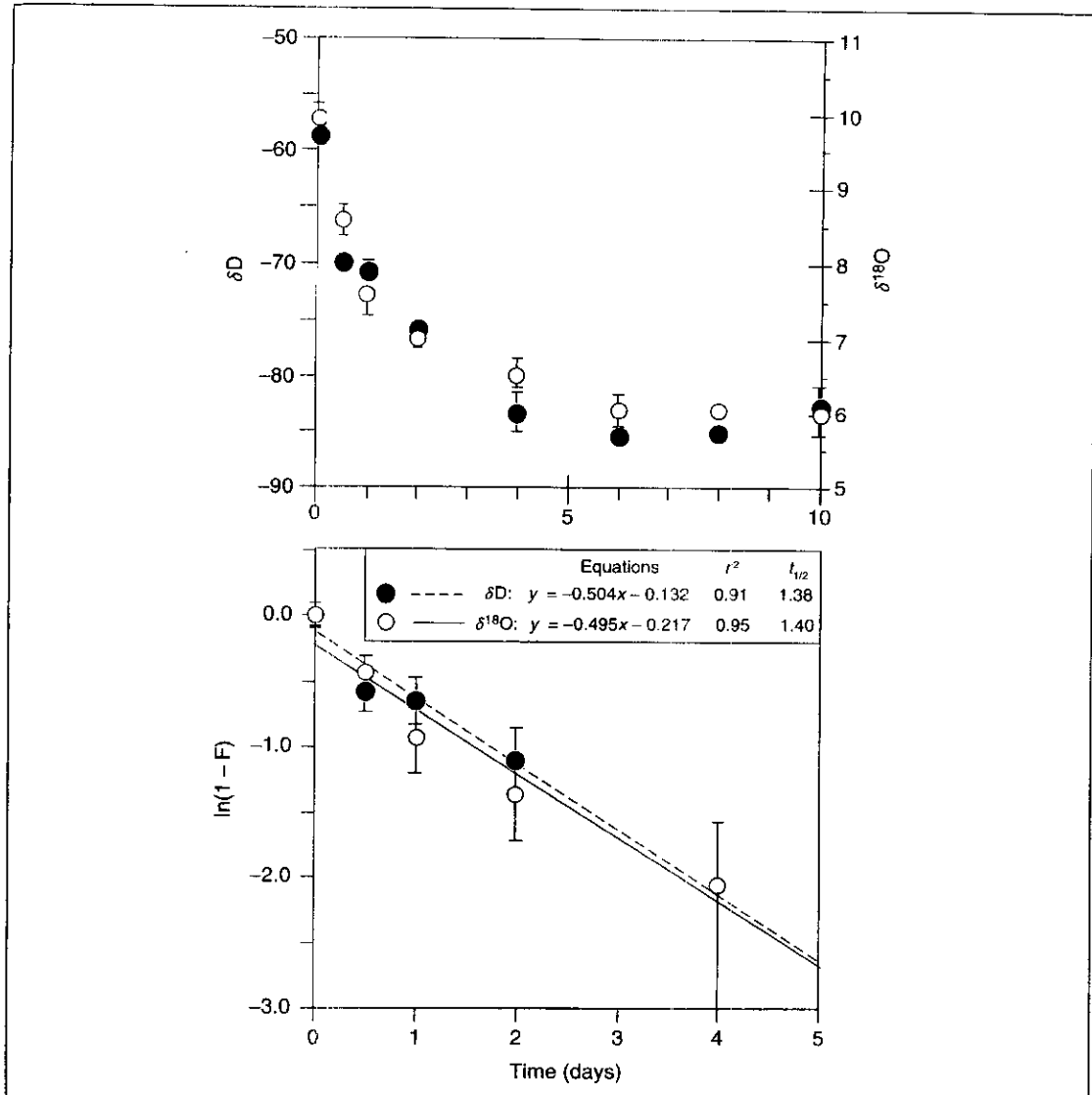


FIGURE 11.1 Dry-down experiment showing δD and $\delta^{18}O$ values for hair that was first equilibrated with water enriched in heavy isotopes and then dried in a freeze drier. (A) Measured δD and $\delta^{18}O$ values of hair dried in a freeze drier for varying lengths of time. (B) The same data plotted as the reaction progress variable, with rate constants for water loss derived from the δD and $\delta^{18}O$ data, respectively.

correspond to the half-life which refers to the physiological process only where half the material is replaced by other material by physiological processes and does not include transit. Thus, the traditional "mean retention time" would be the sum of the transit time and the half-life for turnover of fecal carbon.

