# International Symposium on Managing Soils for Food Security and Climate Change Adaptation and Mitigation

Edited by L.K. Heng, K. Sakadevan, G. Dercon and M.L. Nguyen

Soil and Water Management and Crop Nutrition Subprogramme
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture,
Department of Nuclear Sciences and Applications,
International Atomic Energy Agency, Vienna, Austria


Food and Agriculture Organization of the United Nations
Rome, 2014

# From Fertilizer to Food: Tracing Nitrogen Dynamics in Conventional and Organic Farming Systems Using $^{15}N$ Natural Abundance

P.M. Chalk[1,*], C.T. Inácio[2], and A.M.T. Magalhães[2]


## ABSTRACT

Synthetic nitrogen (N) fertilizers differ markedly from organic N fertilizer sources in relative isotopic composition at natural abundance levels ($\delta^{15}N$). The objective of this paper is to provide an overview of the applications of $\delta^{15}N$ techniques to study the dynamics of synthetic fertilizers, animal excreta and composts in the soil-plant-atmosphere continuum. However, isotopic fractionation processes often complicate the interpretation of results. These fractionation processes and the factors affecting the $\delta^{15}N$ signatures of organic N fertilizers are reviewed. Published data from short-, medium- and long-term experiments with annual crop rotations and in pastures subject to organic N inputs are also examined and analyzed with respect to changes in delta nitrogen-15 ($\delta^{15}N$) signatures of the soil, the crop or pasture, the soil biota and leachates. The use of $\delta^{15}N$ to differentiate organic and conventional plant products is briefly covered. There are few data on the dynamics of N during the storage of animal excreta or the composting of agricultural wastes as shown by $\delta^{15}N$ values in the organic, inorganic or gaseous N phases. The major N loss process is ammonia ($NH_3$) volatilization. Reviewed data show significant relationships between bulk $\delta^{15}N$ signatures of stored manure and cumulative $NH_3$ loss or bulk $\delta^{15}N$ of livestock manure composts and N concentration. These significant relationships suggest that $\delta^{15}N$ may have wider applications in estimating the efficiency of N conservation during storage or composting. In addition, the combined use of bulk $\delta^{15}N$ and delta oxygen-18 ($\delta^{18}O$) signatures of nitrous oxide ($N_2O$) evolved during storage and composting, together with the isotopomer-derived site preference of $N_2O$, are emerging technologies for identifying $N_2O$ production pathways. $\delta^{15}N$ in combination with appropriate statistical analysis is a promising diagnostic tool for differentiating organic and conventional plant products.


**Key words:** *animal excreta, compost, crop rotations, delta $^{15}N$, isotopic fractionation, pastures.*

## INTRODUCTION

In conventional farming systems the use of both synthetic and organic N fertilizers is permitted, whereas in organic systems only organic N sources may be used as fertilizer. The principal organic N fertilizer sources are animal excreta (manure and urine) and composts which may be derived from both animal wastes and crop residues. Organic fertilizer may also consist of human excreta or composts made from household or municipal wastes, including sewage sludge, but domestic- or municipally-derived organic fertilizers are not considered in this paper.

There are fundamental differences between synthetic and organic N fertilizer sources with respect to their ability to supply N for crop growth. Organic N sources must be biologically mineralized to inorganic N before they become available for plant uptake, whereas synthetic N fertilizers (ammonium and nitrate salts) are highly soluble in water and are readily available for plant uptake. Urea is the principal synthetic N fertilizer used in agriculture, and it is also quickly taken up by plants following its rapid enzymatic hydrolysis to ammonium in soil. Therefore, organic N fertilizers are often referred to as slow-release N sources, and are considered to be somewhat better synchronized with crop demand for N. However, organic fertilizers contain a mixture of both organic and mineral ($NH_4^+$ and $NO_3^-$) forms of N.

Both organic and synthetic sources are subject to several N loss processes which reduce their effectiveness to supply N for plant growth. Animal excreta must be collected and stored in intensive animal feeding operations before application to crops or pastures, and gaseous N losses ($NH_3$, $N_2O$, $N_2$, and other oxides of N ($NO_x$)) may occur during storage. Similar loss processes may occur during the composting of agricultural wastes. Nitrogen losses may occur following N fertilizer application to soil either via gaseous N emissions or nitrate leaching. Therefore it is important to estimate the N fertilizer use efficiency, and where possible to quantify the N loss pathways of synthetic and organic fertilizer sources during each phase of the continuum.

