Stable C and N Isotopes: A Tool to Interpret Interacting Environmental Stresses on Soil and Plant

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Natural abundances of stable isotopes of nitrogen and carbon ($\delta^{15}N$ and $\delta^{13}C$) are being widely used to study N and C cycle processes in plant and soil systems. Variations in $\delta^{15}N$ of the soil and the plant reflect the potentially variable isotope signature of the external N sources and the isotope fractionation during the N cycle process. N_2 fixation and N fertilizer supply the nitrogen, whose $\delta^{15}N$ is close to 0‰, whereas the compost as an organic input generally provides the nitrogen enriched in ^{15}N compared to the atmospheric N_2. The isotope fractionation during the N cycle process decreases the $\delta^{15}N$ of the substrate and increases the $\delta^{15}N$ of the product. N transformations such as N mineralization, nitrification, denitrification, assimilation, and the NH_3 volatilization have a specific isotope fractionation factor (α) for each N process. Variation in the $\delta^{13}C$ of plants reflects the photosynthetic type of plant, which affects the isotope fractionation during photosynthesis. The $\delta^{13}C$ of C3 plant is significantly lower than, whereas the $\delta^{13}C$ of C4 plant is similar to that of the atmospheric CO_2. Variation in the isotope fractionation of carbon and nitrogen can be observed under different environmental conditions. The effect of environmental factors on the stomatal conductance and the carboxylation rate affects the carbon isotope fractionation during photosynthesis. Changes in the environmental factors such as temperature and salt concentration affect the nitrogen isotope fractionation during the N cycle processes; however, the mechanism of variation in the nitrogen isotope fractionation has not been studied as much as that in the carbon isotope fractionation. Isotope fractionation factors of carbon and nitrogen could be the integrated factors for interpreting the effects of the environmental factors on plants and soils.

Key words: *stable isotope, isotope fractionation, environmental stress*

Numerous studies focused on the effects of environmental conditions on soils and plants have been performed, among which the carbon and nitrogen processes in the ecosystem, especially the soil-plant systems, have been extensively studied. In general, the natural abundance of stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) are used as a reliable tool for studying nitrogen and carbon dynamics in plants and soils and reflects the interaction between isotope composition of source and isotope fractionation. Measurements of $\delta^{15}N$ revealed isotopically different N sources in the Chinese cabbage [Yun *et al.*, 2006] and NO_3^- leached into the groundwater [Choi *et al.*, 2002]. Variation in $\delta^{15}N$ among the soil nitrogen forms,

resulting from the isotope fractionation during the enzymatic processes, provides information on the transformation of N in soil and the soil environment [Choi and Ro, 2003]. Variation in $\delta^{15}N$ of the plant nitrogen reflects the potentially variable $\delta^{15}N$ values of the external N sources and the isotope fractionation, which occurs during assimilation, transport, and loss of N [Evans *et al.*, 1996; Robinson *et al.*, 1998; Yun *et al.*, 2006]. In contrast, $\delta^{13}C$ has been used to screen C3 genotypes for the potential water use efficiency [O'Leary, 1993]. A robust theory [Farquhar *et al.*, 1982] is available with which to interpret the $\delta^{13}C$ variations among the C3 plants in terms of the measurable physical and physiological processes [Robinson *et al.*, 2000]. Plant $\delta^{13}C$ reflects mainly the extent to which the primary CO_2 assimilation is limited by carboxylation and/or CO_2 diffusion in the leaves [Farquhar *et al.*, 1989]. Environmental stresses modify $\delta^{13}C$ largely in predictable ways by breaking the balance between stomatal

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Table 1. Absolute isotope ratios of international standards for carbon and nitrogen

Standard	Ratio	Accepted value ($\times 10^{-6}$)	Source
PDB	$^{13}C/^{12}C$	11237.2 ± 2.9	Craig, 1957
Air nitrogen	$^{15}N/^{14}N$	3676.5 ± 8.1	Junk and Svec, 1958

conductance and carboxylation. Whole-plant $\delta^{13}C$ is dominated by these processes.

General Characteristics of Stable Isotopes

Isotopes are atoms whose nuclei contain the same number of protons but a different number of neutrons and can be divided into two fundamental types, stable and radioactive species. The number of the stable isotopes is about 300, whereas that of the radioactive ones is over 1200. The term stable is relative one, depending on the detection limits of the decay time.