- There are several examples in animal physiological studies that have identified multiple turnover pools (Ayliffe *et al.* 2004). Such behavior is expected in the soil carbon system, but most experiments have not been of sufficient sampling detail to determine multiple carbon turnover pools. Experimental design should allow the multiple turnover pool concept to be examined.

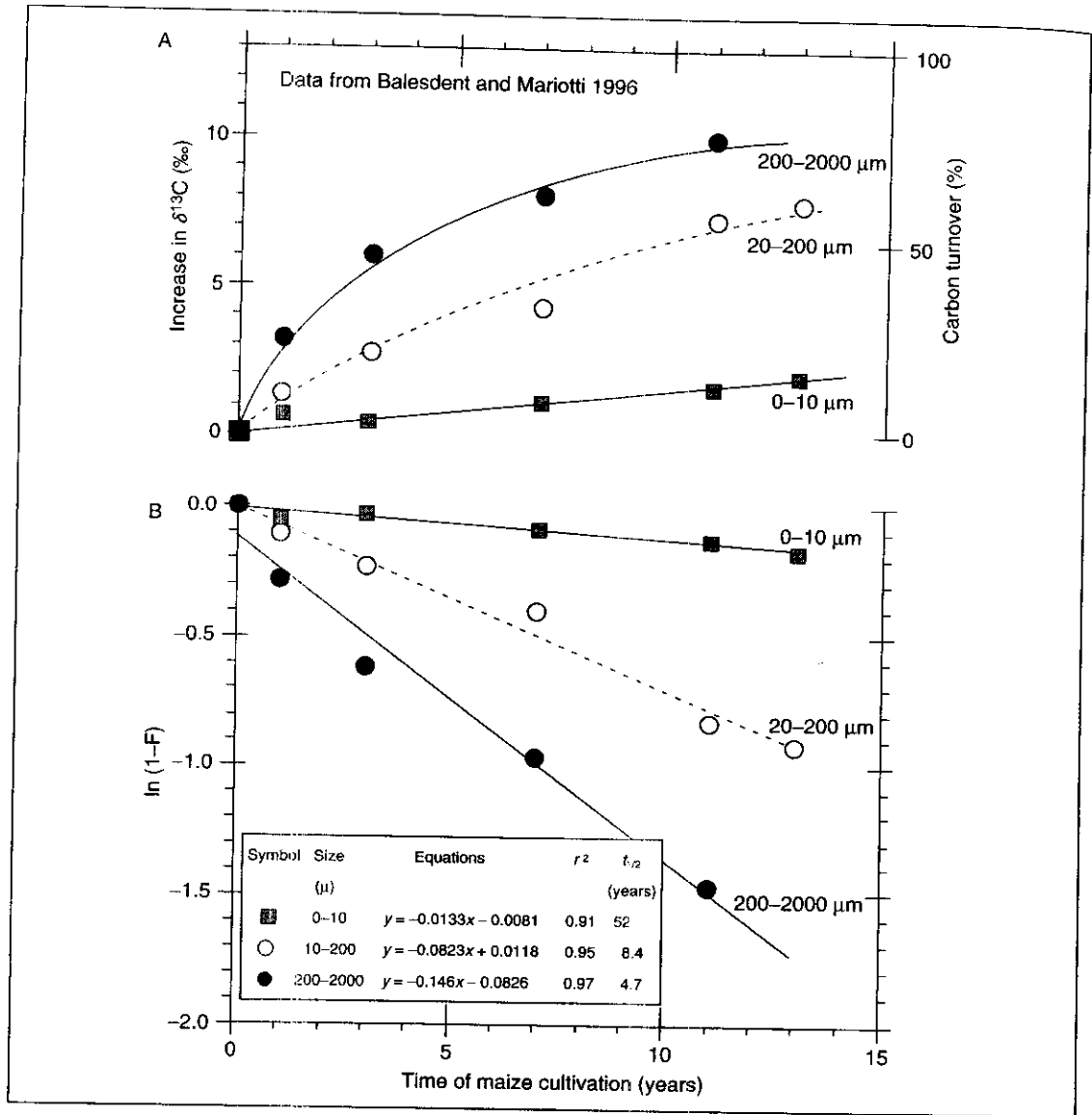


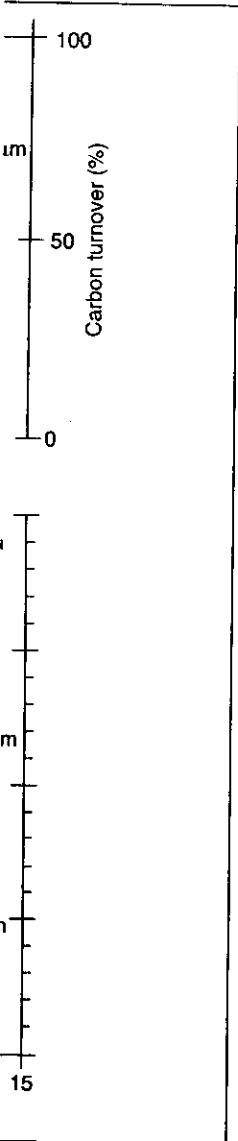
FIGURE 11.2 Stable isotope values and the reaction progress variable for soils converted from C_3 to C_4 biomass. Panel (A) is the original data of Balesdent and Mariotti (1996). Panel (B) shows the same data plotted as the reaction progress variable, with rate constants derived from first-order reaction kinetics.

IV. DISCUSSION

The purpose of this chapter is to illustrate the possibilities of using the reaction progress model to stable isotope studies. We provide four examples of the reaction progress model to ecological applications. The reaction progress model has advantages over the traditional exponential fit of data, principally because of the linearization of the variable:

$$\ln(1 - F) = -\lambda t$$

This makes the recognition of multiple pools (Cerling *et al.* 2007) easier than the conventional method. Data from related experiments, such as approaching an equilibrium state from two directions or using different stable isotopes (Figure 11.1), can be used together to calculate rate constants. This is particularly important when data sampling intervals are limited. Very short turnover pools can often be inferred using the reaction progress model: the nonzero intercept of the 200- to 2000-mm size fraction in the example 2 (Figure 11.2B) suggests that a very short turnover pool may be present, but that the sampling interval is too sparse to capture it. Delay, or transit, times can also be determined from this visualization, and can be separated from turnover times (Figure 11.3B).



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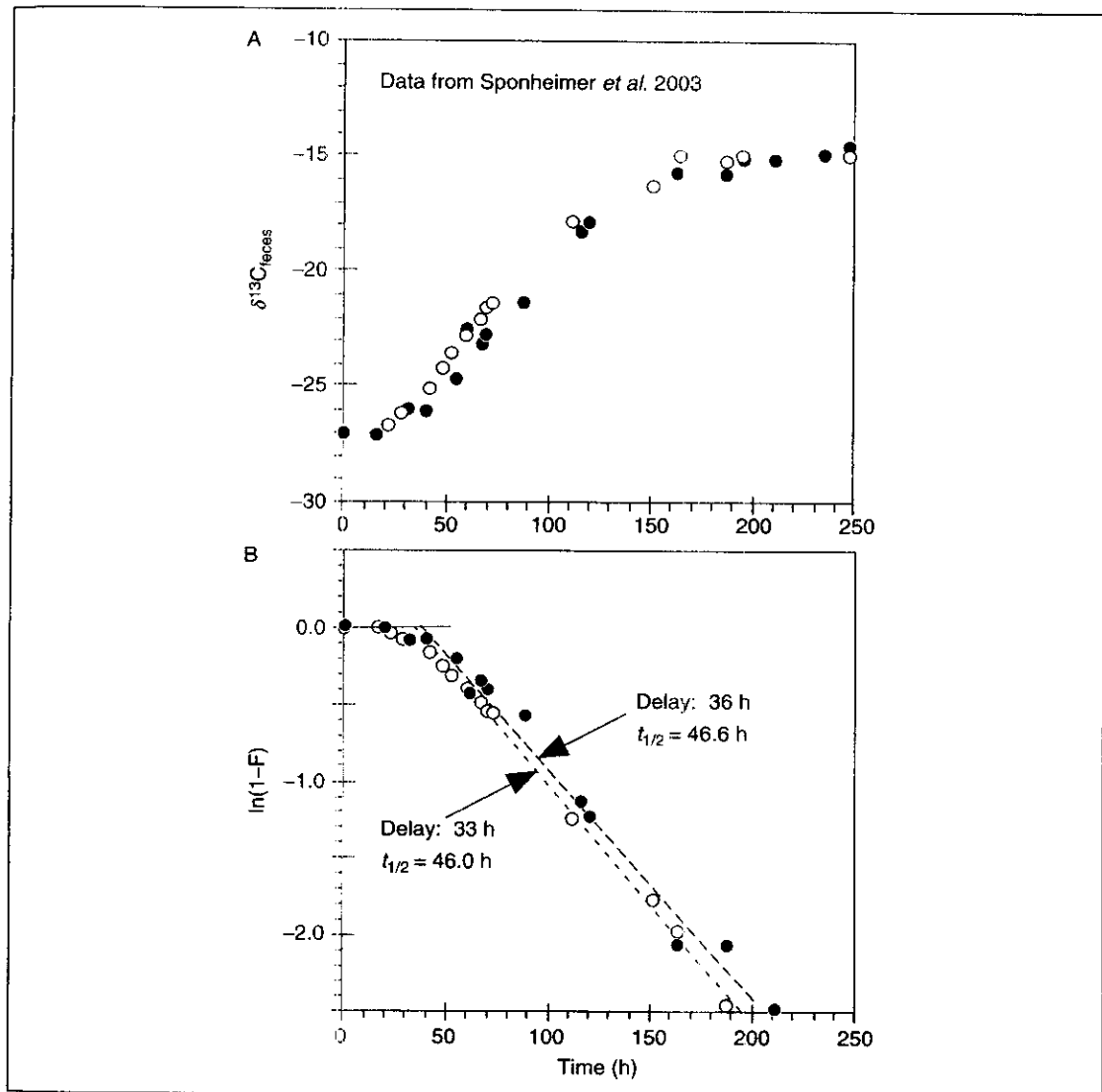