The use of the $^{15}N$ stable isotope is a basic tool for studying the dynamics of N in farming systems (Chalk, 1997). Both naturally-occurring differences in the relative abundance of $^{15}N$ in N sources, or the use of N sources artificially-enriched in $^{15}N$ can be used to trace the fate of fertilizer N. The literature on the application of $^{15}N$ to study N dynamics in animal excreta or compost-amended soils was reviewed by Dittert, Georges and Sattelmacher (1998) and Chalk, Magalhãese and Inácio (2013), respectively. The emphasis of these reviews was on quantifying post-application N use efficiency and N transformations in soil, rather than on N transformations prior to soil application i.e. during storage or composting. The objective

1 International Atomic Energy Agency, Wagramerstrasse 5, PO Box 100, 1400 Vienna, Austria
2 EMBRAPA-Soils, National Center of Soil Research, Brazilian Agricultural Research Corporation, Rua Jardim Botânico 1024, Rio de Janeiro, Brazil
* E-mail address of the corresponding author: chalkphillip@gmail.com

L.K. Heng, K. Sakadevan, G. Dercon and M.L. Nguyen (eds), Proceedings — International Symposium on Managing Soils for Food Security and Climate Change Adaptation and Mitigation. Food and Agriculture Organization of the United Nations, Rome, 2014: 339–348

**TABLE 1. $\delta^{15}N$ signatures of animal diets and excreta**

| Animal | Diet | $\delta^{15}N$ (‰) | | | Reference |
|---|---|---|---|---|---|
| | | Diet | Faeces | Urine | |
| Jersey cow | Pasture | +0.6 | +2.6 | –1.6 | Steele and Daniel (1978) |
| Angus steer | Silage | +0.6 | +2.1 to +2.5 | –2.1 to –2.8 | |
| Llama | Alfalfa | +0.4 | +3.3 | +0.1 | Sponheimer *et al.* (2003) |
| | Bermuda grass | +5.8 | +8.8 | +3.7 | |
| Swine | Not specified | +1.7 ± 1.0 | +2.4 ± 0.9 | –0.1 ± 1.1 | Mariappan *et al.* (2009) |
| Dairy cow | Silage[1] | +1.8 to +8.4 | +4.2 to +8.8 | –0.9 to +4.1 | Cheng *et al.* (2011) |

[1] Nine individual silages were made from forage grasses (3 types), red clover, red clover mixed with corn or oats in different proportions

of the present paper is to provide an overview of the applications of the $^{15}N$ natural abundance technique to trace N transformations that occur during pre- and post-application of animal excreta and composts. The application of $\delta^{15}N$ to differentiate organic and conventional foodstuffs is only briefly covered, as this topic was comprehensively reviewed for plant products by Inácio, Chalk and Magalhães (2013).

## Units of $^{15}N$ concentration

Stable isotopic values close to the natural abundance of the designated isotope are expressed by the notation (δ) in units of parts per thousand (per mil or ‰) relative to the international standard for that element (Chalk, 1995). Since N has only two stable isotopes ($^{14}N$ and $^{15}N$), then:

$$\delta^{15}N\ (‰) = \{[(^{15}N / {}^{14}N)_{sample} / (^{15}N / {}^{14}N)_{standard}] - 1\} \times 1\,000 \quad (1)$$

where the international standard is atmospheric $N_2$ ($\delta^{15}N$ = 0‰, by definition).

The $\delta^{15}N$ value can be either negative or positive depending whether it is depleted or enriched in $^{15}N$ relative to the standard.

Stable isotopic values of artificially-enriched samples are expressed as absolute abundance in units of atom $\%^{15}N$ (Chalk, 1995).

$$\text{Atom } \%^{15}N = (\text{number of } ^{15}N \text{ atoms / total number of } ^{15}N + {}^{14}N \text{ atoms}) \times 100 = [^{15}N / (^{14}N + {}^{15}N)] \times 100 \quad (2)$$

Thus as can be seen from Equation 2, it is incorrect to substitute atom $\%^{15}N$ for the $^{15}N/^{14}N$ ratio in Equation 1, as is sometimes seen in the earlier literature (e.g. Selles and Karamanos, 1986), and although a close approximation will be obtained it will nevertheless be an underestimate (Chalk, 1995).

Since the absolute $^{15}N$ abundance of atmospheric $N_2$ is 0.3663 ± 0.0004 atom % (Junk and Svec, 1958) then:

$$^{15}N \text{ enrichment (atom } \%^{15}N \text{ excess)} = \text{atom } \%^{15}N - 0.3663 \quad (3)$$

It is quite common in the literature to see atom $\%^{15}N$ incorrectly designated as $^{15}N$ enrichment. Atom % excess values are used to trace the pathways of $^{15}N$-enriched fertilizers added to soil.

The relationship between the $^{15}N/^{14}N$ ratio and atom $\%^{15}N$ is given by:

$$^{15}N/^{14}N = \text{atom } \%^{15}N/(100 - \text{atom } \%^{15}N) \quad (4)$$

For atmospheric $N_2$:

$$^{15}N/^{14}N = 0.3663/(100 - 0.3663) = 0.00367647$$

## $\delta^{15}N$ signatures of synthetic and organic fertilizers

Synthetic N fertilizers (ammonium salts and urea) are derived from ammonia ($NH_3$) produced by the Haber-Bosch process, which involves the catalytic reduction of atmospheric $N_2$ at high temperature and pressure by $H_2$ derived from methane or natural gas. Therefore the $\delta^{15}N$ signatures of synthetic fertilizers are expected to be close to that of atmospheric $N_2$ (0‰ by definition). A review of published data (Inácio, Chalk and Magalhães, 2013) shows that synthetic N fertilizers have slightly positive or negative $\delta^{15}N$ values within the range of –3.9 to + 5.9‰.

