Chin. Geogra. Sci. 2011 21(2) 1–20
doi: 10.1007/s11769-011-0000-0
www.springerlink.com/content/1002-0063

Application of Stable Isotope Techniques in Studies of Carbon and Nitrogen Biogeochemical Cycles of Ecosystem

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Abstract: Stable isotope techniques have been proved useful as tools for studying the carbon (C) and nitrogen (N) biogeochemical cycles of ecosystem. This paper firstly introduced the basic principles and the distribution characteristics of stable isotope, then reviewed the recent advances and applications of stable isotope in the C and N biogeochemical cycles of ecosystem. By applying the ^{13}C natural abundance technique, ecologists are able to understand the photosynthetic path and CO_2 fixation of plants, the CO_2 exchange and C balance status of ecosystem, the composition, distribution and turnover of soil organic C and the sources of organic matter in food webs, while by using the ^{13}C labeled technique, the effects of elevated CO_2 on the C processes of ecosystem and the sources and fate of organic matter in ecosystem can be revealed in detail. Differently, by applying the ^{15}N natural abundance technique, ecologists are able to analyze the biological N_2-fixation, the N sources of ecosystem, the N transformation processes of ecosystem and the N trophic status in food webs, while by using the ^{15}N labeled technique, the sources, transformation and fate of N in ecosystem and the effects of N input on the ecosystem can be investigated in depth. The applications of both C and N isotope natural abundance and labeled techniques, combined with the elemental, other isotope (^{34}S) and molecular biomarker information, will be more propitious to the investigation of C and N cycle mechanisms. Finally, this paper concluded the problems existed in current researches, and put forward the perspective of stable isotope techniques in the studies of C and N biogeochemical cycles of ecosystem in the future.

Keywords: stable isotope; isotope fractionation; isotope natural abundance; biogeochemical cycle; carbon; nitrogen

Citation: Sun Zhigao, Mou Xiaojie, Li Xinhua, Wang Lingling, Song Hongli, Jiang Huanhuan, 2011. Application of stable isotope techniques in studies of carbon and nitrogen biogeochemical cycles of ecosystem. *Chinese Geographical Science*, 21(0): 000–000. doi: 10.1007/s11769-011-0000-0

1 Introduction

The stable isotope techniques initially originated from physics in the 1930s, and became an important part of geology in the 1940s. The original studies of stable isotope mainly focused on the isotope evolvement approach, isotope fractionation mechanism and isotope composition of various substances (Cai *et al*., 2002), and few researches have been devoted to the applications of isotopes in the biogeochemical fields. The studies on the fractionation of ^{13}C and ^{12}C during photosynthesis in the 1960s ultimately made the stable isotope become an effective means in the analysis of biogeochemical processes (Park and Epstein, 1961). In the past 20 years, the stable isotope techniques have been prosperously applied in the ecological fields due to its special advantages (such as safety, accuracy and noninterference). Isotopes function as natural dyes or colors,

Received date: 2010-09-27; accepted date: 2010-12-29
Foundation item: Under the auspices of Knowledge Innovation Programs of Chinese Academy of Sciences (No. KZCX2-YW-223), National Natural Science Foundation of China (No. 40803023), Key Program of Natural Science Foundation of Shandong Province (No. ZR2010DZ001), Talents Foundation of Chinese Academy of Sciences (No. AJ0809BX-036)
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generally tracking the circulation of elements (Fry, 2006). Because hydrogen (H), carbon (C), nitrogen (N), oxygen (O), and sulfur (S) are five important elements in biosphere, researchers have paid special attention to the application of HCNOS stable isotope technique in the element cycles of ecosystem. In general, the applications of stable isotope techniques in the biogeochemistry mainly focus on two aspects: 1) quantitatively or semi-quantitatively analyze the elements cycling processes in long temporal scale and large spatial scale by applying the stable isotope natural abundance method; 2) identify the definite elements cycling mechanisms in short temporal scale and small spatial scale by applying the stable isotope labeled method (Gu, 2007). Presently, element biogeochemical process is an important research field of global change (Fang *et al*., 2002), and the scientific background of global change affords many advantages for the application of stable isotope techniques in the biogeochemistry of ecosystem.

In this paper, the basic principles and the distribution characteristics of stable isotopes (C and N are emphasized) are firstly introduced. Then, the recent advances and applications of stable isotope in the C and N biogeochemical cycles of ecosystem are reviewed in detail. Finally, the limitations existed in current research are analyzed, and the suggestions for the future research are put forward.

2 Basic Principles of Stable Isotope

Isotopes are atoms with the same number of protons but different numbers of neutron and thus isotopes have different atomic mass (or mass number). Different isotopes have identical chemical behavior, with the exception that due to their larger masses, heavier isotopes tend to react somewhat more slowly than lighter isotopes of the same element because the molecules bearing the lighter isotope have higher vibrational energies, a phenomenon called the kinetic isotope effect (Chang and Choi, 2009). As the existence of kinetic isotope effect, the natural abundance of element isotope in the substrate can become gradually enriched during the element cycling processes if the reaction is incomplete, and such changes are called isotope fractionation. The second mechanism that causes isotopic fractionation is called equilibrium isotope effect. In exchange reactions when equilibrium is reached, heavy isotopes concentrate where bonds are the strongest. In addition, physical processes (such as diffusion, evaporation and distillation) and biochemical processes (such as photosynthesis, animal assimilation and trophic transfer in food chain) discriminate against heavy isotopes and thus cause significantly isotopic fractionation (Chang and Choi, 2009). Based on the differences of isotopic fractionation in each part of ecosystem, ecologists usually utilize the stable isotopes to understand the element biogeochemical processes and trace the source and fate of matters in ecosystem (Peterson and Fry, 1987).

The isotope ratio (δ) is usually used to denote a difference measurement made relative to standards during the analysis. The isotope compositions of the standards are given by the International Atomic Energy Agency (LAEA), and are used routinely in the calculation of δ values where they appear as R_{standard} term (Fry, 2006):

$$\delta^{H}X = [(R_{\text{sample}} / R_{\text{standard}} - 1)] \times 1000 \quad (1)$$

where $\delta^{H}X$ represents the isotope ratio of the heavy isotope (^{2}H, ^{13}C, ^{15}N, ^{18}O, or ^{34}S) of element X (H, C, N, O, or S), and R is the ratio of the heavy isotope to the light isotope for the element (^{2}H/^{1}H, ^{13}C/^{12}C, ^{15}N/^{14}N, ^{18}O/^{16}O, or ^{34}S/^{32}S). The heavy isotope percent is also termed atom percent (at.%), which refers to the percentage contribution of heavy isotope (such as ^{15}N) to the total number of N atoms in a sample.

The isotope enrichment factor (ε) is generally used to describe fractionation difference, which is derived from fractionation factor (α) (Fry, 2006).

$$\varepsilon = (\alpha - 1) \times 1000 = ({}^{L}k / {}^{H}k - 1) \times 1000 \quad (2)$$

where ${}^{L}k$ and ${}^{H}k$ are the reaction rates or kinetic 'k' constants for the light (L) and heavy (H) isotope molecules, respectively.

In the circulation processes of HCNOS, fractionation and mixing generally combine to produce regular and characteristic isotope distribution in ecosystem (Fry, 2006), which is favor for the ecologists to grasp the relative information on the transformations of these elements.

3 Isotopic Characteristics of Carbon and Nitrogen

3.1 Carbon stable isotope

The δ^{13}C values in the main pools of global C cycle (atmosphere, terrestrial ecosystems and ocean) are sig-

nificantly different. The $\delta^{13}C$ value of atmospheric CO_2 is decreasing in response to inputs of ^{13}C depleted CO_2 from fossil fuel plus biomass burning and decomposition. Over the past 100 years, the decrease rate may have been almost 1‰, from –7‰ to –8‰ (Fry, 2006). Photosynthesis is one of the important reactions governing isotope circulation and distribution in the biosphere. Carbon fixation (CO_2) by the dominant C_3 (Calvin cycle) plants involves a net fractionation of about 20‰ between the atmospheric CO_2 and plant biomass (average $\delta^{13}C$ value: –28‰), while C fixation (HCO_3^-) by the C_4 (Hatch-Slack) plants involves a net fractionation of about 5‰ (average $\delta^{13}C$ value: –13‰). Comparatively, the net fractionation occurred in the process of C fixation (CO_2 and HCO_3^-) by the CAM (Crassulacaean Acid Metabolism) plants ranges from 2% to14%, with the average $\delta^{13}C$ value of –16‰ (Gu, 2007). Besides photosynthesis, many physiological and environmental factors (such as substrate concentration, ^{13}C natural abundance and plant growth rate) also can affect the composition of stable isotope in plants. As the plants prefer to absorb $^{12}CO_2$ in the process of photosynthesis, the lower $\delta^{13}C$ values are generally observed in plants. In addition, the isotope fractionation effect generally decreases as the intensity of photosynthesis increasing, and the ecosystems with higher productivity generally have lower $\delta^{13}C$ values (Gu, 2007). Soil organic matter (SOM) globally contains more C than either the atmosphere or living plant biomass and in general is similar or slightly enriched with ^{13}C in comparison with the dominant vegetation. Although either differential preservation or mineralization of soil components with different $\delta^{13}C$ values does lead to gradual shifts in soil ^{13}C content, on average there is little fractionation of respired CO_2. The $\delta^{13}C$ values in animal are mainly determined by the composition of stable C isotope in foods, and there is little or no C isotope fractionation during animal assimilation and growth (DeNiro and Epstein, 1978). Thus, animal C isotopes are generally used to reflect the time-integrated average diet, and index food sources and C flow in ecosystem.