The isotope ratio R (heavy isotope/light isotope) is a very useful variable for reporting certain isotope signature. However, R is generally not used to report natural abundances of the stable isotopes, because the absolute abundances of the heavy isotopes are, in many cases, very low (Table 1). Moreover, the natural variations in the abundance of the stable isotopes are generally quite small. For many stable isotope systems, therefore, it is a general practice to report the difference between the isotope composition of the sample and an accepted standard in terms of "delta" (δ) values, defined by the formula:

$$\delta = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000$$

where the R values refer to the isotope ratios, for example, either $^{13}C/(^{13}C+^{12}C)$ or $^{13}C/^{12}C$ for carbon and $^{15}N/(^{15}N+^{14}N)$ or $^{15}N/^{14}N$ for nitrogen. For each element, the former definition of R is generally used when the subject is the source identification, the latter when the subject is the isotope discrimination. Depending on the element of interest, this formula defines $\delta^{13}C$ or $\delta^{15}N$. Multiplying by1000 converts the δ values to per mil (‰).

Isotopic standards are chosen for the purpose of convenience, and are assigned the value of 0‰ on the δ scale by the definition. An ideal standard would be a homogeneous material available in a large quantity, safe and easy to process, and easily obtainable. The isotope signature of a standard would be similar to those of the samples undergoing investigation. The most common standard for carbon is PDB, represented by a Cretaceous belemnite from the Peedee Formation of South Carolina [Craig, 1957]. The $^{15}N/^{14}N$ ratio of air nitrogen is generally used to calibrate the nitrogen isotope data.

Table 1 summarizes the absolute isotope ratios of the standards used internationally.

Isotope Effect

Variations in the atomic mass of an element can bring about differences in the physicochemical properties, which are referred to as isotope effects. The electronic structure of an element essentially determines its chemical behavior, whereas its physical properties are dependent on the nucleus. Because all isotopes of an element contain the same number and arrangement of electrons, a similarity in the chemical behavior is expected. However, the chemical behavior is not completely the same among isotopes of a given element; certain differences exist in the physicochemical properties due to the mass differences. The replacement of any atom in a molecule by another isotope produces a very small variation in the chemical behavior. For example, the addition of one neutron can decrease the reaction rate considerably. When the element is lighter, such effect of mass difference on the reaction rate is more evident.

Differences in the physicochemical properties of the isotopes arise as a result of the quantum mechanical effects [Hoefs, 1997]. The scheme of the potential energy of a diatomic molecule as a function of the distance between the two atoms is shown in Fig. 1. According to the quantum theory, the energy of a molecule is restricted to certain energy levels. The lowest level is not at the minimum of the energy curve, but has the value of 1/2 hv, where h is Plank's constant and v is the frequency with which the atoms in the molecule vibrate. This value is known as the zero point energy. Thus, even in the ground state at the temperature of absolute zero, the vibrating molecule possesses certain zero point energy of the molecule. The fundamental frequency depends on the mass of the isotopes. Only the vibration motions cause the chemical isotope effects; rotational and translational motions have no effect on the isotope discrimination. Therefore, zero point energy will be different between isotope species in the molecules with the same chemical formula. The molecule of the heavy isotope discrimination will have a lower zero point energy than that of the light isotope. This phenomenon is shown schematically in Fig. 1, where the upper horizontal line (E_L) represents the dissociation energy of the light molecule and the lower
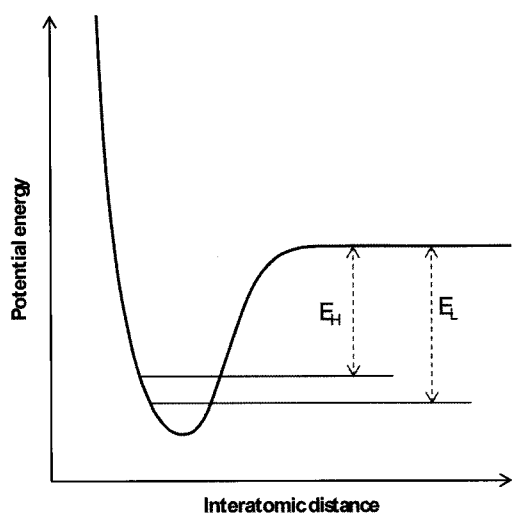
Fig. 1. Schematic potential energy curve for the interaction of two atoms in a stable molecule or between two molecules in liquid or solid [Bigeleisen, 1965; Hoefs, 1997].

line (E_H) that of the heavy one. E_L is actually not a line, but an energy interval between the zero point energy level and the steady-state energy level. This means that the bonds formed by the heavy isotope are stronger than those involving the light isotope. Thus, generally, in the course a chemical reaction, the molecules bearing the light isotope will react slightly faster than those carrying the heavy isotope.