Organic N fertilizers are generally naturally enriched in the stable isotope $^{15}N$ compared with synthetic N fertilizers. A review of published data (Inácio, Chalk and Magalhães, 2013) shows that total N in manures and composts varies with $\delta^{15}N$ values in the range of +2.0 to +16.7 and +4.9 to +45.2‰, respectively. Animal excreta consist of both solid (dung) and liquid (urine) components, except for poultry where there is no urinary component. Dung and urine components often show marked differences not only in the relative amounts and N concentrations, but also $\delta^{15}N$ signatures.

Steele and Daniel (1978) reported that dairy and beef cattle urine was depleted in $^{15}N$ relative to the animal diet while manure was enriched (Table 1). Sponheimer *et al.* (2003) reported similar data for llamas, with faeces enriched in $^{15}N$ relative to both low- and high-protein diets, whereas urine was depleted in $^{15}N$ (–2.1‰) relative to the low-protein diet, but was not significantly different from intake $\delta^{15}N$ on the high protein diet (Table 1). Mariappan *et al.* (2009) similarly reported swine urine depleted in $^{15}N$ relative to diet and faeces (Table 1). More recently, Cheng *et al.* (2011) reported both positive and negative $\delta^{15}N$ values for the urine of dairy cows, while manure was always positive (Table 1). Urine was invariably depleted in $\delta^{15}N$ relative to diet, while faeces were either similar to or enriched in $\delta^{15}N$ relative to diet (Cheng *et al.*, 2011). A highly significant positive linear relationship was found between the $\delta^{15}N$ values of the feed (range of +2 to +8.5‰) and faeces (range of +4 to +9‰) (Cheng *et al.*, 2011).

Organic fertilizers contain both organic and inorganic [ammonium ($NH_4^+$) and nitrate ($NO_3^-$)] forms of N. The inorganic N concentrations are generally low compared with the total N of the manure or compost, but $\delta^{15}N$ values can be quite variable (Table 2). The inorganic N fraction is often enriched in $^{15}N$ compared with the total N, indicating non-uniform labelling due to isotope fractionation processes. The unusually high $\delta^{15}N$ values for inorganic N in cattle feedlot manure (Kim *et al.*, 2008; Table 2) may be indicative of substantial N losses during storage.

**TABLE 2. Concentration and $^{15}$N natural abundance of total and inorganic forms of N in organic N fertilizers**

| Material | N fraction | N concentration[1] (g/kg) | $\delta^{15}N$[1] (‰) | Reference |
|---|---|---|---|---|
| Cattle manure | Total | 4.4–10.7[2] | +7.9[3] | Choi *et al.* (2006) |
| | $NH_4^+$ | 0.001–0.067[2] | +9.9[3] | |
| | $NO_3^-$ | 0.007–0.009[2] | +16.6[3] | |
| Cattle feedlot manure (sawdust bedding) | Total | 9.5 | +11.4 | Kim *et al.* (2008)[4] |
| | $NH_4^+$ | 2.0 | +39.8 | |
| | $NO_3^-$ | 0.22 | ~+26 | |
| Swine manure compost | Total | 23.1 (1.2) | +15.3 (0.2) | Yun *et al.* (2011) |
| | $NH_4^+$ | 0.33 (0.014) | +12.5 (1.3) | |
| | $NO_3^-$ | 0.12 (0.008) | +22.6 (0.1) | |

[1] Data in parentheses are standard deviations of the mean
[2] Range of values over 4 yr
[3] Mean values over 4 yr
[4] Data are time zero at the beginning of composting for 90 d

**TABLE 3. Discrimination factors (ε) for some N cycle processes**

| Process | Discrimination factor (‰) | |
|---|---|---|
| | Högberg (1997)[1] | Robinson (2001) |
| Ammonification (organic N → $NH_4^+$) | ≈0 | 0–5 |
| Nitrification ($NH_4^+$ → $NO_2^-$ → $NO_3^-$) | 15–35 | 15–35 |
| Ammonia volatilization ($NH_4^+$ → $NH_3$↑) | 29 | 40–60 |
| Denitrification ($NO_3^-$ → $NO_2^-$ → $N_2O$↑ → $N_2$↑) | 0–33 | 28–33 |
| $N_2O$ and NO production during $NH_4^+$ oxidation | | 35–60 |
| Biological $N_2$ fixation | 0–2 | 0–6 |
| Inorganic N assimilation by plants | 0–20 | 0–19 ($NO_3^-$) |
| | | 9–18 ($NH_4^+$) |

