Chapter 9

NITROGEN

Contents

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9.1 Introduction

Nitrogen is a trace phase in rocks and the major component of air. Estimates for the distribution of nitrogen between the major reservoirs – rocks, air, terrestrial – vary wildly, with some compilations suggesting that 98% of nitrogen is hosted by rocks (Hübner, 1986) to about ½ in rocks, with the remainder in the atmosphere (McDonough and Sun, 1995)[1]. Regardless of this discrepancy, there is roughly equal amounts of organic matter-hosted nitrogen in plants, soil, and the ocean (Hübner, 1986). Although minor in abundance, organic nitrogen is of tremendous importance, because almost all nitrogen isotope fractionation occurs by metabolic or metabolically-related processes. Over the eons, this has led to a range of nitrogen isotope compositions that span well over 100‰. Even in the mantle, the substantial range of $\delta^{15}N$ values has been attributed by some to subduction of surficial material (Beaumont and Robert, 1999; Marty and Dauphas, 2003).

The two stable isotopes of nitrogen are ^{14}N and ^{15}N, with a $^{14}N/^{15}N$ ratio in air of 272. Because the ratio is constant, air nitrogen is taken as our standard given by[2]

$$\delta^{15}N \text{ (‰ vs AIR)} = \left(\frac{\left(^{15}N/^{14}N\right)_{sample}}{\left(^{15}N/^{14}N\right)_{AIR}} - 1 \right) 1000 \qquad 9.1.$$

A reference gas of N_2 from air is easily made by removing CO_2 and water from air cryogenically, and removing O_2 by reaction with copper oxide. The remaining gas will be N_2 with a trace of Ar. Solid reference samples are also available from NIST and the IAEA (Appendix 1).

Nitrogen is a trace element in rocks, and because nitrogen isotope ratios have traditionally been some of the most difficult to measure, nitrogen isotope geochemistry has not been thoroughly embraced by the geochemical community. Analytically, nitrogen is difficult to transfer in vacuum lines, because it cannot simply be frozen with liquid nitrogen. Instead, it needs to be *adsorbed* on zeolite-filled cold fingers. Also, at low nitrogen levels, even small leaks will compromise an analysis. Contamination with CO will have a drastic effect on measured $\delta^{15}N$ ratios due to the interference at mass 29 ($^{13}C^{16}O$).

Many analytical problems have been eliminated with the coupling of the elemental analyzer and mass spectrometer, which allows for combustion and analysis of N-bearing compounds to be made in continuous flow mode (Bebout et al., 2007, see section 2.8.3). Nitrogen analyses of many solids can now be made rapidly and with little

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[1] The very high estimate for nitrogen in the mantle is not in agreement with contemporary models for how the volatile elements were delivered to a newly-forming Earth.

[2] In the agricultural literature, the $\delta^{15}N$ value is defined as $\delta^{15}N(\text{AIR ‰}) = \left(\frac{\left(\text{at}\%\,^{15}N\right)_{sample}}{\left(\text{at}\%\,^{15}N\right)_{AIR}} - 1 \right) 1000$, which

is nearly, but not quite, identical to the definition in equation 9.1.

effort (except at low concentrations). This has raised the status of nitrogen as an important isotopic tracer, especially for pollution studies and within the biological community. It must be stressed however, that for nitrogen dissolved in water, sophisticated wet-chemical procedures are generally required to convert the nitrogen-bearing ion ($\left(NH_4^+\right)$, $\left(NO_3^-\right)$, etc.) to a solid form suitable for analysis (Kendall, 1998), although exciting new methods that employ bacterial denitrification have drastically simplified the procedure (Sigman et al., 2001; Coplen et al., 2004).

9.2 The nitrogen cycle

Nitrogen forms a number of oxidation states from +5 $\left(NO_3^-\right)$ to -3 $\left(NH_4^+\right)$. A simplified nitrogen cycle is shown in Fig. 9.1. Diatomic nitrogen is removed from air by microorganisms (particularly *Rhizobium* sp.) living symbiotically in higher plants or lichens. This process is called *nitrogen fixation*; a typical reaction is

$$N_{2\,(g)} + 3\ H_2O_{\,(g)} \xrightarrow{\ nitrogenase\ } 2\ NH_{3\,(g)} + 3/2\ O_{2\,(g)} \qquad\qquad 9.2.$$
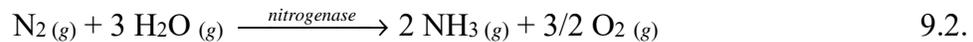
Nitrogen fixation is an energy consuming process. The ammonia or ammonium ion is extremely important in fertilizer, to the point where 5-14 $\times$ 10^{10} kg/yr of nitrogenous fertilizers are produced by industrial nitrogen fixation alone.

Assimilation or *immobilization* are the processes where NH_4^+ or NO_3^- are incorporated in living tissue. The reverse of this is the degradation of organic matter by heterotrophic bacteria and release of NH_4^+ in a process called *mineralization* (also called *ammonification*).

Nitrification is the oxidation of ammonia to NO_3^- by nitrifying organisms (*e.g.*,
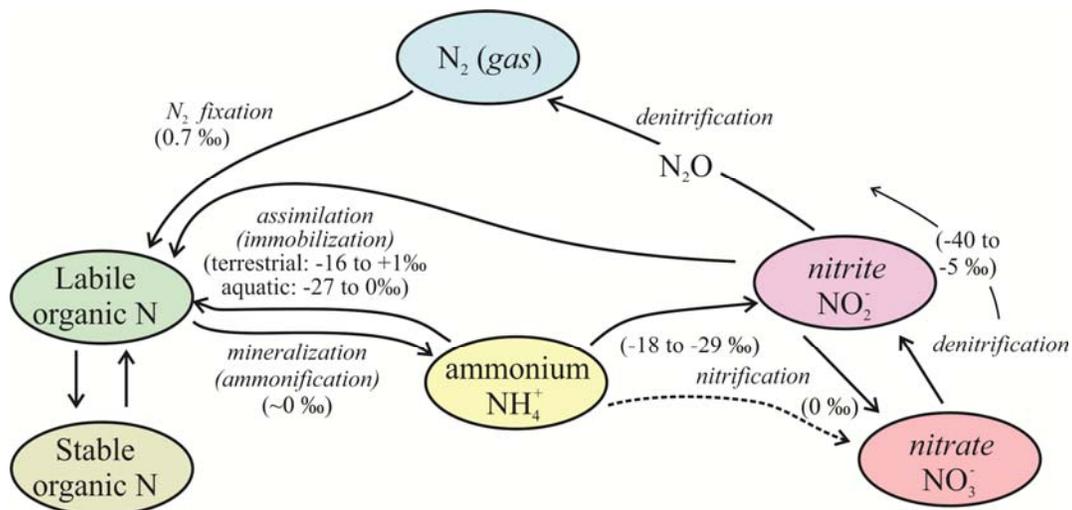