Isotope Fractionation

The partitioning of isotopes between two substances or two phases of the same substance with different isotope ratios is called isotope fractionation. The main phenomenon-producing isotope fractionations are isotope exchange reactions and kinetic processes, which depend on the differences in the reaction rates of the isotopic molecules.

Isotope exchange reactions are a special case of the general chemical equilibrium and can be written:

$$aAM_1 + bBM_2 \overset{\leftarrow}{\rightarrow} aAM_1 + bBM_1$$
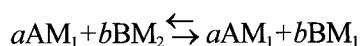
where A, B, and M indicate elements, the subscripts 1 and 2 indicate the light and the heavy isotopes, respectively, and a and b are stoichiometric coefficients. For this reaction, the equilibrium constant is expressed by the following equation:

$$K = \frac{\left(\dfrac{AM_2}{AM_1}\right)^a}{\left(\dfrac{BM_2}{BM_1}\right)^b}$$

where the terms in parentheses in the equation may be, for example, the molar ratios of any species. Using the

methods of statistical thermodynamics, the isotopic equilibrium constant may be expressed in terms of the partition function Q of the various species [Criss, 1999]:

$$K = \frac{\left(\dfrac{Q_{AM2}}{Q_{AM1}}\right)^a}{\left(\dfrac{Q_{AM2}}{Q_{AM1}}\right)^b}$$

The partition function is defined by:

$$Q = \sum_i g_i e^{-E_i/kT}$$

where the summation is over all the allowed energy levels, E_i, of the molecules, and g_i is the degeneracy or statistical weight of the ith level, k is the Boltzmann constant, and T is the temperature. The purpose of calculating the partition function ratio of the isotopic molecule is that introduction of the ratios of their partition functions into those of the corresponding isolated atoms is very convenient [Urey, 1947]. The partition function of a molecule can be separated into factors of each type of energy: translation, rotation, and vibration:

$$\frac{Q_2}{Q_1} = \left(\frac{Q_2}{Q_1}\right)_{trans} \left(\frac{Q_2}{Q_1}\right)_{rot} \left(\frac{Q_2}{Q_1}\right)_{vib}$$

The differences in the translation and rotation energy are similar among the compounds appearing at the left- and the right-hand sides of the exchange reaction equation, except for hydrogen, in which the rotation must be taken into account. This leaves differences in vibration energy term which can be separated into two components. The first is related to the zero-point energy difference, and accounts for most of the variations in the temperature. The second represents the contributions of all the other bound states and is not very different from unity.

The equilibrium constant can be related to the isotopic fractionation factor (α). The fractionation factor is defined as the ratio of the numbers of any two isotopes in one chemical compound A divided by the corresponding ratio of another chemical compound B:

$$\alpha_{A-B} = \frac{R_A}{R_B}$$

where α is the nth root of K,

$$\alpha = K^{1/n}$$

n refers to the number of atoms transferred in the exchange reaction. When only one atom is exchanged, the equilibrium constant is identical to the fractionation factor. In others, K and α differ by a power that relates to the stoichiometry of the reaction.

The second main group of the phenomena-producing

isotope fractionations is the kinetic isotope effect, which is associated with the incomplete and the unidirectional processes such as evaporation, dissociation reactions, biological reactions, and diffusion. The theory of kinetic isotope fractionations has been discussed by Bigeleisen and Wolfsberg [1958], Melander [1960], and Melander and Saunders [1980]. Knowledge on the kinetic isotope effects can provide information on the reaction pathways.

The unidirectional chemical reactions generally show an enrichment of the lighter isotope in the reaction products. The isotope fractionation introduced during the course of a unidirectional reaction may be considered in terms of the ratio of the rate constants for the isotope substances. Thus, for the two competing isotopic reactions, the following equations are applied:

$$A_1 \xrightarrow{k_1} B_1, \quad A_2 \xrightarrow{k_2} B_2$$
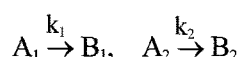
the ratio of rate constants for the reaction of light and heavy isotope species k_1/k_2, as in the case of equilibrium constants, is expressed in terms of two partition function ratios, one for the two reactant isotopic species, and one for the two isotopic species of the activated complex or transition state A*:

$$\text{Kinetic isotope effect} = \frac{k_1}{k_2} = \frac{\left(\dfrac{Q_{(A_2)}}{Q_{(A_1)}}\right)}{\left(\dfrac{Q^*_{(A_2)}}{Q^*_{(A_1)}}\right)}\left(\frac{v_1}{v_2}\right)$$

The factor v_1/v_2 in the expression is a mass term ratio for the two isotopic species. The determination of the ratio of the rate constants is, therefore, principally the same as the determination of the equilibrium constant. This theory is based on the idea that there is a critical intermediate called the activated species or the transition state during a chemical reaction [Hoefs, 1997).