The ^{13}C contents of components of the C cycle of freshwaters vary widely depending on the source of dissolved C in the waters. These sources include carbonate rock weathering, mineral springs, atmospheric CO_2, and organic matter respiration (Fry, 2006). Terrestrial organic matters are the main C sources of rivers or lakes, and the $\delta^{13}C$ values in freshwater plants are generally lower than those in organic matters due to the respiration and transformation of organic matter and plants absorption. Where large amounts of organic matters are imported and respirations are strong, the values for dissolved inorganic C may approach –20‰, and algae that further fractionate during C uptake can measure –45‰. The exchange of CO_2 between the atmosphere and the surface of ocean involves an equilibrium chemical fractionation between atmospheric CO_2 (–8‰) and the total CO_2 in surface ocean water (about 1‰). The withdrawal of C to form carbonates involves small isotope fractionations whereas uptakes of dissolved inorganic C in planktonic photosynthesis involves larger kinetic fractionation that results in algal values of about –24‰ to –19‰ (Fry, 2006).

3.2 Nitrogen stable isotope

Most N in the biosphere presents as N_2 gas in the atmosphere, and this massive reservoir is well mixed with an isotope composition that is essentially constant at 0‰. There is a wide range reported for N isotope values for ammonium (NH_4^+-N) and nitrate (NO_3^--N) in precipitation from about –20‰ to 10‰, and some of the more negative values are related to soil and anthropogenic emissions in highly industrialized areas (Fry, 2006). Thus, the $\delta^{15}N$ values can be used to trace human pollutant plumes and identify the sources and fates of N that human activities are currently adding to many ecosystems. The main N cycling processes in the plant-soil system are illustrated with different fractionation factor (α) values (Table 1), and as a result, the different N pools have significantly different $\delta^{15}N$ signatures. The N_2-fixing, mineralization, immobilization, NO_3^--N leaching and plant uptake processes generally cause very little N isotope fractionation (Chang and Choi, 2009). Because the rate of N supply often limits reactions such as plant growth and bacterial mineralization (under these conditions, all available N can be consumed), slow rates of N supply, plants growth rates and limiting amounts of substrate N are often important for understanding N isotope distributions (Gu, 2007). As the N uptake processes cause little isotopic fractionation, the $\delta^{15}N$ values of non-N_2-fixing plants generally reflect the signature of the N sources. The nitrification, denitrification and ammonia (NH_3) volatilization processes generally induce the most dramatic fractionations since

these processes are virtually unidirectional in the soil, i.e., reduction of NO_3^--N to NH_4^+-N is rare and gaseous products of denitrification and NH_3 volatilization have little chance to be converted to the substrates (Handley and Raven, 1992; Chang and Choi, 2009). In addition, as the N stable isotopes in animal are enriched systematically during trophic transfers, the $\delta^{15}N$ values are generally used to indicate the trophic position of consumers, food chain length and trophic niche width (Gu, 2009).

Some cumulative and large fractionations do occur in the N cycling processes of ocean. A cumulative faster loss of ^{14}N than ^{15}N during particulate N decomposition results in ^{15}N increases of 5‰ to 10‰ with increasing depth in the ocean. Both nitrification and denitrification in the sea proceed with substantial isotope effects (ε = 10‰ to 40‰), and where NO_3^--N is abundant, assimilation by phytoplankton proceeds with a smaller effect (ε = 4‰ to 8‰) (Fry, 2006). Lakes or rivers appear more variable in N isotope composition than the large world ocean. Large N isotope contrasts might occur between lakes (or rivers) in which primary production is limited by N (little fractionation by phytoplankton) versus phosphorus (P) (large fractionation during N uptake by phytoplankton). The phytoplankton generally have different $\delta^{15}N$ values than terrestrial vegetation, and the N isotopes may function as source markers for autochthonous and allochthonous organic matter (Fry, 2006).

4 Application of Stable Isotope in Carbon Cycle

4.1 Application of ^{13}C natural abundance technique

4.1.1 Photosynthetic path and CO_2 fixation

Photosynthesis is the most important process governing the C isotope fractionations in nature. In the photosynthetic process, the plant leaves prefer to assimilate $^{12}CO_2$ and exclude $^{13}CO_2$, and the phenomena is more obvious as the stomata in leaves completely open (Farquhar *et al*., 1989). Carbon fixation (CO_2) by the C_3, C_4 and CAM plants involves different C fractionations as mentioned above, with the average $\delta^{13}C$ values in plants of –28‰, –13‰ and –16‰, respectively. Smith and Epstein (1971) firstly indicated that the $\delta^{13}C$ could be used to identify the C_3 and C_4 photosynthetic paths of plant. Compared with the conventional methods (morphology-anatomy (Kranz structure) and biochemical analysis (RuBPCase and PEPCase activity)), the $\delta^{13}C$ analysis is more effective and accurate. Yin and Wang (1997) determined the photosynthetic paths of the the plants in Northeast China based on the three methods (89 C_4 species and 144 C_3 species), and primarily analyzed the relationship between photosynthetic path and habitat. Tang and Liu (2001) identified the photosynthesis paths of the plants in Inner Mongolia according to the $\delta^{13}C$ analysis, and 82 C_4 species and 198 C_3 species were determined. Although the $\delta^{13}C$ values in plants are controlled by genetic factor, the environmental conditions also can induce the values to be changed (3‰–5‰) (O'Leary, 1981). These environmental conditions mainly include illumination (Zimmerman and Ehleringer, 1990), temperature (Panek and Waring, 1997), moisture (Saurer *et al*., 1995), CO_2 concentration (Williams *et al*., 2001), mineral nutrition (Guehl *et al*., 1995), precipitation (Anderson *et al*., 2000) and atmospheric pollutant (Martin and Thbrston, 1988). Zimmerman and Ehleringer (1990) found that the $\delta^{13}C$ values in Pananian orchid (*Catasetum viridiflaum*) were correlated with the irradiance levels in habitat, and the values in shade orchid were generally lower than those in sun orchid. Polley *et al*. (1993) indicated that the $\delta^{13}C$ values in some C_3 plants gradually decreased with increasing CO_2 con-

Table 1 Fractionation factor for various processes in the N cycle

Process	Fractionation factor (α)	Process	Fractionation factor (α)
Mineralization	≈ 1.000	Diffusion of NH_4^+, NH_3, NO_3^- in solution	≈ 1.000
Nitrification	1.015–1.035	Immobilization of NH_4^+-N	1.000–1.002 [2)]
Denitrification	1.020–1.033	Immobilization of NO_3^--N	1.000–1.002 [2)]
NH_3 volatilization	1.020–1.029	Uptake of NH_4^+-N by plants	≈ 1.000
N_2-fixing	0.998–1.002	Uptake of NO_3^--N by plants	≈ 1.000
$NH_4^+ \leftrightarrow NH_3$ in solution	1.020–1.027 [1)]	NO_3^--N leaching	≈ 1.000

Notes: 1) Equilibrium fractionation factor, other examples represent kinetic fractionations; 2) Values < 1.002 are probably more appropriate in most natural situations

Sources: Shearer and Kohl, 1986; Handley and Raven, 1992; Högberg, 1997; and Chang and Choi, 2009

centration, and they had significantly negative correlation. Some environmental factors not only affect the $\delta^{13}C$ values in plants, but also can alter the photosynthetic path of plants. The *Mesembryanthemum crystallinum* would adopt CAM photosynthetic path as the habitat is drought, but as the moisture in the habitat was better, the plants would adopt C_3 photosynthetic path, and the transformations were mainly correlated to the water status in leaf (Winter *et al*., 1982). Smith and Osmond (1987) even indicated that water stress induced the leaves and twigs of *Eriogonum inflatum* to adopt C_3 and CAM photosynthetic paths, respectively. Conversely, the information of time-integrated $\delta^{13}C$ contained in the annual tree rings has been widely applied to reconstruct the key palaeoenvironment factors (such as precipitation, temperature and humidity) during the growing season in which the wood is formed. Saurer *et al*. (1995) compared the $\delta^{13}C$ values in tree rings of beech, pine and spruce, and found that the $\delta^{13}C$ values were generally high in the trees growing in the arid habitat. Saurer *et al*. (1997) studied the $\delta^{13}C$ variations in cellulose of rings of beech (*Fagus sylvatica*) in the Swiss Central Plateau to distinguish climatic (precipitation) from local effects, and proved the total precipitation in May, June and July to have the strongest effect on the $\delta^{13}C$ ($R = -0.73$).