Fig. 9.1. Simplified diagram of the nitrogen cycle. Note that nitrification is generally thought of as the conversion of ammonium to nitrate, but that nitrite is an intermediate step. Likewise, denitrification is the conversion of nitrate to N_2 and/or N_2O gas, but that nitrite is an intermediate phase. Numbers in parentheses indicate average fractionations ($\delta^{15}N_{product}$ - $\delta^{15}N_{source}$) associated with each process.

chemotrophic bacteria). Nitrification is the two-step process given by a first oxidation, such as *Nitrosomonas*, $NH_4^+ \rightarrow NO_2^-$ and a second step *Nitrobacter*, $NO_2^- \rightarrow NO_3^-$. Oxygen comes from both H_2O and O_2 for this nitrification process. Nitrification is an energy-releasing process and is used by organisms as an energy source. A typical reaction sequence is (Kaplan, 1983)

$$NH_3 + 3/2\ O_2 \rightarrow HNO_2 + H_2O \qquad\qquad 9.3a$$
$$KNO_2 + \tfrac{1}{2}\ O_2 \rightarrow KNO_3 \qquad\qquad 9.3b.$$

Denitrification is the process whereby NO_3^- and NO_2^- are converted to N_2O and ultimately N_2 gas by anaerobic bacteria, some fungi and aerobic bacteria. Denitrification accompanies degradation of organic matter, *e.g.* glucose,

$$5\ C_6H_{12}O_6 + 24\ NO_3^- + 24\ H^+ \rightarrow 30\ CO_2 + 42\ H_2O + 12\ N_2(g) \qquad 9.4.$$

Denitrification tends to occur in deeper layers of soil or in poorly aerated soils where $p(O_2)$ is low. In the ocean, denitrification is most active in stagnant water masses and where $p(O_2)$ is low. Correspondingly, denitrification increases with depth in the ocean. Atmospheric N_2 would be exhausted in 100 million years, if it were not for denitrification processes.

9.3 Nitrogen isotope fractionation

The nitrogen isotope fractionations attending the various processes shown in Fig. 9.1 are difficult to quantify because most of the transformations are metabolically driven and therefore kinetically controlled. They are not equilibrium reactions. As we have seen for biologically-mediated carbon reduction and will see in Chapter 10 for sulfate reduction, the magnitude (and even sign) of fractionation can be highly variable, depending upon the availability of nutrients and reaction rates. For example, nitrification, given by the multi-step transformation *organic nitrogen* $\rightarrow NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$ may have different isotopic fractionations associated with each step, and within each step the fractionations can be variable, depending on ambient conditions. The $\delta^{15}N$ value of the product nitrate will be anywhere from -12 to -29‰ lighter than the ammonium from which it forms (Kendall, 1998).

Nitrogen isotope fractionation occurs during the transformation from the reactant to the product. The most significant fractionation effects in the low temperature nitrogen system are going to be kinetic. Following the idea for equilibrium fractionation, we can use the α terminology, recognizing that the fractionations are not equilibrium and do not follow basic thermodynamic rules. In this form, $\alpha_{p\text{-}s} = R_p/R_s$, where $R = {}^{15}N/{}^{14}N$ and p and s are the products and the substrate source of nitrogen (e.g., Kendall, 1998). Equilibrium fractionation in stable isotope geochemistry is often reported as $1000ln\alpha$, where $1000ln\alpha_{a\text{-}b}$ is very similar to $\delta_a - \delta_b$ (section 2.6). Because nitrogen isotope fractionations in nature are generally kinetically controlled, it is common to see fractionations reported using the '*enrichment factor*' notation ε, given by

$$\varepsilon = 1000\,(\alpha\text{-}1) \qquad\qquad\qquad 9.5a.$$

The use of an ε signifies that there is no suggestion of a reversible equilibrium process. Regardless of the subtleties that these different equations might convey, in practice they are the same. The ε and Δ values are almost identical (Kendall, 1998). The enrichment factor is also sometimes given by

$$\varepsilon = \left(\frac{\delta_p - \delta_r}{\delta_r + 1000} \right) 1000 \qquad\qquad 9.5b,$$

where δ_p and δ_r are the delta values of the product and reactant, respectively. These two definitions of ε are not identical, but very close, except when δ_s values are very large (*e.g.*, in ^{15}N-enriched tracer experiments). ε is also nearly identical to δ_p - δ_s or Δ^{15}N$_{\text{product – reactant}}$. To be consistent with the rest of this book, we will use the simple difference between the δ values of the products and reactants, in which Δ^{15}N $\approx \varepsilon$.

9.3.1 Nitrogen fixation

Nitrogen fixation is generally considered as a single process in terms of isotopic fractionation, because δ^{15}N values are measured on the product plant or bacterium, regardless of the pathway from N$_2$ (g) to organic matter. Nitrogen isotope fractionation associated with fixation is generally small. Hoering and Ford (1960) measured fractionations (δ^{15}N$_{\text{fixed}}$ – δ^{15}N$_{\text{Air}}$) ranging from +3.7 to -2.2‰ (n = 4) and considered the average fractionation between atmospheric N$_2$ and fixed nitrogen in organic matter to be near 0‰. A compilation by Fogel and Cifuentes (1993) ranges form -3 to +1‰; one by Hübner (1986) gives an average value of -0.7 ± 1.6‰ (Fig. 9.2). The scatter does not indicate some sort of analytical error or uncertainty, but rather that real variations in fractionation for this and all other pathways exist.
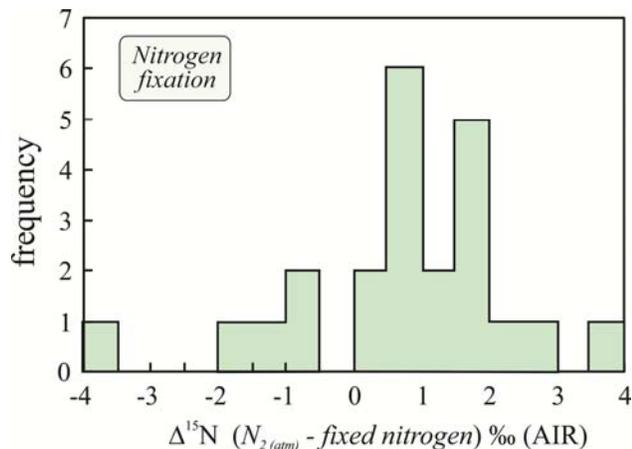

Fig. 9.2. Fractionation associated with N$_2$ fixation. The average value is 0.72‰ (meaning that the organisms have δ^{15}N values less than 0‰).