Carbon Isotope Fractionation of Plants

Plants fractionate carbon isotopes during photosynthesis.

The fractionation varies depending on the photosynthetic type, the environment, and the genotype, and this variation can be used to study a variety of issues in the plant physiology [Troughton, 1979; Vogel, 1980; O'Leary, 1988; Farquhar et al., 1989; Rundel et al., 1989]. The physical and biochemical phenomena for carbon isotope fractionation are generally well understood, and this understanding provides information on a variety of problems in plant physiology and ecology.

Before the industrial activity, the atmospheric carbon dioxide (CO_2) has a $\delta^{13}C$ value of -8‰, and each year this value becomes slightly more negative as a result of the combustion of fossil fuels and deforestation [Keeling et al., 1979]. Since the isotope fractionations reflect the isotopic difference between the source and the product, discrimination can be expressed based on the isotopic difference between them,

$$\text{discrimination} = \frac{\delta^{13}C_a - \delta^{13}C_p}{1 + \delta^{13}C_p/1000}$$

where $\delta^{13}C_a$ is the $\delta^{13}C$ value of air and $\delta^{13}C_p$ is that of the plant. The second term in the denominator is quite small; therefore the discrimination can be expressed as the difference between the $\delta^{13}C$ of the air and the plant.

Carbon isotope fractionation in plants can be understood by the individual physical and chemical processes that contribute to the carbon assimilation processes (Table 2).

Thermodynamic and kinetic isotope fractionations. Isotope fractionations may be produced by thermodynamic and kinetic process. Thermodynamic fractionations reflect the differences in the equilibrium constants among the isotopic species and are generally smaller than the kinetic fractionations. For example, the kinetic isotope fractionation associated with the carboxylation of the ribulose bisphosphate is close to 29‰, whereas the equilibrium fractionation for this process is negligible [O'Leary and Yapp, 1978].

Isotope fractionations in physical processes. Diffusion

Table 2. Carbon isotope fractionations associated with photosynthesis

Process	Fractionation (‰)	Reference
Solubility of CO_2 in water	1.1	O'Leary, 1984
Hydration of CO_2	-9.0	Mook et al., 1974
CO_2 diffusion in air	4.4	O'Leary, 1981; Hersterberg and Siegenthaler, 1991
CO_2 diffusion in aqueous solution	0.7	O'Leary, 1984
Spontaneous hydration of CO_2	6.9	Marlier and O'Leary, 1984
Carbonic anhydrase-catalyzed hydration of CO_2	1.1	Paneth and O'Leary, 1985
Carboxylation of PEP	2.2	O'Leary et al., 1981
Carboxylation of RuBP	29.0	Roeske and O'Leary, 1984

processes often show small isotope discriminations. The isotope fractionation during the diffusion of $^{12}CO_2$ and $^{13}CO_2$ in air is 4.4‰. Diffusion of CO_2 dissolved in water produces a smaller isotope fractionation (0.7‰) than that of CO_2 in air (4.4‰), because the hydrogen bond of water needs much work for diffusion, and the work is independent of the mass of CO_2. The dissolution of CO_2 in water shows an equilibrium isotope fractionation of −1.1‰; that is, $^{13}CO_2$ is less soluble than $^{12}CO_2$. Because this fractionation is an equilibrium fractionation, it must be included in any treatment involving the CO_2 uptake. Therefore, this value is usually included in the value used for CO_2 carboxylation of plant.

Isotope fractionations in enzymatic processes. The first step of the CO_2 fixation is catalyzed by ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) in C3 plants:

$$CO_2 + RuBP \rightarrow 2(3\text{-PGA})$$
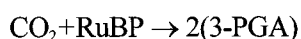
The isotope fractionation by this enzyme is a major source of the large isotope fractionation shown by the C3 plants and is 29‰ compared with the dissolved CO_2.
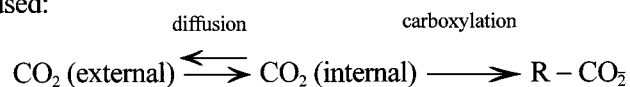
The inter-conversion of CO_2 and HCO_3^- occurs readily in plants and is generally under the control of carbonic anhydrase, which probably keeps the reaction near equilibrium. At equilibrium, the fractionation is −9.0‰, with respect to the ^{13}C concentration in HCO_3^-. Kinetic fractionation can be associated with the carbonic anhydrase-catalyzed hydration of CO_2, but this is not probable under most conditions. This hydration is not included in the C3 fractionation model at all because RuBisCO uses CO_2 rather than HCO_3^-.