4.1.2 CO_2 exchange and carbon balance of ecosystem

Stable C isotopes have been proved useful as tools for assessing the global C balance, and the significance of the terrestrial C sink and its contribution to the global C fluxes relative to the oceans. Since 1990, the stable C isotope composition of tropospheric CO_2 ($\delta^{13}C_{trop}$) has been used in general circulation models (GCMs) to calculate the global C budget and analyze the position and quantity of C source/sink. When C isotope ratios ($\delta^{13}C$) of atmospheric CO_2 are incorporated into global inverse models, the simulations suggest that large C sinks occur in the Northern Hemisphere (Francey *et al*., 1995). However, lacking sufficient data from terrestrial ecosystems, important physiologically based input parameters such as the ratio of internal to atmospheric CO_2 concentration [CO_2] (C_i/C_a) and the C isotopic composition of respired CO_2 have been estimated by using models for the dominant plant species within different biomes. As the regional and temporal changes of terrestrial C flux may induce the fluctuations of [CO_2] and $\delta^{13}C$ in troposphere, better quantification of ecophysiological parameters and understanding of regional and temporal variation in these parameters could prove useful in identifying potential mechanisms constraining atmosphere/terrestrial ecosystem dynamics. Since there seems to be no significant discrimination during respiration, respired CO_2 has a $\delta^{13}C$ close to that of the organic substrate. This information has been successfully used to describe the mixing of tropospheric and respired CO_2 within the canopy of terrestrial ecosystems (Lloyd *et al*., 1996). Farquhar *et al*. (1989) proposed a relationship between the C isotope discrimination of a leaf (Δ_{leaf}) and its C_i/C_a. Ehleringer *et al*. (1993) revealed close relationships between leaf C discrimination and the ratio of CO_2 to water fluxes at the leaf level. Lloyd and Farquhar (1994) extended this application and modeled C isotope discrimination of entire canopies. Buchmann *et al*. (1998) further put forward the concept of ecosystem C isotope discrimination (Δ_e, a measure for C exchange between terrestrial ecosystems and the troposphere), which was used to describe the C dynamics of the entire ecosystem.

$$\Delta_e = \frac{\delta^{13}C_t - \delta^{13}C_r}{1 + \delta^{13}C_r} \tag{3}$$

where $\delta^{13}C_t$ is the C isotope ratio of the troposphere and $\delta^{13}C_r$ is the C isotope ratio of respired CO_2. Thus, the familiar concept of C isotope discrimination at the leaf level is transferred to the ecosystem level. Because the Δ_e values are based on $\delta^{13}C$ of tropospheric CO_2 and on field measurements of canopy air to estimate $\delta^{13}C$ of respired CO_2, detailed information on canopy profiles of [CO_2] and $\delta^{13}C$ is needed. Several factors affect the canopy [CO_2] and its $\delta^{13}C$, such as turbulent mixing with the troposphere (Lloyd *et al*., 1996), stand structure (Buchmann *et al*., 1998), C exchange responses of over- and under-story plants to abiotic factors, as well as soil C exchange as influenced by soil respiration and litter decomposition (Buchmann *et al*., 1997). Presently, the Δ_e variations have been assessed in different biomes, ranging from boreal (15.9‰–19.3‰) to temperate (16.1‰–20.3‰) to tropical forests (19.5‰–21.1‰). Keeling (1958) found the relationship between $\delta^{13}C_a$ (C isotope ratio of the atmospheric CO_2) and $1/C_a$ (reciprocal of atmospheric [CO_2]), and established the Keeling plot approach to partition the net ecosystem C fluxes into C uptake during photosynthesis and C release during respiration.

$$\delta^{13}C_a = C_b \times (\delta^{13}C_b - \delta^{13}C_s)(1/C_a) + \delta^{13}C_s \quad (4)$$

where C_b and C_s are the background values of atmospheric [CO_2] and the added source [CO_2], respectively, and $\delta^{13}C_b$ and $\delta^{13}C_s$ are the C isotope ratios of the corresponding parts. The intercept of Keeling plot represents the spatial and temporal integration of $\delta^{13}C$ of respired CO_2. Presently, the Keeling plot approach has been widely applied to the C flux researches of forest ecosystem (Buchmann *et al*., 1997), grassland ecosystem (Ometto *et al*., 2002) and agro-ecosystem (Buchmann and Ehleringer, 1998). As the respired CO_2 can be partly recycled by plant and the $\delta^{13}C$ of respired CO_2 is much lower than that of the atmospheric CO_2, the recycle amount of respired CO_2 can be quantified through analyzing the $\delta^{13}C$ of plant tissues and ambient atmospheric CO_2 in different heights of canopy. Sternberg (1989) improved the Keeling formula, and the recycle of CO_2 was considered.

$$\delta_F = \{(\delta_a - \delta_R) \times [CO_2]_a \times (1 - f_s) / [CO_2]_F\} + \delta_R + f_s \times \delta \quad (5)$$

where δ_F, δ_a and δ_R are the isotope compositions of environment, atmosphere and respired CO_2, $[CO_2]_a$ and $[CO_2]_F$ are the CO_2 concentrations of troposphere and ambient environment, δ is the C isotope discrimination during photosynthesis, and f_s is the recycle index of CO_2. If the recycle of CO_2 does not occur ($f_s = 0$), then the above formula is changed into Keeling formula. At the global scale, the judgment of global C pool distribution and the relative contributions of terrestrial and ocean ecosystems to the global C cycle can be studied by combing stable C isotope techniques, the Keeling plot approach and terrestrial ecosystem models. However, application of stable isotope techniques and the Keeling plot approach to ecological research are sometimes constrained by the heterogeneity of terrestrial ecosystems when the study scale is transferred to the global level.

4.1.3 Composition, distribution and turnover of soil organic carbon

Stable C isotopes have been proved useful for studying the C cycle processes in soil in long temporal and large spatial scale, which are especially appropriate for the research of C distribution, C dynamic, C stock and C turnover in soils of different ecosystems (Del Galdo *et al*., 2003). Because soil organic carbon (SOC) mainly originates from terrestrial higher plants and the fractionation of SOC decomposition in soil is much lower than that of CO_2 fixation by plant photosynthesis (Nadelhoffer and Fry, 1988), the $\delta^{13}C$ of SOC is close to its derived plant. This information has been successfully used to reflect the isotope composition characteristics of CO_2 fixation by plant in local region, and the contributions of different parts to the isotope composition of SOC (Ehleringer *et al*., 2000). The $\delta^{13}C$ of SOC in C_3 and C_4 ecosystems are different, and the average $\delta^{13}C$ of SOC in degraded grassland is very high (de Camargo *et al*., 1999). The transformation of C_3 and C_4 plants in agro-ecosystem, forest ecosystem and grassland ecosystem generally has continuous effects on the $\delta^{13}C$ of SOC and soil respired CO_2 (Buchmann and Ehleringer, 1998), indicating that the $\delta^{13}C$ of SOC records the transformation history between C_3 and C_4 ecosystems. At present, the $\delta^{13}C$ of SOC are used to determine the sources of SOC, identify the contributions of C_3 and C_4 plants to the soil C stock, and restore the dominant vegetation and the historical pattern of ecosystem (Tieszen *et al*., 1997; Ehleringer *et al*., 2000). As the transformation of different plants comprises the ^{13}C transfer signals of the derived organic C, the isotope mass balance equation is usually applied to estimate the proportions of SOC derived from C_3 and C_4 plants (de Camargo *et al*., 1999).

$$\%C_3 \times \delta^{13}C_3 + \%C_4 \times \delta^{13}C_4 = 100 \times \delta^{13}C_{sample} \quad (6)$$

where $\%C_3$ and $\%C_4$ are the proportions of SOC derived from C_3 and C_4 plants, respectively, and $\delta^{13}C_{sample}$ represents the ^{13}C composition of soil sample. A typical restoration research of the historical pattern of ecosystem was reported by Delegue *et al*. (2001) who studied the ebb and flow of savannah and forest across landscapes in Gabon (Africa) over thousands of years through analyzing the $\delta^{13}C$ values of soils from six sites where C_4 savannah grasses (–12‰) and forest trees (–29‰) contributed to SOC. Strong latitudinal gradients in C_3/C_4 composition occur across grasslands throughout the world and are linearly correlated with growing season temperature (Ehleringer *et al*., 1997). Consequently, C_4-dominated grasslands occur at lower latitudes, while C_3-dominanted grasslands occur at higher latitudes (Ehleringer *et al*., 2000). Similar aboveground C_3/C_4 gradients exist along elevational transects, mirroring the changes in temperature with elevation (Bird *et al*., 1994; Tieszen *et al*., 1997). Wang *et al*. (2003) found that the $\delta^{13}C$ values of C_3 plants in the Qinghai-Xizang Tibetan Plateau generally increased with altitude (1.37‰/km), and temperature and CO_2 partial pressure were the major factors causing the increase of $\delta^{13}C$. Latitude and elevation are to a large degree inter-

changeable (Ehleringer *et al*., 2000). Along the elevational transect in Papua New Guinea, Bird *et al*. (1994) observed a similar pattern as was seen in the latitudinal grassland gradients: Along these elevation gradients (0–4000 m) C_3 and C_4 end members were –26‰ and –13‰, respectively. Therefore, the spatial and temporal distribution pattern of C_3 and C_4 plants and the history of climate change can be easily revealed through studying the distribution of $\delta^{13}C$ of SOC and the relationship between $\delta^{13}C$ and climate parameters (Yu *et al*., 2005). The $\delta^{13}C$ values of SOC in profile generally increase with increasing soil depth, and the $\delta^{13}C$ enrichment of SOC is more significant in C_3 ecosystems (de Camargo *et al*., 1999). Many researches show that the $\delta^{13}C$ enrichment is not related to the physical and chemical properties of soil, but related to the isotope fractionations of litter decomposition and humification (Del Galdo *et al*., 2003). As the decomposition of SOC in different depths is situated in different phases (Melillo *et al*., 1982), the distribution of $\delta^{13}C$ in soil profile can be used to study the turnover rates and transformations of SOC.