9.3.2 Mineralization

The fractionation associated with the breakdown of organic matter to soil ammonium is small (Δ = 0±1‰). As pointed out by Kendall (1998), mineralization is defined by some researchers as the breakdown of organic matter and conversion to nitrate. Under such circumstances, fractionations can be large and variable, but the differences are not due to the mineralization step itself, rather the nitrification of ammonium to nitrate.

9.3.3 Assimilation

Assimilation by microorganisms causes a strong and variable discrimination, favoring ^{14}N (Fig. 9.3). There is no appreciable difference between assimilation of NH_4^+, NO_2^-, and NO_3^-. Higher plants show much smaller fractionations, averaging only -0.25‰ ($\delta^{15}N_{plant} - \delta^{15}N_{assimilant}$). A compilation of data for ammonium assimilation by aquatic algae spans a very large range between -27 to 0‰ (Fogel and Cifuentes, 1993). The wide range of delta values can be modeled in terms of kinetic processes where rates are controlled by the availability of nitrogen, enzymes responsible for NH_3 fixation, and diffusion of NH_3 through the cell walls. Velinsky *et al*. (1991) found that ammonium assimilation in anoxic waters was strongly dependent on NH_4^+ concentrations. In waters with NH_4^+ concentrations of 40μM, the fractionation between particulate organic matter and NH_4^+ was modeled to be -20 to -30‰. In waters with concentrations of only 9μM, fractionations were -5 to -15‰.

9.3.4 Nitrification

Nitrification is a two step process from NH_4^+ through NO_2^- and finally NO_3^- (Equation 9.3). The second part of the reaction (Equation 9.3b $NO_2^- \rightarrow NO_3^-$) is quantitative, meaning all nitrite is converted to nitrate, and so there can be no nitrogen
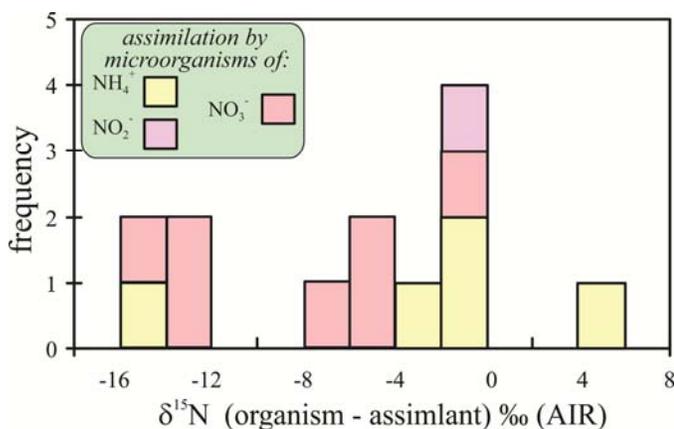

Fig. 9.3. Nitrogen isotope fractionation during assimilation by microorganisms. On the basis of limited data, the fractionation is similar regardless of whether ammonium, nitrate, or nitrite is the assimilant. Data from (Hübner, 1986).
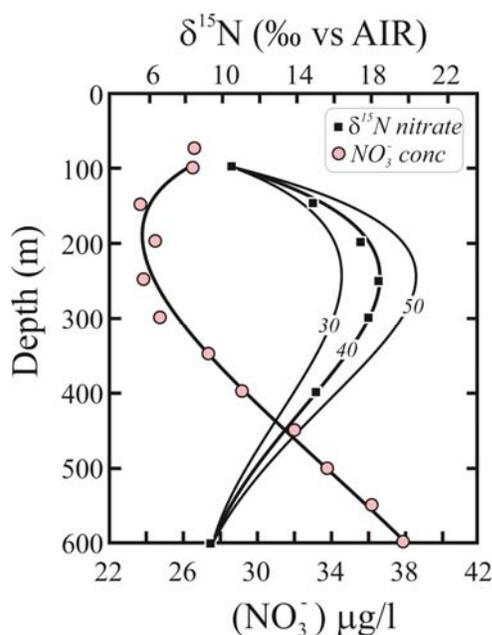

Fig. 9.4. Depth profile and dissolved nitrate concentration and $\delta^{15}N$ value in a depth profile from the eastern tropical North Pacific Ocean. As nitrate is converted to N_2, the nitrate concentration decreases and its $\delta^{15}N$ value increases, because the $\delta^{15}N$ value of the evolved N_2 gas is ~40‰ lighter. The data are modeled assuming $\Delta^{15}N_{NO_3\text{-}N_2}$ of 30, 40 and 50‰ as shown by curves through isotope data. After Cline and Kaplan (1975).

isotope fractionation associated with this step. Published estimates for the fractionation of ammonium to nitrite (Equation 9.3a) range from -18 to -29‰, with the nitrite (and ultimately nitrate) having lower $\delta^{15}N$ values than the ammonium precursor. The fractionation depends on the proportions of ammonium and nitrate after reaction. Obviously, if all ammonium is converted to nitrate in a 'closed system', then the $\delta^{15}N$ value of the nitrate will be identical to that of the original ammonium reservoir.

9.3.5 Denitrification

Laboratory experiments give a $\Delta^{15}N$ $_{N_2 \text{ gas} - \text{dissolved nitrate}}$ value of -17 to -20‰. Measured fractionations from soil samples are often less, between -12 to -14‰ (e.g., Blackmer and Bremner, 1977). Mariotti *et al.* (1982) found the fractionation $\Delta^{15}N$ $_{N2O - NO2^-}$ to range from -33‰ to -11‰. Cline and Kaplan (1975) measured the concentration and $\delta^{15}N$ values of dissolved nitrate in a water column from the eastern tropical North Pacific Ocean. They were able to model the variations in $\delta^{15}N$ values in terms of diffusion theory if the $\Delta^{15}N$ $_{N2}$ $_{(\text{gas} - \text{dissolved nitrate})}$ value is -40‰ (Fig. 9.4).