[1] Values given as α were converted to ε by Equation 8

## Isotopic fractionation processes affecting the $\delta^{15}N$ signatures of organic N fertilizers

### *Units*

Isotopic fractionation can occur as a result of physical (e.g. diffusion), chemical (equilibria or ion exchange) or biological (enzymatic) processes. It can be expressed by the fractionation factor (α) where $\alpha = \delta_A / \delta_B$ in an equilibrium reaction, where A is a reactant and B is a product. Isotopic fractionation can also be expressed as discrimination (Δ or ε) in units of per mil (‰).

$$\varepsilon(‰) = (\delta_s - \delta_p)\,[1 + (\delta_p\ 1\ 000)] \quad (5)$$

where $\delta_s$ is substrate and $\delta_p$ is product

An approximation of the above equation is

$$\varepsilon(‰) = \delta_s - \delta_p \quad (6)$$

The fractionation factor (α) is approximated by:

$$\alpha = (\varepsilon\ /1\ 000) + 1 \quad (7)$$

or

$$\varepsilon = (\alpha - 1) \times 1\ 000 \quad (8)$$

Thus for a fractionation factor (α) of 1.020, ε of the product = –20‰ relative to the substrate (Högberg, 1997).

### *Processes*

Fractionation factors for all physical, chemical and microbially-mediated transformations of N in soil are significant, especially $NH_3$ volatilization, nitrification and dissimilatory $NO_3^-$ reduction (biological denitrification) (Högberg, 1997; Robinson, 2001). These three processes are the major N transformations affecting the natural $^{15}$N isotopic composition of the principal organic N fertilizers, animal wastes and composts. Nitrogen isotope discrimination (ε) factors for the major N cycle processes (Högberg, 1997; Robinson, 2001) are given in Table 3.

Volatilization of $NH_3$ involves several steps (i–iv) in which isotopic fractionation can occur (Högberg, 1997):

(i) Equilibrium effect (A ↔ B in solution)

$$^{14}NH_3 + {}^{15}NH_4^+ \leftrightarrow {}^{15}NH_3 + {}^{14}NH_4^+$$

$NH_4^+$ is more enriched with $^{15}$N than $NH_3$ at equilibrium. i.e. = α = 1.020 – 1.027

(ii) Kinetic effects

1. Diffusion of $NH_3$ in solution to the site of volatilization (α ≈ 1 000)
2. Volatilization of $NH_3$ (α = 1.029)
3. Diffusion of $NH_3$ away from the site of volatilization (α ≈ 1 000)

The compound effect of these processes on the net fractionation can be large (Högberg, 1997) since α for equilibrium effect and for volatilization of ammonia are greater than 1.02. Ammonia volatiliza-

tion is quantitatively the most significant N loss process during storage of animal excreta, the voiding of excreta on grazed pastures and during the composting of agricultural wastes.

The first step in nitrification, the enzymatic oxidation of $NH_4^+ \rightarrow NO_2^-$ was shown to have a large fractionation factor (1.015 to 1.036) in pure cultures of *Nitrosomonas* (Högberg, 1997). Thus a decrease in $NH_4^+$ concentration with a concomitant increase in its $\delta^{15}N$ signature and the production of $NO_3^-$ relatively depleted in $^{15}N$ all point to active nitrification (Wrage *et al*., 2004). According to Högberg (1997) the second step in nitrification ($NO_2^- \rightarrow NO_3^-$) is not normally rate-limiting and should therefore not lead to further fractionation. However, this is frequently not the case in urine patches and during the composting of animal excreta where the oxidation of nitrite is inhibited by the high pH resulting in the temporary accumulation of nitrite (e.g. Clough *et al*., 1998; Sasaki *et al*., 2006).

Denitrification can be a significant fractionation process during the storage of animal excreta and the composting of agricultural wastes. The overall process or so-called "total denitrification" ($NO_3^- \rightarrow NO_2^- \rightarrow N_2O\uparrow \rightarrow N_2\uparrow$) has a fractionation factor of 1.028 – 1.033 (Robinson, 2001) although Högberg (1997) gives a much wider range (Table 3). Robinson (2001) also shows a large discrimination factor for $N_2O$ and NO produced during nitrification, of the same order of magnitude as for $NH_3$ volatilization (Table 3).