4.1.4 Sources of organic matter in food webs

Stable isotopes analysis ($\delta^{13}C$, $\delta^{15}N$) has become a powerful tool for the study of food web ecology in recent years (Gu, 2009). Many researches show that there is little or no C isotope fractionation during consumer assimilation and growth (DeNiro and Epstein, 1978), and the average ^{13}C enrichment factor is only 0.4‰ (Post, 2002). Differently, the stable N isotopes of consumers are enriched systematically during trophic transfers, and the average ^{15}N enrichment factor is 3.4‰ (Post, 2002). Therefore, $\delta^{13}C$ is generally used to index food sources and C flow in ecosystem, while $\delta^{15}N$ is applied to indicate the trophic position of consumers. The organic matters of different sources generally possess distinct stable isotope signals ($\delta^{13}C$, $\delta^{15}N$) (Peterson and Fry, 1987), and these signals provide the probability for us to distinguish different sources. When the consumer has two food sources, a single stable isotope (such as $\delta^{13}C$) approach can be used to determine the definite source, and the following two-source mixing model is applied to determine the contributions of different sources (Fry, 2006).

$$f_1 = (\delta^{13}C_{\text{sample}} - \delta^{13}C_{\text{source1}}) / (\delta^{13}C_{\text{source1}} - \delta^{13}C_{\text{source2}}) \quad (7)$$

$$f_1 + f_2 = 1$$

where $\delta^{13}C_{\text{sample}}$, $\delta^{13}C_{\text{source1}}$ and $\delta^{13}C_{\text{source2}}$ are the C isotope ratios of sample, source 1 and source 2, respectively, and f_1 and f_2 are the fractional contribution of source 1 and source 2, respectively. Ramsay and Hobson (1991) studied the $\delta^{13}C$ in polar bear (*Ursus maritimeus*) tissues and found that the amount of food that polar bears consumed from terrestrial food webs appeared negligible, even though some bears spend 1/3 or more of each year on land during the seasons of greatest primary productivity. Magnusson *et al*. (1999) determined the contributions of C_3 and C_4 plants to higher trophic levels in a central Amazonian savanna by comparing the $\delta^{13}C$ of potential food plants to the $\delta^{13}C$ of different consumers. Although the grasshopper (*Tropidacris collaris*) completed its entire life cycle within the savanna and ate a variety of plants, about 90% of its dietary C was derived from C_3 plants. Both species of leaf-cutter ants (*Acromyrmex latticeps nigrosetosus* and *Atta laevigata*) obtained the major part of their diet (–70%) from C_3 plants. Two species of termite (*Nasutitermes* sp. and *Syntermes molestus*) obtained most of their diet from C_4 grasses. As termites were the major prey items for many of the lizards and frogs, more than 50% of their dietary C from food chains originating in C_4 grasses. In contrast with single isotope approach, dual-isotope (such as ^{13}C and ^{15}N) approach may provide significantly more power to distinguish the different sources (Peterson *et al*., 1985) and reconstruct the species′ ecological history. Hilton *et al*. (2006) studied the causes of population decline of rochhopper penguin (*Eudyptes chrysocome*) through employing the stable isotope analyses of $\delta^{13}C$ and $\delta^{15}N$ in time-series of feathers. The $\delta^{13}C$ and $\delta^{15}N$ signatures decreased significantly over time in penguin, indicating that the population decline might be associated with a shift towards lower primary productivity in the ecosystem in which they fed, and with a shift to a diet of lower trophic status and lower quality. The sulfur (S) isotope (^{34}S) is an additional tool for studying energy flow of ecosystem, particularly when used with ^{13}C and ^{15}N (Peterson *et al*., 1985). The use of a combination of the stable isotopes of S, C, and N allows the flow of organic matter and trophic relations in ecosystem to be traced while eliminating many ambiguities that accompany the use of a single isotopic tracer. For the S isotope to be useful as a tracer in food web studies, we must know whether there are large changes in isotopic composition

between organism′s diet and its body tissue. When there are three or more potential sources, the multiple isotopes ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) approach is generally applied to distinguish different sources. If there are three potential sources and the $\delta^{13}C$ and $\delta^{15}N$ is applied, the contributions of different sources can be determined by the following equations (Fry, 2006).

$$f_1 + f_2 + f_3 = 1$$

$$f_1 \times \delta^{13}C_1 + f_2 \times \delta^{13}C_2 + f_3 \times \delta^{13}C_3 = \delta^{13}C_{\text{sample}} \quad (8)$$

$$f_1 \times \delta^{13}N_1 + f_2 \times \delta^{13}N_2 + f_3 \times \delta^{13}N_3 = \delta^{13}N_{\text{sample}} \quad (9)$$

where the three sources are denoted by the subscripts 1–3, and f is the fractional contribution of each source. $\delta^{13}C_{\text{sample}}$, $\delta^{13}C_1$, $\delta^{13}C_2$ and $\delta^{13}C_3$ are the C isotope ratios of sample, source 1, source 2 and source 3, respectively, and $\delta^{15}N_{\text{sample}}$, $\delta^{15}N_1$, $\delta^{15}N_2$ and $\delta^{15}N_3$ are the N isotope ratios of sample, source 1, source 2 and source 3, respectively. Peterson *et al*. (1985) determined the contributions of three potential food sources (upland C_3 plants, *Spartina alterniflora* (C_4 plant) and plankton) of ribbed mussel (*Geukensia demissa*) in a New England marsh by using multiple isotope approach ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$). *S. alterniflora* and plankton were the main food sources for the ribbed mussel, and the contribution of upland plants was little. Mussels in the interior portions of the marsh had an isotopic composition that reflected a diet of 80% *S. alterniflora*, whereas the diet of mussels near Buzzards Bay consisted of up to 70% plankton and only 30% *S. alterniflora*. Moncreiff and Sullivan (2001) studied the trophic importance of epiphytic algae in a northern Gulf of Mexico, and found that the epiphytic algae was the primary source of organic matter for higher trophic levels in seagrass (*Halodule wrightii*) beds of Mississippi Sound, while the contribution of *H. wrightii* to the food web appeared to be minimal. Although the use of multiple isotopes allows us to distinguish three or more potential food sources, there are often many sources (5–10, or even more) and not enough tracers (Phillips and Gregg, 2003). Therefore, when there are more sources, the multiple isotope approach only can distinguish the main sources by dividing these potential sources into different groups according to the isotope values of similar sources.

4.2 Applications of ^{13}C labeled technique

4.2.1 Effects of elevated CO_2 on carbon processes of ecosystem

The ^{13}C labeled technique has been proved effective as a tool for studying C cycling processes. At the ecosystem scale, the ^{13}C labeled experiment ($^{13}CO_2$) is generally hard to be carried out for the labeled chemicals are still very expensive and the cost is high. However, a relative research has been reported by Hagedorn *et al*. (2004b) who made use of large CO_2 enrichment experiments in natural forest ecosystems to analyze the potential as sinks for elevated CO_2 of soil stock and the turnover rates of C in different soil pools. Three years of CO_2 (^{13}C-depleted CO_2) enrichment had little effect on dissolved organic matter (DOC) concentrations, but significantly decreased the $\delta^{13}C$ values in DOC. Compared with DOC, soil CO_2 responded strongly to the addition of $^{13}CO_2$, indicating that a large fraction of C was cycling rapidly through the plant and soil system and was respired back to the atmosphere. In some small experiments, the ^{13}C labeled technique is widely used to understand the effects of elevated CO_2 concentration on the C uptake and allocation in plant (Stewart and Metherell, 1999), the C turnover of ecosystem (Hungate *et al*., 1997), and the stability and fate of C in soil (Hungate *et al*., 1997; Hagedorn *et al*., 2003). Hagedorn *et al*. (2003) estimated the influence of two forest soils (acidic loam and calcareous sand) on the net input of new C into soils under CO_2 (^{13}C-depleted CO_2) enrichment. The overall effects of CO_2 enrichment on soil C were small in both soils, and the potential of soils for C sequestration was limited as only a small fraction of new C input into soils would become long-term soil C. Moreover, some researches have successfully applied dual stable isotope (^{13}C and ^{18}O) technique to partition soil CO_2 efflux into three components (rhizosphere respiration, litter decomposition, and SOM oxidation), and found that the components responded differently to elevated CO_2 and elevated temperature (Lin *et al*., 1999; 2001)

4.2.2 Source and fate of organic matter in ecosystem

The source and fate of organic matter in ecosystem is one of the most important research fields of ecology. In terrestrial ecosystem, the ^{13}C labeled technique has been applied to trace the sources of different C components (Hagedorn *et al*., 2004a) and the microbial decomposition of organic matter (Thompson, 1996) through adding ^{13}C labeled-carbohydrate into soils. Hagedorn *et al*. (2004a) identified the origin of DOC in forested mineral soils, and suggested that DOC was produced during incomplete decomposition of recalcitrant native C in the

soils, whereas easily degradable new components were rapidly consumed by microbes and thus made only a minor contribution to the dissolved C fraction. The ^{13}C labeled technique is also used to investigate the transfer of C among plants (Simard *et al*., 1997) and the fate of organic matter in plants (Amiard *et al*., 2003). Simard *et al*. (1997) found that the C transfer between *Betula papyrifera* and *Pseudotsuga menziesii* was primarily through the direct hyphal pathway, and the source-sink relationships regulated such C transfer under field conditions. Amiard *et al*. (2003) indicated that, at the end of the 4 h labeling period on defoliated plants (*Lolium perenne* L.), 77% of the ^{13}C-fructose incorporated was still located in leaf sheaths, and only 4% and 0.9% were, respectively, allocated to stem and roots, while 18% was imported by the growing leaves.