Denitrification has large isotope fractionation effects due to the 'distillation' of N_2 gas. In shallow aquifers, N_2 gas produced by denitrification can be lost by diffusion to the atmosphere. This is a classic Rayleigh fractionation process with a large coefficient of fractionation. If a large amount of N_2 is produced, the nitrogen isotope composition of the remaining nitrate can change significantly. Figure 9.5 illustrates the magnitude of this effect. It also shows how we can use nitrogen isotopes to evaluate the amount of nitrate that has been removed from a system. In a contaminated aquifer, this obviously is an important tool for water quality studies.

Fig. 9.5. Variations in the $\delta^{15}N$ values of dissolved nitrate and N_2 gas from water wells from the Kalahari desert. The data can be modeled by Rayleigh fractionation process, with a $\Delta^{15}N$ value between N_2 gas (product) and dissolved nitrate (reactant) of -35‰. When only a small amount of nitrate remains, its $\delta^{15}N$ value gets extremely heavy, while the sum of the N_2 approaches the original nitrate value. Solid lines are instantaneous nitrate value; dashed lines are *total* N_2 value for a given proportion of N_2 produced. After Heaton (1986).

9.4 The characteristic $\delta^{15}N$ value of various materials

Now that we have the chemistry and fractionation factors for a number of chemical transformations involving nitrogen, it should be a relatively straightforward task to make sense of the variability and range of $\delta^{15}N$ values of the different reservoirs. For example, from Figure 9.1, it is clear that the fractionation between N_2 gas and labile
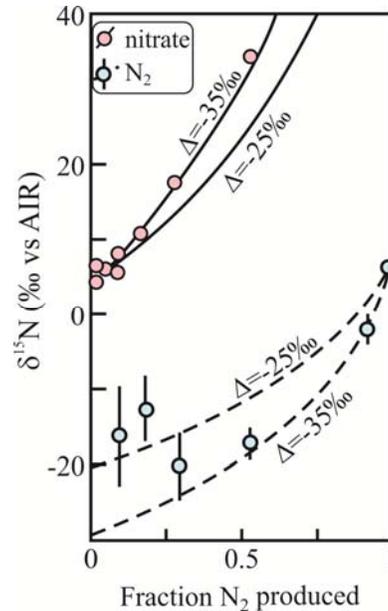
organic nitrogen is close to zero. So we should expect that nitrogen-fixing plants[3] have $\delta^{15}N$ values close to zero, and indeed, this is the case. Especially when growing in nitrate-poor soil (so that the only source of nitrogen is N_2), nitrogen fixing plants have $\delta^{15}N$ values that are within 2‰ of air (Shearer and Kohl, 1986). As shown below, we can use similar logic to explain the $\delta^{15}N$ values of a number of different reservoirs.

9.4.1 Plants and soil

Nitrogen-fixing plants have $\delta^{15}N$ values close to zero. Other plants cannot fix N_2 and instead incorporate nitrogen by assimilation of NH_4^+ or NO_3^- from soil. The $\delta^{15}N$ values of plants are strongly dependent on those of the soil, which are in part controlled by the plants. In order to predict the $\delta^{15}N$ value of plants it is necessary to know the range of $\delta^{15}N$ values of soil *and* the mechanisms of uptake from the soil. $\delta^{15}N$ values of soil range from -10 to +15‰, with most soils between +2 and +5‰ (Kendall, 1998). The positive values are loosely tied to a preferential loss of ^{14}N during decomposition of particulate nitrogen sources. Unfortunately, specific factors controlling soil $\delta^{15}N$ values are complex and defy quantification. Even the source of extracted nitrogen are variable: tree roots preferentially assimilate soil nitrate while microorganisms tend to incorporate soil ammonium (Nadelhoffer and Fry, 1988). Nevertheless, some general guidelines can be established.

- Denitrification is most intense in poorly drained or poorly oxidized soils, as nitrate-consuming organisms become active only when oxygen levels are low. The subsequent loss of N_2 gas – the product of denitrification – increases the $\delta^{15}N$ value of any remaining nitrate (Fig. 9.5).
- Soils with abundant leaf litter tend to have lower $\delta^{15}N$ values than in surrounding regions with less foliage. An explanation for this trend is that the preferential uptake of ^{14}N by plants results in higher $\delta^{15}N$ values of the soil. In heavily vegetated areas, the ^{15}N-depleted plant material is returned to the soil as leaf litter.
- Anthropogenic activity can strongly affect the $\delta^{15}N$ of soil. In one study, the average $\delta^{15}N$ value of cultivated soils are 5.0±3.5‰ compared to 6.8±6.4‰ for uncultivated soils, due to addition of nitrogenous fertilizers with low $\delta^{15}N$ values (Hübner, 1986). In some soils, there are variations with depth, while in others, no such correlation is found. Factors include drainage, total N-content of soil and precipitation rate.

In nitrogen-limited soils, the $\delta^{15}N$ value of plants is close to that of the soils, as no discrimination is possible. In nutrient-rich soils, the fractionation between plants and dissolved inorganic nitrogen can be several per mil. Trees tend to have slightly lower $\delta^{15}N$ values than soil due to the negative fractionation during assimilation. Heterotrophic fungi, on the other hand, may have $\delta^{15}N$ values higher than those of the soil (Högberg, 1997). Overall, tree leaves have a range of $\delta^{15}N$ values of -8 to +3‰ (Peterson and Fry, 1987). There is a strong global-scale variation in the $\delta^{15}N$ values of soils that is related to the mean annual precipitation and mean annual temperature (Fig. 2 in Amundson et al.,

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[3] Nitrogen fixing plants are those that are able to assimilate N_2 gas directly. In fact, nitrogen fixation is a symbiotic relationship with bacteria that live on the roots, but the isotopic effect is the same, nevertheless.

2003), although the trends cannot be explained in terms of only a few simple processes.

9.4.2 Other terrestrial reservoirs

- *Fertilizers*

    Fertilizers generally have a $\delta^{15}N$ range of -4 to +4‰, the low values related to an atmospheric N_2 source. Organic fertilizers range from +6 to +30‰, related to the processes occurring in animal wastes (Kendall, 1998). The $\delta^{15}N$ value of animals increases by ~3‰ at each higher trophic level (see section 9.5). The most important factor for this increase is the excretion of isotopically light urine. Therefore, there is an enrichment in ^{15}N from plants to animals. Animal waste gets further enriched in ^{15}N by subsequent volatilization of isotopically light ammonia.