A PEP carboxylase carries out the initial carboxylation in the C4 plants and catalyzes the following reaction:

$$PEP + HCO_3^- \rightarrow OAA + P_i$$
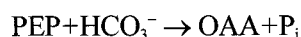
The isotope fractionation is 2.2‰. However, when the reaction starts from CO_2, the isotope fractionation becomes −5.7‰ (−9.0+2.2+1.1).

A quantitative approach to carbon isotope fractionation. For a quantitative approach to the isotope fractionation in a C3 plant, a simple two-step CO_2 uptake scheme can be used:

$$CO_2 \text{ (external)} \underset{\text{diffusion}}{\overset{}{\rightleftharpoons}} CO_2 \text{ (internal)} \overset{\text{carboxylation}}{\longrightarrow} R - CO_2$$

The first step describes the CO_2 diffusion through the stomata into the carboxylation site and the second step corresponds to the irreversible transformation of the internal CO_2 by the carboxylase. The isotope fractionation associated with diffusion is 4.4‰, whereas that associated with carboxylation is 29‰ in the C3 plants

(30‰ when CO_2 dissolution is considered).

Two limiting cases can be considered. First, if the stomata are nearly closed, the overall CO_2 assimilation rate is limited by the diffusion process of the first step, and the internal CO_2 concentration becomes low because the carboxylation process uses virtually all carbons available. Therefore, the carboxylation isotope fractionation of the second step is not expressed. The isotope fractionation is small, approaching 4.4‰ when the stomata are almost closed and $\delta^{13}C$ for a C3 plant should be similar to −12‰ (−8-4.4). On the other hand, if the stomata are relatively open, an easy transfer of CO_2 occurs between the external and the internal pools; thereby the internal CO_2 concentration and the external CO_2 concentration reaches an equilibrium. In this case, if the diffusional fractionation is not considered, the observed fractionation approaches the carboxylation fractionation, and therefore the $\delta^{13}C$ of the leaf would then approach −38‰ (−8-30). The behavior of the plants, thus, is an intermediate between these two extremes.

Isotope fractionation in C3 plants. For C3 plants, isotope fractionation can be expressed using the following equation:

$$\text{fractionation} = \left[a + (b-a)\frac{p_i}{p_a} - d \right]$$

where a and b are the discriminations due to diffusion (4.4‰) and carboxylation (30‰ when corrected for the equilibrium effect on CO_2 dissolution), and p_i and p_a are the internal and the external CO_2 pressures, respectively. The isotope fractionation varies with variation in the internal CO_2 concentration, which reflects the relative inhibition on the stomatal diffusion and the carboxylation. The d involves contributions from respiration, isotopic changes due to the carbon export, CO_2 fixation by PEP carboxylase, among other factors. The variations in d within a single series of studies can usually be neglected.

Isotope fractionation in C4 plants. Farquhar [1983] adapted the same kind of method used for C3 photosynthesis to account for isotope fractionation in C4 photosynthesis:

$$\text{fractionation} = a + (b_4 + b_3\phi - a)\frac{p_i}{p_a}$$

where a is the fractionation due to diffusion (4.4‰), b_4 is the fractionation due to PET carboxylase from gaseous CO_2 (−5.7‰), b_3 is the fractionation due to RuBisCO (30‰), and $\emptyset$ is the fraction of CO_2 released in the bundle sheath that leaks to the mesophyll, where it may be either used by PEP carboxylase or released to the atmosphere. Farquhar *et al.* [1989] suggest that this value is often near 0.37; a more recent study suggested that the value of 0.21

is more appropriate [Henderson *et al.*, 1992].

Isotope Fractionations in the Nitrogen Cycle Processes

Nitrogen isotope fractionation. Variations in $\delta^{15}N$ among samples reflect the N isotope fractionations, which occur because more energy is needed to break or form chemical bonds involving ^{15}N than ^{14}N. By definition, if a substrate and a product maintain the same $^{15}N/^{14}N$ ratio, no isotope fractionation occurs; that is, the isotope fractionation factor (α), the $R_{product}$: $R_{substrate}$ ratio, is in unity. Deviations in α from the unity indicate that isotope fractionation occurs. Apparent isotopic fractionation factor ($\alpha_{s/p}$) associated with a decrease in NH_4^+-N concentration during nitrification process can be calculated using the following equation [Mariotti *et al.*, 1981]:

$$\ln\left(\frac{10^{-3}\delta_s + 1}{10^{-3}\delta_{s,0} + 1}\right) = \left(\frac{1}{\alpha_{s/p}} - 1\right)\ln f$$

where f is the unreacted fraction of the substrate (NH_4^+-N) at time t, and $\delta_{s,0}$ and δ_s are the $\delta^{15}N$ of NH_4^+-N at time 0 and t, respectively. From the slope ($1/\alpha_{s/p}-1$) of the straight line on a log-log scale, $\alpha_{s/p}$ can be calculated and converted into $\varepsilon_{p/s}$ using the following equation to be expressed on the δ scale:

$$\varepsilon_{p/s} = 1000(1/\alpha_{s/p}-1)$$

$\varepsilon_{p/s}$ is the difference in $\delta^{15}N$ between a substrate and its immediate product. For a given reaction, $\varepsilon_{p/s}$ is not constant [Handley *et al.*, 1999; Adams and Sterner, 2000), but rather depends on the external conditions, for example, temperature. Representative $\varepsilon_{p/s}$ values that have been measured for the major N cycle processes are shown in Table 3.

The $\delta^{15}N$ of residual substrate (δ_s) changes during an N process can be expressed by the following equation [Mariotti *et al.*, 1981]:

$$\delta_s = \delta_0 + \varepsilon \ln f$$

where δ_0 is the initial $\delta^{15}N$ of the substrate. The products become simultaneously ^{15}N-depleted relative to substrate. The $\delta^{15}N$ of the accumulated product is:

$$\delta_p = \frac{\varepsilon f \ln f}{1 - f}$$

If all the substrates are consumed, $f=0$ and $\delta_p=\delta_0$, the final product has the same $\delta^{15}N$ as the initial substrate.

This Rayleigh model [1896] applies to the unidirectional reactions in closed systems. Although the natural systems are neither closed nor unidirectional, ecosystem $\delta^{15}N$ variations can be interpreted using the Rayleigh equations, but with critical differences. Furthermore, in an ecological context, f is the extent to which the N source has been consumed or remained. If $f=1$, the N supply is effectively infinite relative to the demand for N by the potential sinks. If $f=0$, the N source has been totally consumed by the various sinks. In natural systems, unlike closed vessels, a consumed N source can be replenished, and f varies between 0 and 1 depending on the variation of the source supply and the consumption over time. In addition, if the supply of an N source is effectively infinite relative to demand, the $\delta^{15}N$ of source N remain unaffected by the fractionations. Fractionation depends on the process, the external conditions, and the amount of the N source consumed.

Variations in natural abundances of ^{15}N in soil-plant system. Most N in soils is not immediately available to plants. Furthermore, the $\delta^{15}N$ of the soil total-N is generally reflected by that of the stable N [Johannisson and Högberg, 1994], thus is not a good indicator of the $\delta^{15}N$ of the N available to plants. On the contrary, the

Table 3. N isotope fractionations (ε) during major N cycle processes [Handley *et al.*, 1999]

Process	ε (‰)
N_2 fixation via nitrogenase	0-6
NH_3 volatilization	40-60
N_2O and NO production during NH_4^+ oxidation (nitrification)	35-60
N_2O and N_2 production during NO_3^- reduction (denitrification)	28-33
NO_3^- assimilation into organic N by plants	0-19
NH_4^+ assimilation into organic N by plants	9-18
NO_3^- or organic N assimilation by microbes	13
NH_4^+ assimilation by microbes	14-20
NH_4^+ production from organic matter decomposition (ammonification)	0-5
NO_3^- production during nitrification	15-35
Organic N assimilation by animals (deamination and transamination)	1-6

biologically active nitrogen pools could change dramatically over a short time period. Plants $\delta^{15}N$ can be an indicator to integrate $\delta^{15}N$ of the available N sources.