$N_2O$ production pathways have been investigated using the dual isotope measurements of $\delta^{15}N$ and $\delta^{18}O$ of the $N_2O$ emitted from soil (e.g. Yamulki *et al*., 2000). Several studies have reported that $\delta^{15}N$ values of soil-emitted $N_2O$ can be as low as –56‰ and as high as +3‰, with $\delta^{18}O$ values varying between –21‰ and +57‰ (Yamulki *et al*., 2001; Bol *et al*., 2003). The $\delta^{15}N$ values of soil-emitted $N_2O$ are thus lower than the corresponding tropospheric (+18.7‰) and stratospheric (+21.3‰) values (Yamulki *et al*., 2001; Bol *et al*., 2003). Nitrous oxide produced by nitrification or denitrification in soils is depleted in $^{15}N$ relative to its substrate ($NH_4^+$ and $NO_3^-$, respectively), but as noted above, the fractionation is larger for nitrification-derived $N_2O$. Therefore, a shift in $N_2O$ production from nitrification to denitrification increases both $\delta^{15}N$ and $\delta^{18}O$ values up to 20–50‰ and 10–25‰, respectively (Yamulki *et al*., 2001; Bol *et al*., 2003). However, if urine is the main source of nitrification, a shift in the $\delta^{15}N$ signature (but not in the $\delta^{18}O$ signature) would be expected, because the oxygen in urea [$CO(NH_2)_2$] would be hydrolyzed to $CO_2$ (Yamulki *et al*., 2001).

When $N_2O$ is further reduced to $N_2$ during total denitrification, $\delta^{15}N_2O$ becomes more enriched relative to the $N_2$ product. Thus simultaneous measurements of the $\delta^{15}N$ signatures of emitted $N_2O$ and $N_2$ permits the amount of $N_2O \rightarrow N_2$ to be calculated, and hence improves estimates of the relative contribution of nitrification and denitrification to total $N_2O$ emissions from soils (Bol *et al*., 2003).

The intramolecular distribution of N isotopes in $N_2O$ is an emerging tool for defining the relative importance of microbial sources of this greenhouse gas (Sutka *et al*., 2006). Since $N_2O$ has two N atoms within the asymmetric molecule (central and outer N), $^{15}N$ is distributed across three principal isotopomers, $^{15}N^{15}NO$, $^{15}N^{14}NO$, $^{14}N^{15}NO$. A technique developed by Sutka *et al*. (2006) enables the individual measurement of $^{15}N^{14}NO$ and $^{14}N^{15}NO$. The difference in $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ is the so-called site preference ($SP = \delta^{15}N^{\alpha} - \delta^{15}N^{\beta}$, where $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ represent the $^{15}N/^{14}N$ ratios at the centre and outer N atoms, respectively). The difference in the site preference for $N_2O$ from hydroxylamine oxidation (~33‰) and nitrite reduction (~0‰) found in a pure culture study (Sutka *et al.,* 2006) can be used to differentiate the relative contributions of nitrification and denitrification to $N_2O$ emissions. Yamulki *et al.* (2001) considered that site preference can provide a more fundamental and sensitive analysis of $N_2O$ sources and production processes compared with the bulk $\delta^{15}N$ analysis of $N_2O$.

## Factors affecting the $\delta^{15}N$ signatures of organic N fertilizers

### *Storage of animal excreta*

Many types of storage facilities for animal excreta exist on farms worldwide. In the UK, the principal types of storage facilities for dairy, cattle and swine excreta are middens (piles or heaps with a permeable or impermeable base), slurry tanks (below or above ground) constructed of steel or concrete, and lagoons with pervious or impervious linings (Nicholson and Brewer, 1997).

Hristov *et al.* (2009) and Lee *et al.* (2011) studied the cumulative amounts and $\delta^{15}N$ signatures of $NH_3$ emitted from dairy manure during simulated storage under laboratory conditions. $\delta^{15}N$ values of $NH_3$ volatilized increased quadratically ($r^2 = 0.92$; ***$p < 0.001$) from –31‰ (1 d) to –15‰ (14 d) while the $\delta^{15}N$ of total N remaining in manure also increased quadratically ($r^2 = 0.96$) from +5.6 to +7.2‰ (Hristov *et al*., 2009). A highly significant positive linear relationship was observed between cumulative $NH_3$ loss and the $\delta^{15}N$ signature of the stored manure ($r^2 = 0.76$; ***$p < 0.001$) over the range of $\delta^{15}N$ in manure of 4 to 8‰ (Hristov *et al.,* 2009).

Ammonia volatilization was most significant during the first 2–3 d of storage and 90 percent of emitted $NH_3$ came from the urine component of the faeces as a consequence of rapid urea hydrolysis (Lee *et al*., 2011). A sigmoidal curve ($r^2 = 0.96$, ***$p < 0.001$) best described the $\delta^{15}N$ of volatilized $NH_3$ during incubation for 30 d (Lee *et al*., 2011). $NH_3$ was highly depleted in $\delta^{15}N$ at the beginning of the manure storage process, and $\delta^{15}N$ values of manure reached a plateau which coincided with the decline in $NH_3$ volatilization. Therefore, $\delta^{15}N$ of volatilized $NH_3$ is a promising tool for estimating cumulative ammonia losses during storage of animal excreta, but further testing is required with different excreta under different and more realistic conditions of storage.