- *Rain*

    The sources of nitrogen in rain are volatilization of ammonia, nitrification and denitrification of soils and anthropogenic sources. Hoering first measured the $\delta^{15}N$ values of NH_4^+ and NO_3^- in rain from the roof of the chemistry laboratory at the University of Arkansas, USA (Hoering, 1957). He found that, although there was significant variability in the $\delta^{15}N$ value of each component ($\delta^{15}N$ NH_4^+ = -0.1 to 9.0‰; $\delta^{15}N$ NO_3^- = -7.2 to +3.4), the fractionation between the two phases could be explained in terms of a kinetic fractionation between ammonium and nitrate. The range of $\delta^{15}N$ values of nitrate in rain has since been found to cover a range of ~ -10 to +9‰. Heaton (1986) gives an average of -5‰ (for South Africa) while a compilation by Kendall (1998) ranges from ~-3 to +9‰, with a strong mode at 0 to +2‰. In general, ammonium is lighter than nitrate by several per mil. The measured fractionation between NH_4^+ and NO_3^- in rain is dependent on the concentrations of the ions in precipitation. Variations from site to site are huge, because inputs can be so different. Pure air has $\delta^{15}N$ values of NH_3 and NO_2 of -10.0 ± 2.6‰ and -9.3 ± 3.5‰, respectively (Hübner, 1986). The effects of mixing different reservoirs are clear when just a few 'endmember' sources are considered. Barnyard-derived NH_3 has a $\delta^{15}N$ value of +25‰, NO_x from automobile exhaust is ~ +3.7‰, and fumaroles from southern Kamchatka have $\delta^{15}N$ values of $(NH_4)_2SO_4$ as low as -31‰ (Hübner, 1986). Freyer (1978) measured $\delta^{15}N$ values of NH_4^+ in rain water of -12.0 ± 1.9‰ from Jülich Germany. Published values of NH_4^+ in rain water range from -9.7 to +6.9. Not surprisingly, variations can be large between storms and even within individual storms, because the sources of nitrogen – fossil-fuel burning, ocean denitrification, etc. – themselves have a large range of $\delta^{15}N$ values. Peterson and Fry's average estimate (1987) for precipitation are -18 to +8‰ for NH_4^+ and -15 to +3‰ for NO_3^-.

- *Fossil fuels*

    Peat and coal average +0.8‰ with a standard deviation of 1.6‰, ranging up to 6.3‰ (Hoering, 1955; Wada et al., 1975; Stiehl and Lehmann, 1980), similar to modern peats and bogs. Crude oils are generally in the range of +1.0 to +6.7‰, while natural gas

has far more variation (-10.5 to 14.4‰) (Hoering and Moore, 1958). The $\delta^{15}N$ value of natural gas changes quite drastically with the distance of migration from its source. An example from north Germany shows a systematic increase from -8.7 to +18.0‰ as migration distances increase, likely due to Rayleigh fractionation attending denitrification (Stahl, 1977).

9.4.3 Nitrogen in the oceans

The fact that the $\delta^{15}N$ values of most ocean materials are positive is easily explained in terms of the nitrogen cycle in the ocean. Nitrogen is one of the most important limiting nutrients in the ocean, so that productivity is limited by availability of metabolically available nitrogen[4]. The major inputs are river runoff, rain and fixation of molecular N_2 by marine blue-green algae (Fig. 9.6). Outputs or sinks of nitrogen in the ocean include burial in sediment and denitrification. We assume that the nitrogen cycle is balanced, with inputs equaling outputs, but how variable this is over time is not known. The fluxes of each exchange path are shown in Fig. 9.6. It is clear that the average values are not known well enough to quantify. Even more intractable is an attempt to quantify isotopic mass balance because the $\delta^{15}N$ values of each source are quite variable, as are the fractionations accompanying transfer from one reservoir to another.
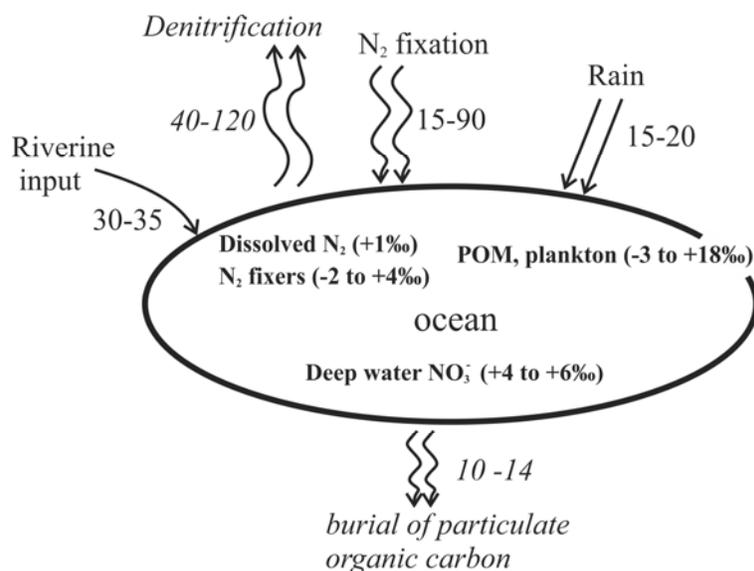

Fig. 9.6. Nitrogen system for the oceans. Sources and sinks (and their fluxes in 10^{12} gm-N_2/yr) are shown as normal and italicized text, respectively. $\delta^{15}N$ values of ocean materials are in bold text. Data from following sources: (Kaplan, 1983; Macko et al., 1984; Berner and Berner, 1987; Peterson and Fry, 1987; Fogel and Cifuentes, 1993).