In aquatic ecosystem, the origin of organic matter mainly includes autochthonous production and allochthonous production. For a long time, ecologists consider that the running of aquatic ecosystem is mainly driven by autochthonous production, and the produced organic matter is transferred in food web through animal ingestion and detrital pathway. The opinion ignores the fact that large amounts of allochthonous organic matter are imported into aquatic ecosystem or considers that the allochthonous organic matter can not be utilized by biology due to its recalcitrant decomposition (Pace *et al*., 2004). At present, the role of allochthonous organic matter in aquatic ecosystem is paid more and more attention, and the traditional opinion is challenged. More and more proofs indicate that the allochthonous organic matter is actually the main energy source of aquatic bacteria, and it also has important contributions to the different nutrient-enriched aquatic ecosystems (Gu, 2007). However, it is difficult to estimate the contributions of autochthonous and allochthonous organic matter to aquatic ecosystem by the traditional C budget method. Although the stable isotope natural abundance technique can estimate the contributions of different C sources to food web, as the isotope ratios of terrestrial and aquatic primary producer are very close, the C sources of food web can not be easily distinguished (Cole *et al*., 2002). The ^{13}C labeled technique has been widely used to acquaint the effects of allochthonous organic matter to the aquatic ecosystem by adding ^{13}C labeled-carbohydrate into water-body. As the aquatic botany can absorb ^{13}C-enriched carbohydrate, the $\delta^{13}C$ values of botany are generally higher than those of allochthonous organic matter. Thus, the role of allochthonous organic matter in different nutrient-enriched aquatic ecosystems can be well understood by analyzing the isotope compositions of biology in different trophic levels. The two-source mixing model is generally applied to calculating the contributions of allochthonous organic matter to aquatic food web (f_{all}) (Fry, 2006; Gu, 2009).

$$f_{all} = (\delta^{13}C_{con} - \delta^{13}C_{aut}) / (\delta^{13}C_{all} - \delta^{13}C_{aut}) \quad (10)$$

where $\delta^{13}C_{aut}$ and $\delta^{13}C_{all}$ represent the autochthonous and allochthonous isotope ratios, respectively, and $\delta^{13}C_{con}$ is the isotope ratio of consumers in aquatic ecosystem. Cole *et al*. (2002) evaluated the roles of allochthonous and autochthonous organic C by manipulating ^{13}C content of dissolved inorganic carbon (DIC) in a lake, and showed that the oxidation of terrestrial DOC was the major source of DIC in the lake, and the zooplankton production was predominantly derived from current autochthonous C sources. Kritzberg *et al*. (2004) examined the importance of autochthonous versus allochthonous DOC in supporting the growth of pelagic bacteria, and indicated that the bacterial biomass consisted of 35%–70% allochthonous C, which confirmed the often-stated hypothesis that autochthonous C alone did not support bacterial production. Although 13% of the DOC standing stock was of recent autochthonous origin, it supported 30%–65% of bacterial production. Similar results were also reported by Pace *et al*. (2004) who found that the internal primary production was insufficient to support the food webs of the studied aquatic ecosystems, 40%–55% of particulate organic C and 22%–50% of zooplankton C were derived from terrestrial sources, indicating that there was significant subsidy of these ecosystems by organic C produced outside their boundaries.

5 Application of Stable Isotope in Nitrogen Cycling

5.1 Application of ^{15}N natural abundance technique

5.1.1 Biological N_2-fixation

Stable N isotope technique has been successfully applied to the research of biological N_2-fixation since the 1950s. ^{15}N natural abundance technique is one of the most effective methods which is based on the difference in $\delta^{15}N$ of N_2-fixing and non-N_2-fixing plants that grow

in the same site (the $\delta^{15}N$ of non-N_2-fixing plant is higher than that of N_2-fixing ones), and based on the understanding that N_2-fixing usually causes little N isotopic fractionation and soil available N is usually enriched in ^{15}N relative to atmospheric N (Chang and Choi, 2009). The percent of N derived from the atmosphere (%*Ndfa*) and total fixed N (TN_{fixed}) can be calculated by the following equations (Shearer and Kohl, 1993):

$$\%Ndfa = \frac{\delta^{15}N_{\text{ref}} - \delta^{15}N_{\text{fixed}}}{\delta^{15}N_{\text{ref}} - \delta^{15}N_{\text{hrdro}}} \times 100 \quad (11)$$

$$TN_{\text{fixed}} = \%Ndfa \times B \times P \times N_{\text{p}} \quad (12)$$

where $\delta^{15}N_{\text{ref}}$ and $\delta^{15}N_{\text{fixed}}$ are the $\delta^{15}N$ values of non-N_2-fixing plants and N_2-fixing plants, respectively; $\delta^{15}N_{\text{hydro}}$ is the $\delta^{15}N$ value of N_2-fixing plants that grow hydroponically with N-free nutrient medium; *B* is plant biomass; *P* is the percent of N_2-fixing plants and N_{p} is the total N content in N_2-fixing plant. The relative researches mainly focused on the identification of N_2-fixing plants (Kohls *et al*., 1994), the N_2-fixing ability or contribution of legumes (Kohls *et al*., 1994; Gathumbi *et al*., 2002), and the effects of N_2-fixing plants on the nutrient cycling of ecosystem (Stock *et al*., 1995). Gathumbi *et al*. (2002) determined the contributions and amounts of N_2-fixation of herbaceous and woody legumes in improved fallows of western Kenya, and indicated that the proportions of N_2 fixed ranged 75%–83%, 63%–74%, 55%–67%, 46%–59%, 36%–54%, 35%–50%, and 36%–51% for *Crotalaria grahamiana*, *Tephrosia vogelii*, *Cajanus cajan*, *Sesbania sesban*, *Calliandra calothyrsus*, *Macroptilium atropurpureum* and *Arachis hypogaea*, and the average amounts of N_2-fixed were 42 kgN/ha, 100 kgN/ha, 91 kgN/ha, 52 kgN/ha, 24 kgN/ha, 64 kgN/ha and 8 kgN/ha, respectively, 9 months after planting. Stock *et al*. (1995) found that the invasion of *Acacia* species (*A. saligna* and *A. cyclops*) not only resulted in organic matter and nutrient enrichment of surface soils in invaded ecosystem, but also enhanced the N mineralization rates, indicating the strong influence of the alien species on the soil N component. Similar results were also reported by Kohls *et al*. (1994) who thought that the *Dryas* taxa served as a source of N for non-N_2-fixing species through the N transfer process.

5.1.2 Nitrogen source identification of ecosystem

Besides biological N_2-fixation, N deposition, anthropogenic N input and N runoff input are also important N sources of ecosystem. These N sources generally have different $\delta^{15}N$ values due to the differences of isotope fractionation effect. Since the composition of particulate suspended and sedimentary organic matter contains information on the mixing of the various organic matter sources and their subsequent degradation history, the differentiation of N sources can be identified based on the elemental, isotopic and molecular biomarker information (Middelburg and Nieuwenhuize, 1998). Michelsen *et al*. (1996) analyzed the $\delta^{15}N$ values in leaves of 23 subarctic vascular plant species and two lichens, as well as in soil, rain and snow, and found that coexisting plant species under severe nutrient limitation might tap several different N sources: NH_4^+-N, NO_3^--N, organic N from the soil, atmoshpheric N_2, and N in precipitation. Abbadie *et al*. (1992) determined four major sources (bulk precipitation, mineralization of humified soil organic matter, atmospheric N_2 fixation, and decomposition of plant litter) of mineral N that could meet the annual requirements of plants. Until now, increased anthropogenic N addition to coastal waters has been a worldwide major agent of change for coastal ecosystems, and the $\delta^{15}N$ in organisms can be used to indicate the sources of N input to ecosystem. McKinney *et al*. (2001) found that the $\delta^{15}N$ in tissue of the ribbed mussel (*Geukensia demissa*) in Narragansett Bay had significant positive correlations with the fraction of residential development in the marsh watersheds, while had significant negative correlations with the fraction of combined agricultural and recreational land use, suggesting that the mussel N isotope signature was influenced by N derived from human activities in the adjoining marsh watershed. Carmichael *et al*. (2004) indicated that even at low N loads, N from land-derived sources moved detectably up the food web in the estuaries of Pleasant Bay. Combined with elemental information and other isotope ratios ($\delta^{13}C$ or $\delta^{34}S$), the $\delta^{15}N$ is generally applied to determining the sources of organic matter. Middelburg and Nieuwenhuize (1998) analyzed the C/N, $\delta^{13}C$ and $\delta^{15}N$ of sedimentary and suspended particulate matter in the Schelde Estuary, and four major pools of organic matte (riverine, estuarine, marine and terrestrial materials) were determined. Although the relationship between $\delta^{13}C$ and $\delta^{15}N$ could separate all four endmembers, the values of $\delta^{13}C$ and C/N ratios overlapped, which could not be used to distinguish between river-

ine and estuarine materials. Moreover, the relationship between $\delta^{15}N$ versus C/N ratio also could not be applied to distinguishing between marine and riverine particles. Similar problem was encountered by Graham *et al*. (2001) when they applied the stable isotope ($\delta^{13}C$ and $\delta^{15}N$) and elemental ratios (C/H and C/N) to investigating the importance of both natural and anthropogenic sources of organic matter in bottom sediments of the Forth Estuary. The main reasons were related to the narrow ranges of $\delta^{15}N$ and C/N over the entire length of the estuary, and the weak correlations between $\delta^{15}N$ or C/N and $\delta^{13}C$ ($R^2 = 0.127$ and $R^2 = 0.037$, respectively). In addition, the original source signatures of organic matter also might be lost or overprinted by biochemical alteration, and the information afforded by $\delta^{15}N$ and C/N ratio were the integration of sources and biogeochemical processes (Wu *et al*., 2002).