The earthen anaerobic lagoon is a common method of on-farm storage of feedlot runoff and slurries from dairy and pig barns in mid-western USA. Mariappan *et al.* (2009) studied the spatial and temporal concentrations and $\delta^{15}N$ signatures of total N and $NH_4^+$ within 13 anaerobic lagoons of variable volumes receiving dairy (one), cattle (two) and swine (eleven) wastes. $\delta^{15}NH_4^+$ varied from +2.0 to +59.1‰, was spatially uniform within the top 1.5 m of the lagoon, and was not statistically different from the total $\delta^{15}N$ value. Based on comparisons with feed and fresh manure and urine, most $^{15}N$ isotopic fractionation occurred after excretion and was affected by management and environmental factors (Mariappan *et al*., 2009). $\delta^{15}NH_4^+$ enrichment increased when $NH_3$ volatilization increased with increasing seasonal temperatures, and lagoons that were frequently pumped out and refilled with fresh waste were not characterized by the high $\delta^{15}N$ values normally associated with animal wastes. Wastes must mature in lagoons in order to develop high levels of $\delta^{15}NH_4^+$ enrichment (> +10‰) (Mariappan *et al.,* 2009).

### Composting of agricultural wastes

The $\delta^{15}N$ signature of corn silage increased by +7.9‰ during aerobic-thermophilic composting (Lynch, Voroney and Warman, 2006; Table 4). Composting created a more homogeneous bulk $\delta^{15}N$ signature compared with the feedstock, as seen by the lower sub-sample standard deviation (Table 4). Kim *et al.* (2008) similarly observed an increase in the $\delta^{15}N$ signature of cattle manure composted with sawdust bedding of +4.2‰, and of +3.4‰ for manure composted with rice hull bedding (Table 4).

**TABLE 4. Natural $^{15}$N abundance in agricultural wastes and derived composts**

| Residue | $\delta^{15}$N (‰) | Reference |
|---|---|---|
| Corn silage | +0.3 ± 1.3 | Lynch, Voroney and Worman (2006) |
| Corn silage compost | +8.2 ± 0.4 | |
| Cattle manure | +7.6 | Kim *et al.* (2008) |
| Cattle manure + rice hull[1] compost | +11.0 | |
| Cattle manure | +11.4 | |
| Cattle manure + sawdust[2] compost | +15.6 | |

[1] $\delta^{15}$N of rice hull = +4.9 ± 0.1‰

[2] $\delta^{15}$N of sawdust = +1.7 ± 0.2‰

According to Lynch *et al.* (2006), the observed compost $\delta^{15}$N enrichment is attributable to a combination of fractionation mechanisms, including microbial isotope discrimination during N turnover, a shift to more complex N compounds, and fractionation during $NH_3$ volatilization, with the relative contributions being unknown. Kim *et al.* (2008) reasoned that $NH_3$ volatilization would be the dominant process in the early thermophilic stage of composting of cattle manure due to the fast hydrolysis of the high concentrations of urea in livestock excreta, followed by slow or insignificant increases in $\delta^{15}$N in the latter stages. This hypothesis remains to be tested.

Kim *et al.* (2008) observed an increase in the $\delta^{15}$N of $NH_4^+$ from +30.2 to +41.7‰ in manure + rice hull compost, and from +39.8 to +47.8‰ in the manure + sawdust compost, while the $\delta^{15}$N of $NO_3^-$ fluctuated during composting within the approximate range of +25 to +45‰. Based on both the temporal changes in the concentrations and isotopic signatures of $NH_4^+$ + $NO_3^-$, Kim *et al.* (2008) concluded that loss of $NH_4^+$ in the early stages of composting through $NH_3$ volatilization and nitrification, and loss of $NO_3^-$ in the latter stage (after 60 d) through denitrification were the primary reasons for the increase in $\delta^{15}$N in the composted manure.

There are presently no data available on the $\delta^{15}$N values of $NH_3$ emissions during composting. However, a weak negative linear relationship was observed between N concentrations and $\delta^{15}$N values of livestock manure composts ($r^2$ = 0.16; *p < 0.05) over the range of 10 to 35 g·N/kg and $\delta^{15}$N of +9 to +22‰ (Lim *et al.*, 2010). It would be expected that treatments designed to conserve N during composting would exert a strong influence on the $\delta^{15}$N values of volatilized $NH_3$ and hence compost N concentrations and $\delta^{15}$N values, but data are not yet available.

In contrast to the lack of data for $NH_3$ there are a few measurements on the natural $^{15}$N abundance of $N_2O$ formed during composting. From 0.5 to 1.6 percent of total N was emitted as $N_2O$ during the composting of livestock waste in turned and static piles, respectively, with the $\delta^{15}$N signature of $N_2O$ increasing markedly from –23.1 to –0.2‰ (Yoh *et al.*, 2003). The bulk $\delta^{15}$N signature of $N_2O$ produced during thermophilic composting of cow manure with orchard grass increased from –20 to –15‰ between day 14 and day 45 (Maeda *et al.*, 2010), but then suddenly decreased at day 45 to reach –35‰ at day 56. The isotopomer methodology was applied to identify the sources of $N_2O$ emissions. It was found that denitrification was the main source of $N_2O$ following the turning of the compost pile, with a concomitant reduction in the concentrations of $NO_2^-$ and $NO_3^-$. An increased value in site preference indicated that nitrification, which occurred mainly in the surface of the pile, partially contributed to $N_2O$ emissions between the turnings (Maeda *et al.*, 2010).