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[4] Nitrogen and phosphorus are strongly correlated with a nitrogen/phosphate ratio of ~15. Both nutrients become exhausted at the same time. The constant ratio is probably tied to a biochemical feedback mechanism. If dissolved nitrate levels become low, nitrogen-fixing blue green algae will produce nitrate, restoring the biochemical ratio. If nitrate values become high, non-nitrifying organisms would have an advantage, consuming nitrate disproportionately, again driving the ratio back to its balanced state.
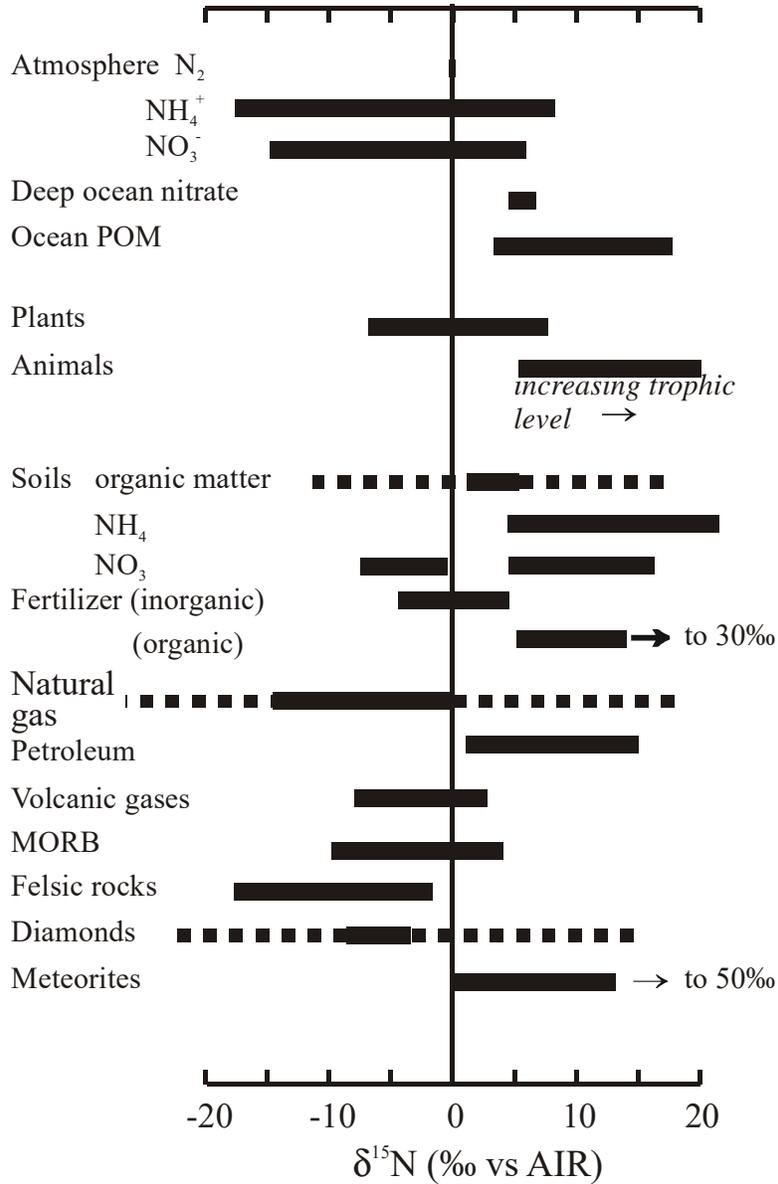
Fig. 9.7. Average $\delta^{15}N$ values for common reservoirs.

In spite of the uncertainties, several gross features are apparent. The major fractionation in the global ocean cycle occurs during denitrification, with N_2 being strongly depleted in ^{15}N relative to its source. All other sources and sinks are associated with rather small fractionation effects (see Fig. 9.1). Clearly loss of light N_2 back to the atmosphere will result in a positive average $\delta^{15}N$ value of the ocean. The positive $\delta^{15}N$ value of organic material in sediments is retained during subduction, seen both in rocks (Bebout and Fogel, 1992) and volcanic fumaroles sourced in oceanic sediments (Fischer et al., 2002).

There is significant spatial variation in the $\delta^{15}N$ value of dissolved nitrate. In the Eastern North Pacific Ocean, $\delta^{15}N$ values range from +6.5‰ in the Antarctic

intermediate water mass (at depth), up to +18.8‰ in the active denitrification zone (Cline and Kaplan, 1975).

Overall, average $\delta^{15}N$ values of various reservoirs are shown in Fig. 9.7. These data are a compilation from many sources. There are always unusual samples that have higher or lower values (Coplen et al., 2002), but the figure should serve as a guide for average ranges that are commonly found for each material.

9.5 Nitrogen isotope ratios in animals

The $\delta^{15}N$ values of animals are related to their diet (DeNiro and Epstein, 1981). The $\delta^{15}N$ value of an animal is generally heavier than the food it eats, and the $\delta^{15}N$ values increase by 3-4‰ for each successive trophic level[5]. Stable nitrogen isotope ratios are therefore an important ecological tool for quantifying trophic position and for reconstructing dietary preferences.

Not all tissues in a body have the same $\delta^{15}N$ value. Milk, blood and muscle tend to have $\delta^{15}N$ values 1-3‰ heavier than the diet, while urinary urea and bile have $\delta^{15}N$ values that are 2 to 4‰ more negative than the diet (Ambrose, 1991). The loss of ^{15}N-depleted urine is probably the primary cause for the elevated overall $\delta^{15}N$ value of animals relative to their diet, although it has been shown that there can be preferential uptake of ^{15}N relative to diet. At each successive trophic level, the $\delta^{15}N$ value of the food source increases, hence the animals' $\delta^{15}N$ values follow suit. The effect is most regular and intense for marine
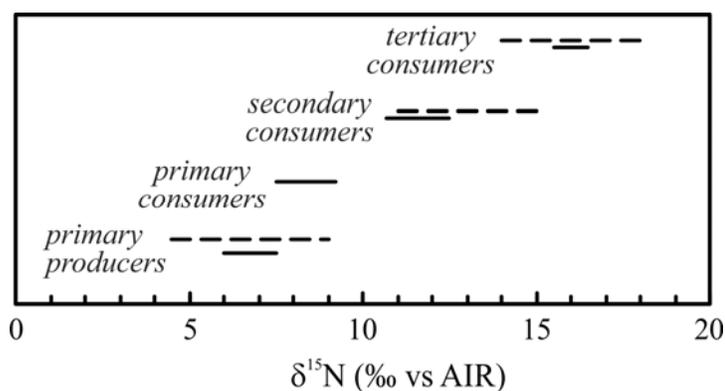

Fig 9.8. Nitrogen isotope compositions of marine plants and animals as a function of trophic level. There is a regular increase of approximately 3‰ per trophic level. Data from (Minagawa and Wada, 1984 (solid); Schoeninger and DeNiro, 1984 (dashed)).

communities. As seen in Figure 9.8, there is a regular increase of ~3‰ per trophic level. The effect on terrestrial communities is not as large and is more variable, controlled by numerous factors. For example, several authors have found that there is a correlation in the $\delta^{15}N$ value of animals and the relative annual rainfall (Sealy et al., 1987). It appears that animals that are more water-stressed excrete a concentrated urine with higher $\delta^{15}N$ values.