In recent years, the use of the $\delta^{15}N$ of plant tissues as a potential technique to identify the use of synthetic fertilizer in agroecosystem has been paid more attention (Bateman *et al*., 2005). The $\delta^{15}N$ of synthetic fertilizer is generally lower than that of organic fertilizer, and the mean $\delta^{15}N$ is –0.2‰ for synthetic fertilizer, 8.1‰ for manure/compost, 2.5‰ for seaweed-based, 5.9‰ for non-manure wastes of livestock, and 7.1‰ for fish-based materials (Bateman and Kelly, 2007). The current researches have showed that crops grown with synthetic fertilizer tend to have consistently lower $\delta^{15}N$ (0–5‰) than those with non-synthetic fertilizer (3‰–15‰) if other conditions are the same (Chang and Choi, 2009). Obviously, the higher the $\delta^{15}N$ and N availability of the organic fertilizer, the higher the $\delta^{15}N$ in crop tissues. However, identifying organic from conventional grown crops applying a critical $\delta^{15}N$ range is not yet a straightforward task since there is overlap in $\delta^{15}N$ signatures between organic and conventional grown crops (Table 2). Although the variation of $\delta^{15}N$ in crops as affected by the $\delta^{15}N$ of applied non-synthetic fertilizer is well demonstrated, the $\delta^{15}N$ of crops also can be influenced by many factors, such as crop species, fertilization frequency, environmental conditions and field management. Currently, since there is no other reliable technique available, the $\delta^{15}N$ technique still can be used as an assistant tool to identify the use of synthetic fertilizers in organic farming (Chang and Choi, 2009).

5.1.3 Nitrogen transformation processes of ecosystem

The N transformation processes of ecosystem mainly include assimilation, mineralization, immobilization, nitrification and denitrification, which are illustrated with different fractionation effects, and as a result, the different N pools have significantly different $\delta^{15}N$ signatures. The comprehension of $\delta^{15}N$ in different N pools may provide the probability for us to trace the N transformation processes in ecosystem (Fry, 2006). Lund *et al*. (2000) indicated that denitrification played a signifycant role in the removal of NO_3^--N in a constructed wetland of California, and nitrification and assimilation by macrophytes also had important impacts. However, Fukuhara *et al*. (2007) showed that the contribution of denitrification to NO_3^--N loss was minor (only accounted for (19.5 ± 7.0) %), whereas that of plant uptake was (80.5 ± 7.0)%, indicating the importance of vegetation in sand dune riparian zone of of *L. Kamisagata*. Since the $\delta^{15}N$ values of N_2-fixing and non-N_2-fixing plants have significant differences (Chang and Choi, 2009), the ^{15}N natural abundance technique is generally applied to analyzing the N processes between N_2-fixing and non-N_2-fixing plants (Van Kessel *et al*., 1994) and the N nutrient status among plant species (Matsushima and Chang, 2007). Van Kessel *et al*. (1994)

Table 2 Ranges of $\delta^{15}N$ in part crops as affected by type of fertilizer applied

Type	$\delta^{15}N$ of crop (‰)	Dominated crop	Reference
No fertilization	+6.8–+7.7	Chinese cabbage	
Synthetic fertilizer			Lim *et al*. (2007)
Urea	+3.2–+3.3	Chinese cabbage	
Ammonium nitrate	+3–+4	Carrots	Bateman *et al*. (2005)
Urea+ammonium phosphate	+0.3–+2.5	Canola, hull-less barley, wheat	
Non-synthetic fertilizer			Choi *et al*. (2006)
Liquid hog manure	+5.6–+8.4	Canola, hull-less barley, wheat	
Solid manure	+2.2–+4.1	Canola, hull-less barley, wheat	
Composted manure	+14.6 ± 3.3	Eggplant, Hot pepper, Brassicas	Choi *et al*. (2003)

found that a portion of the N_2 fixed by *Leucaena leucocephala* during the early stages of tree growth was made available to the understorey species through the decomposition and subsequent incorporation into the available soil-N pool of the abscissed *L. leucocephala* parts, which provided the direct evidence of internal N cycling between a N_2-fixing tree and non-N_2-fixing understorey species. Matsushima and Chang (2007) indicated that the Canada bluejoint grass (*Calamagrostis canadensis*) significantly reduced the $\delta^{15}N$ in the foliar of the co-planted white spruce (*Picea glauca*), which was related to the causes that strong NH_4^+-N uptake by bluejoint might have prevented significant soil N losses and ^{15}N enrichment through nitrification and subsequent denitrification.

Because most ecosystems in nature are considered open systems, they are frequently influenced by lots of disturbance regimes. Disturbance regimes can cause alterations in the rate and pathway of N cycling processes and such changes can result in the changes in the degree of N isotopic fractionation. In turn, changes in $\delta^{15}N$ signatures in various N pools can serve as a tool to infer the changes in the N transformation processes (Chang and Choi, 2009). A typical disturbance effect on the isotopic composition of different N pools in ecosystems is the effect of soil compaction and forest floor (FF) removal. Choi *et al*. (2005a) indicated that soil compaction reduced NH_4^+-N concentrations in the FF (from 48.5 mg/kg to 28.0 mg/kg) and NO_3^--N concentrations in the FF (from 13.8 mg/kg to 6.4 mg/kg) and mineral soil (from 4.3 mg/kg to 2.1 mg/kg), and FF removal tended to decrease NH_4^+-N concentrations in the mineral soil. Similar results were reported by Tan *et al*. (2006) when they understood the effects of soil compaction and forest floor removal on the soil N dynamics and N acquisition by aspen and white spruce. Another typical disturbance effect is the drainage, which will have dramatic effects on the N cycling of wetland ecosystem. Choi *et al*. (2007) found that drainage increased the $\delta^{15}N$ of soil NH_4^+-N from 0.6‰–2.9‰ to 4.6‰–7.0‰ most likely through increased nitrification following enhanced mineralization. Plant uptake of ^{15}N-enriched NH_4^+-N in the drained treatment resulted in higher plant $\delta^{15}N$ (between 0.8‰ and 1.8‰ in the drained and between –3.9‰ and –5.4‰ in undrained plots), and deposition of litterfall N enriched with ^{15}N increased the $\delta^{15}N$ of total soil N in the surface layer in the drained (+2.9‰) as compared with that in the undrained plots (+0.6‰).

Although many researches applied foliar $\delta^{15}N$ signature to inferring soil N availabilities and dynamics (Tan *et al*., 2006), few studies used tree ring $\delta^{15}N$ to explain changes in soil N status. Since the N taken up by plants can have specific $\delta^{15}N$ signatures and those signatures may be preserved in the wood formed in that year, temporal patterns of $\delta^{15}N$ of tree ring samples may be used as isotopic indication of the historical alterations in soil N dynamics and associated environmental changes. Choi *et al*. (2007) compared the annual pattern of $\delta^{15}N$ of black spruce and tamarack rings between drained and undrained plots and found that annual rings provided an isotopically distinct and historically preserved record of changes in soil N dynamics. In general, the decreasing $\delta^{15}N$ in annual growth rings over time is more frequently observed during the past decades (Chang and Choi, 2009). Peñuelas and Estiarte (1997) related the decreasing $\delta^{15}N$ of downy oak over time to decreased N loss, increased N fixation and mineralization over the past century. The $\delta^{15}N$ in rings is also applied to investigating the history of atmospheric deposition. Choi *et al*. (2005b) found that the N concentration increased with decreasing $\delta^{15}N$ of tree rings ($R = -0.84$, $p < 0.01$) during the period (since the 1980s) of increasing NO_x emission (with lower $\delta^{15}N$), which was consistent with the hypothesis that increasing deposition of N depleted in ^{15}N might lead to ^{15}N depletion in tree tissues.