There is very little evidence that isotope fractionation during mineralization of N from larger molecules in soils should be of significance [Högberg, 1997]. The isotope fractionation during the N mineralization process suggested by Létolle [1980] and Dijkstra et al. [2008] did not show the variation in $\delta^{15}N$ by the N mineralization, but was related with the microbial N uptake and the nitrification process. The small differences (<2‰) in $\delta^{15}N$ between the soil total-N and the root N in systems thought to have little nitrification [Nadelhoffer and Fry, 1994; Högberg et al., 1996] also suggest that the N isotope fractionation during the N mineralization is small.
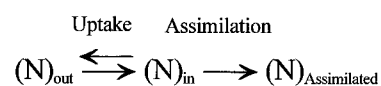
Volatilization of NH_3 involves several steps in which isotope fractionation can occur: the equilibrium between NH_4^+ and NH_3 in solution, diffusion of NH_3 to the site of volatilization, volatilization of NH_3, and diffusion of NH_3 away from the site of volatilization. The mixed effects of these processes on the net fractionation can be enormous and each has been reported to have α value >1.02. The α values vary according to which step is rate-limiting, the pH of the substrate, as well as other factors. When the volatilization of NH_3 is a significant process, for example composting process, the remaining N will be left enriched. The $\delta^{15}N$ of the animal manure could have a $\delta^{15}N$ >10‰ through N loss by NH_3 volatilization and can be used to trace the fate of manure N in the soil-plant system [Kerley and Jarvis, 1996; Choi et al., 2002; Yun et al., 2006]. During senescence, plants and fungi could be the major source of NH_3 volatilization.

Nitrification has been associated with fairly large isotope effects ranging from 1.015 to 1.036 in studies of pure cultures of Nitrosomonas europaea carrying out the first step of the reaction from NH_4^+ to NO_2^- [Delwiche and Steyn, 1970; Mariotti et al., 1981; Yoshida, 1988]. The second step of nitrification from NO_2^- to NO_3^- is not rate-limiting and should not, therefore, lead to further fractionation. In soils, complete nitrification has been estimated to have a fractionation of 1.012-1.029 [Shearer and Kohl, 1986].

Fractionation during denitrification has been found to vary significantly, with fractionation factors of 1.000-1.033 [Wellman et al., 1968; Delwiche and Steyn, 1970; Mariotti et al., 1981; Bryan et al., 1983; Yoshida et al., 1989]. The variations in the fractionation factors have been caused by the differences in the concentrations of the electron donors and acceptors, and the variations in temperature [Shearer and Kohl, 1986; Kohl and Shearer, 1995].

Ion exchange, diffusion, and mass flow can cause isotope fractionation. Several studies reported a small fractionation (<1.005) during the ion exchange [Hübner, 1986; Shearer and Kohl, 1986]. When a solution of NH_4Cl, for instance, was exchanged with the cations on clay, the immediate reaction (0-2 h) increased the $\delta^{15}N$ of the solution, followed by a decline to -1.5‰ of the initial value [Karamanos and Rennie, 1978]. Fractionations during ion exchange can affect the $\delta^{15}N$ profiles in soil [Nadelhoffer and Fry, 1994], but are likely to be small compared with other kinetic biological fractionations. The same applies to the diffusion of inorganic N in the soil solution. No isotopic fractionation is detected during the mass flow.

Plant uptake of inorganic N (NH_4^+ and NO_3^-) occurs in the three steps: uptake of nitrogen from the soil solution, translocation to the site of assimilation, and assimilation of inorganic nitrogen into organic nitrogen. Assimilation of NH_4^+ occurs by the glutamine synthetase-glutamate synthase (GS-GOGAT) pathway in the root to avoid the toxicity due to the accumulation of ammonia. Nitrate assimilation can occur in either the roots or leaves by the nitrate reductase-nitrite reductase pathway. The product of this transformation process is NH_4^+, which enters the GS-GOGAT pathway. When plants were grown in the nutrient solution with NO_3^-, a significant discrimination was observed. The discrimination generally increases with the increasing external NO_3^- concentration. Plants grown with NH_4^+ were shown to have similar patterns. Mariotti et al. [1982] described the discrimination observed in the plant acquisition of nitrogen as:

$$\underset{(N)_{out}}{\overset{\text{Uptake}}{\underset{F_1}{\overset{F_2}{\rightleftharpoons}}}} (N)_{in} \overset{\text{Assimilation}}{\underset{F_3}{\longrightarrow}} (N)_{Assimilated}$$

where $(N)_{out}$ and $(N)_{in}$ are the inorganic nitrogen forms outside and inside of the cell, respectively, and $N_{assimilated}$ is the assimilated nitrogen. F_1, F_2, and F_3 are the flux, efflux, and assimilation rates of nitrogen, respectively. F_1 is the equal to the sum of F_2 and F_3 under steady-state conditions. During N assimilation, the observed discriminations by nitrate reductase and glutamine synthetase are 15 and 17‰, respectively.

N_2-fixation data from Azotobacter and symbiotic N_2-fixers indicate that the fractionation is generally small. Several studies have demonstrated that the fractionation during N_2-fixation is influenced by the bacterial strain [Steele et al., 1983; Bergersen et al., 1986; Ledgard, 1989], and Ledgard [1989] provided evidence that the nutrient supply and the soil moisture also influence the fractionation factor.