## $\delta^{15}$N *s*ignatures of soils, crops, soil biota and leachates under organic and conventional fertilizer regimes

### *Soils and plants*

Experiments of short- (42 to 157 d, medium- (4 to 12 yr) and long-term (30 to 91 yr) duration have been carried out either in the glasshouse or field to compare the temporal changes in the concentrations and $\delta^{15}$N signatures of total soil N and crop N uptake

**TABLE 5. N concentration and natural $^{15}$N abundance of N fertilizer sources and crop N in short-term pot and field experiments with animal excreta, composts and synthetic N fertilizer**

| Crop / scale/ duration | N fertilizer source | | | | Plant material | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | Type[1] | Total N (g/kg) | N rate (kg/ha) | $\delta^{15}$N (‰) | Part | Total N (g/kg) | N uptake (g/plant) | $\delta^{15}$N (‰) | |
| Maize/ pot / 70 d | C | | 0 | | Above ground | | 1.83 | +6.3 | Choi *et al.* (2002) |
| | U | 451 | 150 | –2.3 ± 0.2 | | | 3.00 | +6.0 | |
| | SMC | 20.6 | 150 | +13.9 ± 0.2 | | | 3.44 | +6.7 | |
| Chinese cabbage / pot / 42 d | SMC | 25.3 | 0 | +16.2 | Outer leaf[3] | 24.3 | | +14.1 | Yun and Ro (2009) |
| | | | 0.5[2] | | | 31.1 | | +22.2 | |
| | | | 1.0[2] | | | 40.9 | | +24.5 | |
| | | | 1.5[2] | | | 48.4 | | +24.4 | |
| Maize / field / 157 d | C | | 0 | | Grain | 19 ± 1 | | +6.3 ± 0.3 | Szpak *et al.* (2012) |
| | AS | 211 ± 1 | 700 | –0.7 ± 0.1 | | 16 ± 0 | | +5.8 ± 0.2 | |
| | CD | 24 ± 6 | 75 | +13.9 ± 0.6 | | 16 ± 0 | | +8.1 ± 1.6 | |
| | SG | 82 ± 9 | 200 | +38.1 ± 0.6 | | 15 ± 1 | | +21.2 ± 0.2 | |

[1] C — control; U — urea; SMC — swine manure compost; AS — ammonium sulphate; CD — camelid dung; SG — seabird guano

[2] Unit is g/kg

[3] Lsd (*p < 0.05) between N rates; Total plant N = 2.1 g/kg; $\delta^{15}$N plant material = 1.0‰

under animal excreta, composts and synthetic N fertilizer regimes. A range of annual crops in short-term (single season) experiments or in medium- to long-term crop rotations has been used.

*Short-term (single season) experiments*

Choi *et al.* (2002) found large differences in the $\delta^{15}N$ signatures of urea and swine manure compost treatments applied at the same N rate in a pot experiment, which were reflected in the $\delta^{15}N$ signatures of the foliage of maize at day 30. However, these differences were short-lived and converged to the initial $\delta^{15}N$ value of total soil N of +6.9 ± 0.3‰ after 70 d, despite the increasing N uptake from control, to urea to compost treatments (Table 5).

Yun and Ro (2009) found a highly significant positive linear relationship between the N uptake of Chinese cabbage in a pot experiment and the N application rate of swine manure compost, which had a $\delta^{15}N$ value of total N of +16.2‰. There were small differences in the N concentrations between outer (older) and inner (younger) leaves, and the N concentration increased with N rate (Table 5). However, the $\delta^{15}N$ values were significantly higher for outer compared with inner leaves, which often exceeded the $\delta^{15}N$ value of the total N of the applied compost (Table 5). This apparently anomalous result was due to the non-uniform $\delta^{15}N$ label between the organic and inorganic components of the compost. The higher $\delta^{15}N$ values of the compost $NH_4^+$ and $NO_3^-$ pools exerted a stronger influence than the total compost N.

In contrast to the previous short-term pot experiments, the $\delta^{15}N$ signatures of maize grain in a field experiment mirrored the $\delta^{15}N$ signatures of the organic N inputs (Szpak *et al.*, 2012). The grain in the ammonium sulphate treatment had a lower $\delta^{15}N$ value than the control, while the grain in the dung and guano treatments had much higher $\delta^{15}N$ signatures than the control (Table 5). The authors claimed that the guano had the highest $\delta^{15}N$ value (+38.1 ± 0.6‰) for an organic N fertilizer reported to date (2012), but Choi *et al.* (2007) previously reported similar values of +40.1 ± 3.4‰ and +45.2 ± 4.1‰ for two compost samples.