FIGURE 11.3 Stable isotope values of feces of the alpaca and the reaction progress variable for a diet-switch experiment. The two different animals of this experiment are shown as solid or closed symbols, respectively. Panel (A) is the original data of Sponheimer *et al.* (2003). Panel (B) shows the same data plotted as the reaction progress variable, with the turnover rates shown for each individual.

The reaction progress model has important implications for ecological change. It shows how multiple rate constants can be derived from isotope turnover experiments and how multiple experiments can be combined to derive rate constants of ecological turnover processes. For example, this is likely to be important in examining soil carbon turnover in ecosystems under different climatic conditions. With multiple rate constants identified, other experiments can be designed to identify processes responsible for different turnover pools. The example cited in the chapter, the study by Balesdent and Mariotti (1996), is only one example of many that is needed to understand carbon turnover in soils as it is related to climate variables such as precipitation and temperature.

The delay, or transit, time in biological systems can be readily separated from turnover. Previously, these have been treated together and the computed turnover times have been the sum of the transit and true turnover reactions: this has the result of giving an erroneously long turnover time and also giving no insight into the processes related to the transit process. For example, it has been reported that short-term changes in the carbon isotope ratios of ecosystem respiration and soil respiration are closely correlated with atmospheric vapor pressure deficits (Ekblad and Högberg 2001, Bowling *et al.* 2002, McDowell *et al.* 2004). Mechanistically, these ecosystem-scale changes are thought to be mediated by reductions in leaf stomatal conductance in response to increased water vapor-deficit gradients. A decreased stomatal conductance affects photosynthesis and is translated into changes in the isotope ratios of leaf carbohydrates that are formed and then translocated to the roots where the carbohydrates are subsequently respired. Application of the progress reaction model can help resolve time lag and mixed carbohydrate pools that contribute to the observed changes in the carbon isotope ratio of ecosystem-scale respiration.

Finally, this method is useful in the planning of sample intervals: the simple geometric progression 1, 2, 4, 8, 16, 32, 64, 128, and 256 is too sparse to adequately describe isotope turnover if more than one isotope pool is present. We recommend a sample frequency that increases as $(2)^{1/2}$, this captures more of the detail needed to determine multiple half-lives. The reaction progress model is particularly useful in planning experiments; models can be run to see what sample intervals are important. For example, a system with a significant transit time should include a sampling protocol that will capture both the transit time and the initial isotope turnover that is measured after the transit time interval. A simple geometric sampling protocol could under sample the turnover portion of the pool (*e.g.*, in Figure 11.3, single samples at 1, 2, 4, 8, 16, 32, 64, and 128 h would under sample the turnover portion of the experiment).

V. CONCLUSIONS

The reaction progress model is better suited than exponential fit models for deriving rate constants from stable isotope turnover experiments. This makes it possible to determine multiple turnover pools and identify transit times through the ecological system being studied.

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