5.1.4 Nitrogen trophic status in food webs

The $\delta^{15}N$ not only can trace the sources of organic matter in food webs combined with $\delta^{13}C$ and $\delta^{34}S$, but also can determine the trophic position and trophic niche width of consumers (Gu, 2009). The current researches show that the ^{15}N of consumers are enriched systematically during trophic transfers, and an average increase of 3.4‰ (for vertebrates) is typical (Post, 2002). In food webs where the $\delta^{15}N$ of plants, herbivores and higher-level consumers are determined, if using plants and herbivores as the basal level and basal second level of the food web respectively, the trophic level (*TL*) of a consumer can be correspondingly calculated according to equations (13) and (14):

$$TL = 1 + (\delta^{15}N_{con} - \delta^{15}N_{plant}) / \Delta TL \quad (13)$$

$$TL = 2 + (\delta^{15}N_{con} - \delta^{15}N_{her}) / \Delta TL \quad (14)$$

where $\delta^{15}N_{con}$, $\delta^{15}N_{plant}$ and $\delta^{15}N_{her}$ are the N isotope ratios of consumer, plant and herbivore, respectively. The ΔTL in the denominator in the equations represents the

average permil (‰) increase in per trophic level, most recently estimated as 2.2‰ for invertebrates and 3.4‰ for vertebrates (Fry, 2006). If the $\delta^{15}N$ of top-level consumer is measured, the maximum trophic position (food chain length, FCL) also can be calculated by the above calculations. Vander Zanden and Fetzer (2007) analyzed the *FCL* of global 219 waters and found that the mean FCL (± S.D.) for lake, stream and marine ecosystems were 3.79 ± 0.69, 3.43 ± 0.89 and 3.91 ± 0.90, respectively. Streams had shorter food chains (approximated 3.5 trophic levels) than marine and lake ecosystems (approximated 4 trophic levels). At the global scale, FCL showed weak or no relationships with ecosystem scale, mean annual air temperature, or latitude. As food consumption is known to be an important route of exposure for contaminants uptake in consumers, the trophic level is generally regarded as a very significant factor influencing contaminant levels in aquatic organisms. The determination of trophic position has important significance to the interpretation of contaminant concentrations in consumers (Gu, 2009). Lavoie *et al*. (2010) related the transfer of mercury (Hg) in a Gulf of St. Lawrence food web to the trophic structure, and suggested that Hg would be readily bioavailable to organisms at the base of the benthic food chain, but trophic transfer would be more efficient in each trophic level of pelagic and benthopelagic food chains. Similar results were reported by Swanson *et al*. (2003) when they investigated the invasion of rainbow smelt on the trophic positions and Hg bioaccumulation of Ontario lakes. In addition, the relationship between trophic position and mental accumulation generally differed considerably among metals. Quinn *et al*. (2003) analyzed the trophic transfer of metals (Fe, Cu and Zn) in stream food webs of Montana, and found that Fe declined (biodilution) and Zn increased (biomagnification) with trophic level, but trophic position had no effect on Cu levels in insects.

5.2 Application of ^{15}N labeled technique

5.2.1 Source, transformation and fate of Nitrogen in ecosystem

Besides ^{15}N natural abundance technique, the $^{15}N_2$ enrichment and ^{15}N dilution techniques are also widely applied to studying the biological N_2-fixation (Gu, 2007). In general, the application of $^{15}N_2$ enrichment technique is very limited, and it only can be used in closed condition in laboratory. Gu and Alexander (1993) estimated the fractional contribution of atmosphere-derived N of *Anabaena flos-aquae* in Smith Lake based on both ^{15}N natural abundance and $^{15}N_2$ enrichment techniques, and similar results showed that the algae obtained 58%–75% of its N by N_2 fixation. The principle of ^{15}N dilution technique is based on the difference of ^{15}N abundances (at.%^{15}N) in N_2-fixing and non-N_2-fixing plants when equal quantity and at.%^{15}N of ^{15}N-labeled NH_4^+-N or NO_3^--N is input into soil pool. Since the N_2-fixing plants not only absorb abundant ^{15}N-nutrients from soil N pool, but also fix depleted-^{15}N from atmosphere, the at.%^{15}N in N_2-fixing plants are diluted compared with the non-N_2-fixing plants. Actually, both the ^{15}N dilution and natural abundance techniques have the same principle, and they are applied in most of the current studies. Cadisch *et al*. (2000) found that there was a good agreement in the amount of N_2 fixed between the ^{15}N dilution and ^{15}N natural abundance techniques when the N_2 fixation by *Arachis hypogaea* grown on Ultisol in Lampung was determined, but the ^{15}N dilution technique was much more sensitive to a matching planting time between the reference and fixing plant compared to the ^{15}N natural abundance technique. But Bouillet *et al*. (2008) thought that the most reliable estimation of N_2 fixation was likely to be achieved by using ^{15}N dilution technique when the biological N_2 fixation of mixed-species plantations of *Acacia mangium* and *Eucalyptus grandis* in Brazil was investigated. Some researches even indicated that the ^{15}N dilution technique might overestimate N_2 fixation by the tree legumes (an average of up to 18%) as compared with the result obtained by the ^{15}N natural abundance technique (Hairiah *et al*., 2000). Overall, the choice of a particular method depends on the type and site of the experiment, the available resources and the species and/or system in question (Giller, 2001).

At present, the ^{15}N labeled technique has been widely applied to studying the mineralization, nitrification, denitrification, assimilation and immobilization of N in ecosystem. ^{15}N pool dilution technique is generally used to investigate N mineralization and nitrification, which is based on the measurement of the decrease of at.%$^{15}NH_4^+$ (or at.%$^{15}NO_3^-$) in pool due to the increase of $^{14}NH_4^+$ (or $^{14}NO_3^-$) caused by microbial mineralization (or nitrification) as the $^{15}NH_4^+$ (or $^{15}NO_3^-$) is input. The gross mineralization, gross nitrification, NH_4^+ and

NO_3^- consumption rates are calculated using the following equations described by Kirkham and Bartholomew (1954):

$$m = \frac{M_0 - M}{t} \frac{\log H_0 \times M / H \times M_0}{\log M_0 / M} \quad (15)$$

$$c = \frac{M_0 - M}{t} \frac{\log H_0 / H}{\log M_0 / M} \quad (16)$$

where m is the gross mineralization or nitrification rate (μgN/(g·d)); c is the NH_4^+ or NO_3^- consumption rate (μgN/(g·d)); t is the time (d); M_0 is the initial $^{14+15}N$ pool (μgN/g); M is the post-incubation $^{14+15}N$ pool (μgN/g); H_0 is the initial ^{15}N pool (μgN/g); and H is the post-incubation ^{15}N pool (μgN/g). Gross NH_4^+ immobilization rates are calculated by subtracting the gross nitrification rate from the gross NH_4^+ consumption rate, on the assumption that NH_4^+ consumption through volatilization is zero. Gross NO_3^- immobilization is considered equivalent to gross NO_3^- consumption rate on the assumption that denitrification is negligible. Banning *et al*. (2008) determined the ammonification and nitrification rates of mound and furrow soils in a post-mining forest rehabilitation chronosequence in Western Australia, and suggested that the low organic C environment in mound soils may favor autotrophic nitrifier populations, but the production of NO_3^- was limited by the low gross N ammonification rates (≤ 1 μgN/(g·d)). Gross N transformation rates in furrow soil indicated that the capacity of immobilize N was closely coupled to the capacity to mineralize N, suggesting NO_3^- accumulation *in situ* is unlikely. Moreover, the ^{15}N-labeled technique is also applied to tracing the N uptake of plants and N turnover in ecosystems. Henry and Jefferies (2003) investigated the uptake of free amino acids by the grass *Puccinellia phryganodes* from soils of an Arctic coastal salt marsh, and found that the ^{15}N from both organic and inorganic substrates was incorporated rapidly into plant tissue and at least 5%–11% of $^{13}C^{15}N$-glycine was absorbed intact. The *P. phryganodes* could absorb amino acids intact from the soil despite competition from soil microor ganisms, and the free amino acids might contribute substantially to N uptake in this important forage grass.

The approaches of N fate mainly include soil immobilization, N gaseous loss caused by denitrification (N_2, N_2O), NH_3 volatilization, runoff export (NH_4^+-N, NO_3^--N, DIN and DON) and plant uptake or harvest. Presently, the ^{15}N trace technique has been widely applied in the researches of N fate in different ecosystems, such as streams, lakes, estuaries, forests and croplands (Fry, 2006). After the ^{15}N-labeled fertilizers (such as $^{15}NH_4Cl$, $(^{15}NH_4)_2SO_4$, $K^{15}NO_3$ and $CO(^{15}NH_2)_2$) are imported into ecosystems, their traces are easily identified though determining the changes of ^{15}N signatures in different N pools. Matheson *et al*. (2002) discussed the fate of ^{15}N-NO_3^- in three types of wetland soil microcosm (unplanted, planted (*Glyceria declinata*), and planted with shoot harvest) in Hamilton, and indicated that, in both types of plant-inhabited microcosm, similar proportions of added ^{15}N-NO_3^- were denitrified (61%–63%), soil-immobilised (24%–26%), plant-assimilated (11%–15%) and reduced to ammonium (NH_4^+) (< 1%), while in unplanted microcosms, 49% was reduced to NH_4^+, 29% denitrified and 22% immobilised. Shoot harvest did not affect the fate of ^{15}N-NO_3^-, but it increased the NO_3^- assimilation capacity of shoots. Similar results were reported by Sun and Liu (2008) when the fate of anthropogenic N ($^{15}NH_4{}^{15}NO_3$) in the *Calamagrostis angustifolia* wetland ecosystem of the Sanjiang Plain was determined. Rückauf *et al*. (2004) also evaluated the effect of wetland plant combined with different soil moisture conditions on the fate of ^{15}N-NO_3^-, and showed that, under dry soil moisture conditions, up to 80% of the ^{15}N-NO_3^- added was transformed into organic N compounds, and this transformation process was not affected by plant growth. In contrast, under reflooded conditions, the total gaseous N losses were the highest (77%–95%) and the transformation into organic N compounds was very low (1.8%). Under almost all soil conditions plant growth reduced the N losses by 20%–25% of the ^{15}N-NO_3^- added due to plant uptake.