Use of $\delta^{15}N$ and $\delta^{13}C$ to integrate stress on plants

A continuing search has been conducted on the effect of environmental stresses on crops. Tolerance of plants comprises many physiological processes, which vary quantitatively rather than qualitatively [Yeo, 1998; Zhang et al., 1999]. It is impractical to measure each individual process on many plants. Therefore, one possible approach is to measure the alternative variables, which integrate various physiological processes. Isotope signatures of carbon and nitrogen are one of the most useful alternatives.

$\delta^{13}C$ has been used to screen the C3 genotypes for the potential water use efficiency [O'Leary, 1993]. A robust theory of Farquhar et al. [1982] is available to interpret the $\delta^{13}C$ variations among the C3 plants in terms of measurable the physical and physiological processes. Plant $\delta^{13}C$ reflects mainly the extent to which the primary CO_2 assimilation is limited by carboxylation and/or CO_2 diffusion in the leaves. Whole-plant $\delta^{13}C$ is dominated by these processes. Environmental stresses modify $\delta^{13}C$ in predictable ways by evaluating their effects on the balance between stomatal conductance and carboxylation. In contrast, $\delta^{15}N$ has been used much less extensively in this way. Plant $\delta^{15}N$ reflects the potentially variable $\delta^{15}N$ values of external N sources and isotope fractionations, which occur during the assimilation, transport, and loss of N.

Natural abundances of ^{13}C under stress condition. Several studies have been shown that the $\delta^{13}C$ of the plant well reflected the mixed contribution of stomatal conductance and carboxylation rate during photosynthesis. Whole-plant $\delta^{13}C$ varied significantly depending on the treatments of drought and N starvation [Robinson et al., 2000]. The stresses dominantly affected $\delta^{13}C$ of the plant. Shoot and root $\delta^{13}C$ were both affected significantly by drought, but not by N starvation. Under the drought condition, $\delta^{13}C$ became less negative than that of the controls and this effect was more pronounced in shoots than roots. $\delta^{13}C$ of the reed (*Phragmites australis*) in the saline wetland was less negative than that in the non-saline soil [Choi et al., 2005a]. These results possibly reflected the decrease in the CO_2 diffusion rate by the reduction of the stomatal conductance. Carbon isotope fractionation of the loblolly pine leaves was increased by irrigation and decreased by fertilization [Choi et al., 2005b]. The $\delta^{13}C$ of the tree rings of the red pine increased with increasing tree ring indices [Choi et al., 2005c], suggesting that the tree might have been influenced by such environmental factors as nutrient deficiency and acid rain that affect the carboxylation efficiency.

Natural abundances of ^{15}N under stress condition. Highly significant effects of the treatments were observed in the whole-plant $\delta^{15}N$ [Robinson et al., 2000]. Variations in the whole-plant $\delta^{15}N$ were influenced dominantly by the treatment of drought and N starvation. The plants clearly discriminated against ^{15}N between the source $\delta^{15}N$ and the whole-plant $\delta^{15}N$. However, discrimination could not be quantified due to the temporal variability in the source $\delta^{15}N$. When drought or N starvation significantly affected the whole-plant $\delta^{15}N$, $\delta^{15}N$ almost always became more negative than that of the controls. The mean differences in $\delta^{15}N$ between the control and the stressed plants were 0.6 and 0.3‰ for the drought and the N starvation treatments, respectively. When the loblolly pine was influenced by the irrigation and fertilization, foliar $\delta^{15}N$ did not change by irrigation but was increased by fertilization [Choi et al., 2005b]. The variation in foliar $\delta^{15}N$ reflected well the $\delta^{15}N$ of soil NH_4^+, which was higher than that of soil NO_3^-. The differences in $\delta^{15}N$ between NH_4^+ and NO_3^- probably resulted from the isotope fractionation during the nitrification process and varied significantly by the fertilization.

In conclusion, this paper reviewed the theories on the isotope fractionation of carbon and nitrogen. The effect of the environmental changes on plant and soil can be interpreted using the suggested mechanisms of variation in isotope fractionation. In the case of carbon, the theory related with the stomatal conductance and the carboxylation adjusted well to the plant responses to the environmental changes. Isotope fractionation of nitrogen also varied with the environmental factors and provided information on changes in the N cycle processes. However, the mechanisms of variation in the N isotope fractionation are known less than that in the carbon isotope fractionation. With more progress in the theory of nitrogen isotope fractionation, the use of stable carbon and nitrogen isotopes will provide powerful information on the responses of plant and soil to the environmental changes.

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