The physiology of an organism has a large effect on its $\delta^{15}N$ value, especially the biochemical form of nitrogenous waste (Vanderklift and Ponsard, 2003). The fractionation between an organism and excreted urea is larger than the fractionation between an organism of excreted ammonia. Therefore, it is reasonable to assume, and

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[5] A group of organisms that occupy the same position in a food chain. Each successive trophic level consumes the one below it. Hence: trophic level 1- autotrophs (*e.g.*, plants); trophic level 2 – herbivores; trophic level 3 – carnivores, etc.
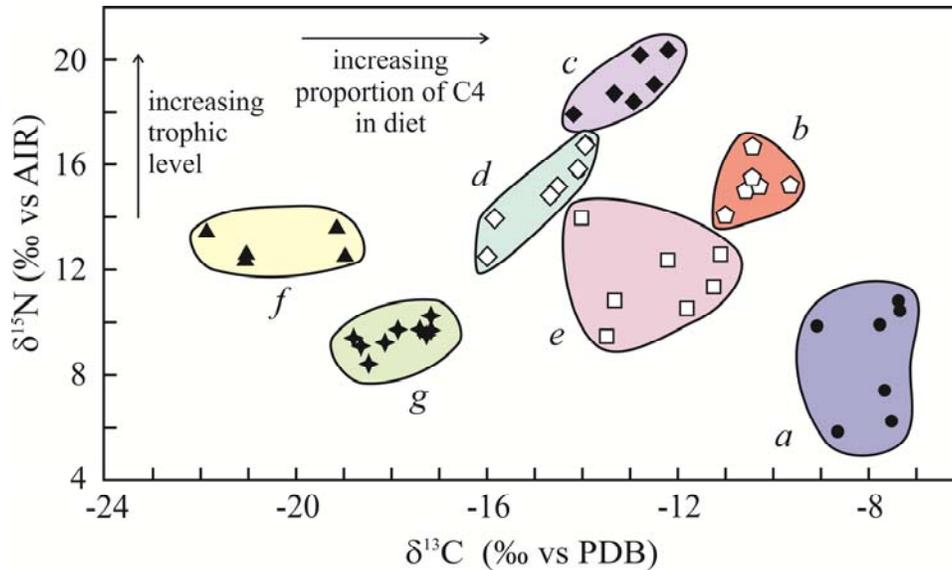
Fig. 9.9. $\delta^{13}C$ and $\delta^{15}N$ values of bone collagen from Native American communities.
- *a)* Western Anasazi, S. Utah (A.D. 1 - 1300)
- *b)* Nantucket Island, Mass. (A.D. 1000 - 1600)
- *c)* N.W. coast Haida and Tlingit salmon fishers (historic)
- *d)* S. California coastal (A.D. 1400 - 1800)
- *e)* Plains Arikara, South Dakota (A.D. 1650 - 1733)
- *f)* S. Ontario, Middle Woodland period (A.D. 1 - 400)
- *g)* Modern Chicago, USA.

See text for details. Compilation and references from Martin (1999) and Schoeller *et al.* (1986).

supported by measurements of natural samples, that urea-excreting organisms have a larger trophic level effect than those which primarily excrete ammonia.

The combination of $\delta^{15}N$ and $\delta^{13}C$ values have been used in well over 100 publications to investigate trophic ecology of birds and mammals (Kelly, 2000). Combined $\delta^{15}N$-$\delta^{13}C$ values from prehistoric bone collagen are a valuable tool for distinguishing different populations and constraining a communities' diet. To a first degree, we can state that the $\delta^{15}N$ values are controlled by the trophic level of diet and the $\delta^{13}C$ values are controlled by the relative dietary proportions of C_3 and C_4 plants. Communities subsisting mainly on an animal diet will inherit the $\delta^{13}C$ value of their prey, perhaps with a subtle offset towards higher values (DeNiro and Epstein, 1978).

Figure 9.9 shows a compilation for a number of North American Native American communities. The combined carbon and nitrogen isotope values are easily explained in terms of assumed diet, and place constraints on diet in cases where ambiguities exist. For example, the Western Anasazi have the lowest $\delta^{15}N$ values and highest $\delta^{13}C$ values. It can be concluded that they had a maize-based diet[6] with only minor animal consumption (Martin, 1999). The Southern Ontario communities (Schwarcz et al., 1985) had a diet consisting of C_3 plants and animals that consumed C_3 plants. The elevated $\delta^{15}N$ values relative to the Anasazi community indicate at least a partial animal diet. The highest $\delta^{15}N$

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[6] Maize is a C_4 plant. See Chapter 7 for a more thorough discussion of C_3-C_4 plants.

values are found in the Northwest coastal communities that consisted in large part on salmon (Schoeninger and Moore, 1992). Salmon are at a high trophic level, and this is reflected in the isotope data of the salmon-eating community.