5.2.2 Ecological effects of nitrogen input on ecosystem

Since the industrial revolution, human activities have altered the global N cycle significantly. It has been estimated that terrestrial biological N-fixation provides about 90 TgN/yr–130 TgN/yr without human activities (Vitousek and Aber, 1997). However, human activities have resulted in the fixation of an additional about 150 TgN/yr by energy production, fertilizer production and cultivation of crops, and much of it (about 60 TgN/yr) are imported into all kinds of ecosystems through N deposition, sewage drainage and runoff (Galloway *et al*., 1995). Loading of excessive N to ecosystems (such as rivers, streams, lakes, forests and estuaries) may cause changes in ecological function, and often has undesirable

environmental and economic consequences (Sun and Liu, 2008). At present, the ^{15}N trace technique has been proved useful as an tool for studying the ecological effects of N input on ecosystem. Jordan *et al*. (1997) studied the N cycling in forest and grass ecosystems irrigated with ^{15}N-enriched wastewater in Falmouth, and found that under conditions of N saturation, $\delta^{15}N$ values for plants were lower, possibly due to discrimination against ^{15}N during uptake of NH_4^+ and NO_3^- by plant roots. Moreover, the capacity of soil N retention fell rapidly as the forest and grass ecosystems became N saturated, and N leaching losses greatly increased. Tietema *et al*. (1998) found that the part of ^{15}N retained in the organic layer was relatively high (20%–45% of applied) at low N inputs (0 kgN/(ha·yr)–30 kgN/(ha·yr) but low (10%–20%) at high N inputs (30 kgN/(ha·yr)–80 kgN/(ha·yr)), suggesting that increased N inputs exceeded the capacity of the microbial population to retain throughfall-N in the organic layer, with the result that N leaching increases. These examples indicated that the N-limitation or N-saturation of ecosystems can be determined through analyzing the relationships between N isotope discriminations and substrate concentrations. Because the plants preferentially utilize ^{14}N, the signifycant ^{15}N isotope discrimination generally occurs in the plants as the substrate concentration is high. Obviously, the $\delta^{15}N$ in plants can be regarded as an effective index to indicate the N saturation of ecosystems (Gu, 2007). N deposition, as an important N input path of ecosystem, has been paid more and more attention to. The effects of N deposition mainly depend on the N saturation of ecosystems. In general, the critical load of N deposition of ecosystems is 25 kgN/(ha·yr), and if the N load ranges from 10 kgN/(ha·yr) to 25 kgN/(ha·yr), the ecosystems would exhibit great responses (Aber *et al*., 1998). The current information suggests that a critical load of 5–10 kgN/(ha·yr) of total N deposition (both dry and wet deposition combined of all atmospheric N species) would protect the most vulnerable terrestrial ecosystems (heaths, bogs, cryptogams) and values of 10–20 kgN/ (ha·yr) would protect forests (Krupa, 2003). Based on these critical values, Nordbakken *et al*. (2003) assessed the effects of N deposition (five different N ($^{15}NH_4$ $^{15}NO_3$) treatments (0 kgN/(ha·yr), 5 kgN/(ha·yr), 10 kgN/(ha·yr), 20 kgN/(ha·yr) and 40 kgN/(ha·yr)) on the bog plants and surface peat in the Kisselbergmosen, and found that the importance of different N sources was species-specific among bog plants. An annual addition of 5 kgN/(ha·yr) was sufficient to significantly increase the N concentration in *Sphagnum* mosses, liverworts and shallow rooted vascular plants, while an annual addition of 40 kgN/(ha·yr) was not sufficient to increase the N concentration in deep rooted plants. The annual addition of 40 kgN/(ha·yr) increased the N content in surface peat at depths of 5 cm and 10 cm, but not at depths of 20 cm and 40 cm, indicating the capacity of the living *Sphagnum* mosses and the surface peat to take up deposited N, and thereby function as a filter. In addition, the effects of N inputs on aquatic ecosystems are also widely studied. Hadwen and Bunn (2005) examined the consequences of low-level nutrient ($^{15}NH_4$ $^{15}NO_3$) input in an oligotrophic dune lake on Fraser Island, and found that, after 10 d of ^{15}N additions, all primary producers and consumers became ^{15}N-enriched, indicating that the added nutrients probably increased primary production and were assimilated and passed through multiple trophic levels. Ashkenas *et al*. (2004) found that all aquatic consumers, both vertebrate and invertebrate, were strongly labeled than their food sources when the N ($^{15}NH_4Cl$) was imported into Mack Creek. Moreover, the increased ^{15}N label in 15 of 17 riparian plants suggested the transfer of aquatic N to terrestrial ecosystem, reflecting that there were strong connections between terrestrial and aquatic ecosystems.

6 Suggestions for Future Research

As a mature technique, stable isotopes have been widely applied to the research of C and N cycles in ecosystems for about 70 years. However, some limitations also existed in current researches. The limitations were analyzed as follows, and the suggestions for the future research are put forward simultaneously:

(1) Since many factors can affect the fractionations of C and N isotopes in the metabolism of plants, microorganisms and animals, some fractionation mechanisms/ fractionation factors are still unclear or uncertainty, which should be further studied. For example, the effects of C isotope fractionations in the metabolism of plants and microorganisms on the composition of $^{13}CO_2$ are unclear, but understanding these fractionation mechanisms is propitious to the identification of canopy photosynthesis, root respiration and SOM decomposition. In addition, as the $\delta^{15}N$ of crops can be influenced

by many factors (such as crop species, fertilization frequency, environmental conditions and field management), in order to improve the feasibility of the $\delta^{15}N$ technique for identifying organic vs. conventional crops in the near future, a crop $\delta^{15}N$ database associated with the factors that are likely to influence crop $\delta^{15}N$ should be established.

(2) The presumption of stable isotopes used for the identification of C and N sources depends on the significant discrepancy existed in different sources. However, not all ecosystems can meet this presumption. Although the use of multiple isotopes allows us to distinguish three or more potential sources, there are often too many sources and not enough tracers in the real world. Moreover, the source identification based on the C and N stable isotopes is very rough, and they only can be used for identifying the general source types, but not the definite source. Thus, in these cases, how to identify more definite C and N sources of ecosystems is still a challenge for ecologists. In the near future, the applications of both isotope natural abundance and labeled techniques, combined with the elemental and molecular biomarker information, can partly solve these problems.

(3) Although the stable isotope labeled technique has many special advantages in the study of source, fate and transformation of C and N, some errors also produced simultaneously due to the uncertainty of environmental changes and the differences of physiological characteristics of plants, microorganisms and animals. The combination of experimental results and model simulation will probably make for revealing the variation mechanisms of $\delta^{13}C$ and $\delta^{15}N$ in ecosystems. However, the integrated mechanism models of $\delta^{13}C$ and $\delta^{15}N$ in boilogy are still scarce. The establishment of C and N isotope mechanism models in different ecosystem scales should be paid more attention to in the near future. Moreover, although the stable isotope techniques and the Keeling plot approach have been widely applied to assessing the significance of the terrestrial C sink and its contribution to the global C fluxes relative to the oceans, the applications are sometimes constrained by the heterogeneity of terrestrial ecosystems as the study scale is transferred to the global level. In the near future, the combination of stable isotope techniques, the Keeling plot approach, C mechanism models and micrometeorology will probably solve these problems.

(4) Compared with the isotope natural abundance technique, the labeled technique is generally applied to the microcosm experiments as the labeled chemicals may have some disturbances on the C and N cycles of ecosystems. In recent years, the natural ecosystems have been significantly affected by the influences of human activities. Loading of excessive C and N to ecosystems may cause changes in ecological function, and often has undesirable environmental consequences. In order to reveal these effects of human activities on the natural ecosystem, the current miniature labeled technique is limited since we can not add large amounts of C and N chemicals to the whole ecosystems. Moreover, the labeled chemicals are still very expensive and the cost may be very high if large quantities need to be applied. Thus, a set of labeled techniques that are suit for applying to the study of whole ecosystems should be explored in the near future.

(5) Although the stable isotope technique has been widely applied to the study of C or N cycles in ecosystems, most of these studies focus on some specific processes, and lack systemic and synthetic study. Moreover, the researches on the relationships between global change and C or N cycles in ecosystem, the environmental effects of C and N in adjacent ecosystems, and the responses of ecosystems to the C and N input are not very comprehensive and deep. Therefore, these relative aspects should be paid more attention to in the future studies based on multiple stable approaches and models.

With the progress of techniques, we believe that the application of stable isotopes in the biogeochemical processes of C and N will become more and more comprehensive and deep, and many real-time ways will be developed to assess, model, and understand what C and N cycles are doing in ecosystems.

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