The effect of trophic level on the $\delta^{15}N$ values of animals has been used for a large number of studies addressing different questions. The breadth of this field is illustrated in a study by Fogel *et al.* (1997) where the recognition that a nursing infant is technically at a higher trophic level than her mother was used to determine the duration of nursing by
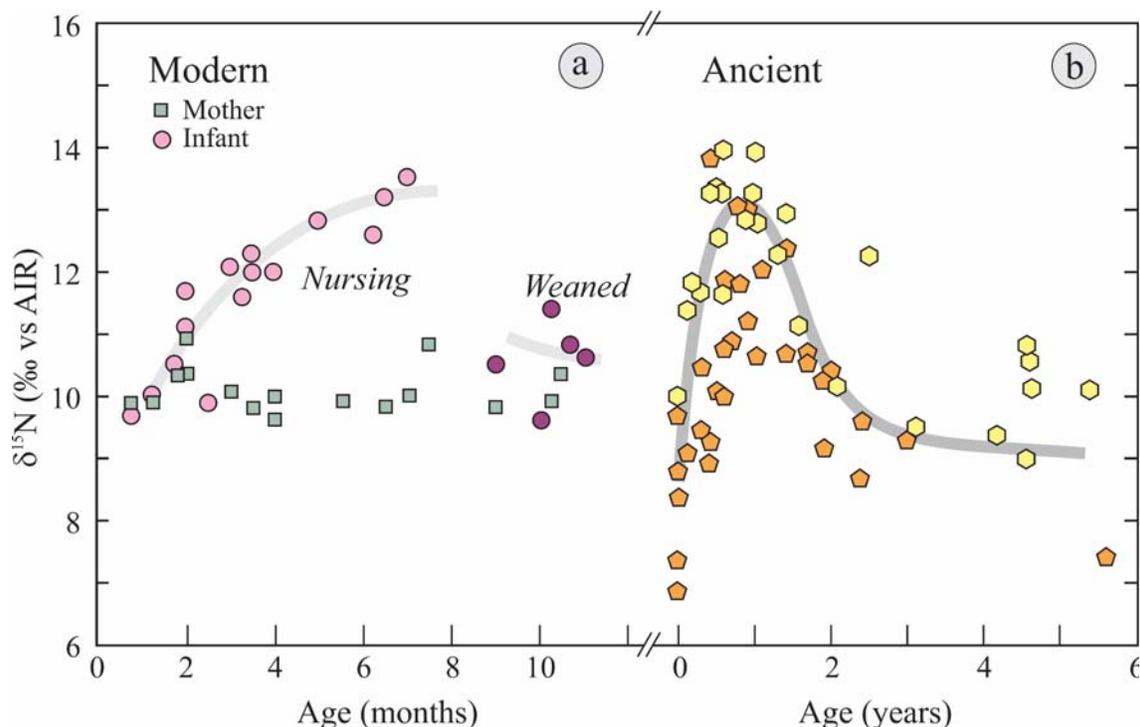

Fig. 9.10. a: $\delta^{15}N$ value of nursing mother and infant. Infant reaches a 3‰ higher $\delta^{15}N$ value, which disappears after weaning. **b:** Native American infants from pre- and post-historic sites, USA. Note different x-axis. After Fogel *et al.* (1997).

prehistoric people. Fig. 9.10a shows the $\delta^{15}N$ values of the fingernails of a mother and her infant from birth through weaning. The $\delta^{15}N$ value of the infant rises after birth to a value ~3‰ higher than the mother. Once the child is weaned, the diet of the two individuals is more-or-less the same, and the higher $\delta^{15}N$ value of the infant is soon lost. This concept was then used for two ancient populations, where the $\delta^{15}N$ values of individuals were measured as a function of age (Fig. 9.10b). In both populations, weaning occurs between one and two years.

9.5.1 Compound specific studies

The isotopic variations seen in individual amino acids are far larger and provide significantly more information than data from bulk samples alone. For example, some amino acids, such as glutamic acid, show an 8‰ increase in $\delta^{15}N$ value with each higher trophic level. In contrast, phenylalanine shown only a 0.4‰ increase with each trophic level (Chikaraishi et al., 2014). This is because the transamination/deamination processes

of glutamic acid always result in cleaving of the carbon-nitrogen bonds, whereas phenylalanine is converted to tyrosine without cleaving of these bonds. The result is that glutamic acid records the increase in $\delta^{15}N$ values of the host (fractionation), whereas, the $\delta^{15}N$ value of phenylalanine cannot change as it moves up into higher trophic levels. The effect is clearly seen in Fig. 9.11. Two food webs were studied: one marine and the other terrestrial. The marine food web has a constant $\delta^{15}N$ value for phenylalanine and a regular increase in the $\delta^{15}N$ of glutamic acid with increasing trophic level. The terrestrial foodweb has a 1-1 correlation between phenylalanine and glutamic acid for each trophic level and a distinct jump to higher glutamic acid values as trophic levels increase. The marine samples suggest a linear food chain with each successive trophic level having essentially identical $\delta^{15}N_{phenylalanine}$ values, whereas the large range of $\delta^{15}N_{phenylalanine}$ values in the terrestrial foodweb suggest that individual species within a given trophic level exploit specific and different food resources (Chikaraishi et al., 2014). The overall $\delta^{15}N$ values of an organism are the sum of all nitrogen-bearing compounds, whereas different amino acids single out processes that are unique to their different chemical behavior during metabolism.
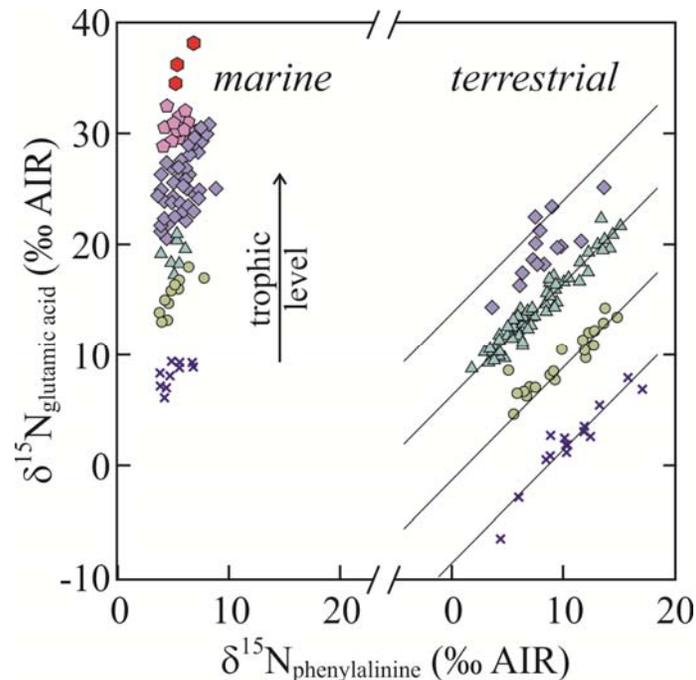

Fig. 9.11. Crossplot of the $\delta^{15}N$ values of isolated amino acids phenylalanine and glutamic acid from a marine (left) and terrestrial (right) food web. The $\delta^{15}N$ values of each food web show an ~8‰ jump with trophic level (from crosses to circles to triangles, squares, etc.). The marine food web has a constant phenylalanine value suggesting a linear and common food chain, whereas individuals in the terrestrial food web have distinct phenylalanine values indicative of distinct food sources at each successive trophic level. After Chikaraishi *et al.* (2014).

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