*Medium-term crop rotations*

Choi *et al.* (2006) applied variable rates of swine manure slurry, cattle manure and urea to a soil in each of four yr (Table 6). There were no significant effects on surface soil total N or its $^{15}N$ natural abundance compared to the control. In contrast, Zhao, Maeda and Ozaki, (2002) found the annual addition of swine manure compost for six yr increased both total soil N and its natural $^{15}N$ abundance compared with the control at site 1, while at site 2 annual addition of cattle manure for 12 yr resulted in increasing soil total N and its $\delta^{15}N$ signatures with increasing rates of manure (Table 6).

*Long-term crop rotations*

Total soil N did not differ between control and animal manure treatments after 91 yr (Bol *et al.*, 2005), whereas it was significantly higher in the manure treatment compared with the control after 42 yr (Gerzabek, Haberhauer and Kirchmann, 2001), and between livestock manure compost and the control after 30 yr (Nishida *et al.*, 2007). In addition, total N either decreased (Gerzabek, Haberhauer and Kirchmann, 2001) or remained unchanged over time in the control or synthetic N fertilizer treatments (Nishida *et al.,* 2007), whereas it increased in manure and compost treatments (Table 7). These differences are most likely due to differences in climatic and edaphic factors as well as the intrinsic differences in the characteristics and rates of addition of the organic N sources.

The $\delta^{15}N$ signatures of total N in surface soil (Table 7) did not differ significantly between control and manure treatments (Gerzabek, Haberhauer and Kirchmann, 2001) but they were higher in manure (Bol *et al.*, 2005), compost and ammonium sulphate treatments (Nishida *et al.*, 2007). The last crop of sugar beet (1985) also had higher $\delta^{15}N$ values in the tops of the manure (+6.5‰) compared with the control (+2.0‰) treatment (Bol *et al.*, 2005). Nishida *et al.* (2007) found that the $\delta^{15}N$ signatures of total N in surface soil decreased in the control and ammonium sulphate treatments over time, but increased in the compost treatment (Table 7). Therefore there were no consistent trends in the relative long-term temporal changes in total soil N and the corresponding $\delta^{15}N$ signatures (Table 7). How-

**TABLE 6. N concentration and natural $^{15}N$ abundance of annual fertilizer amendments and total N in surface soil in medium-term field experiments (crop rotations) with animal excreta, composts and synthetic N fertilizer**

| Annual fertilizer amendment | | | | | Soil | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Type[1] | Time (yr) | Total N (g/kg) | N rate (kg/ha) | $\delta^{15}N$ (‰) | Depth (cm) | Total N (g/kg) | | $\delta^{15}N$ (‰) | | Reference |
| | | | | | | Initial | Final | Initial | Final | |
| C | 6 | | 0 | | 0–20 | | 3.8 | | +6.6[2] | Zhao, Maeda and Ozaki, (2002) Site 1 |
| SMC | | 56.0 | 800 | +14.3 | | | 5.5 | | +9.6[2] | |
| CM | 12 | 25.3 | 163 | +19.1 | | | 4.8 | | +9.4[2] | Zhao, Maeda and Ozaki, (2002) Site 2 |
| | | | 325 | | | | 4.9 | | +10.3[2] | |
| | | | 650 | | | | 5.8 | | +10.4[2] | |
| C | 4 | | 0 | | 0–30 | 1.7 | 1.6 | +4.1 | +4.1 | Choi *et al.* (2006) |
| SMS | | 2.6 – 4.6[3] | 58–172[4] | +4.7 – + 5.6 | | 1.6 | 1.6 | +4.1 | +4.4 | |
| CM | | 4.4 – 10.7 | 82–397 | +6.8 – + 8.5 | | 2.0 | 2.2 | +3.9 | +4.3 | |
| U | | 466 | 39–90 | –0.6 – + 1.9 | | 1.7 | 1.6 | +3.9 | +4.0 | |

[1] C — control; SMC — swine manure compost; CM — cattle manure; SMS — swine manure slurry; U — urea
[2] All values are means of 2 or 3 replicates with a standard error within ± 0.3‰
[3] Unit is g/L
[4] Yr 1–3 (no slurry was applied in year 4)

**TABLE 7. N concentration and natural $^{15}$N abundance of annual fertilizer amendments and total N in surface soil in long-term field experiments (crop rotations) with animal excreta, composts and synthetic N fertilizer**

| Annual fertilizer amendment | | | | | Soil[7] | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Type[1] | Time (yr) | Total N (g/kg) | N rate (kg/ha) | $\delta^{15}$N (‰) | Total N (g/kg) Initial | Total N (g/kg) Final | $\delta^{15}$N (‰) Initial | $\delta^{15}$N (‰) Final | |
| C | 42 | | 0 | | 1.7 | 1.3 ± 0.05 | | +8.5[2] | Gerzabek *et al.* (2001) |
| AM | | 17.6 ± 3.0 | 217 | +7.6[6] ± 1.4 | | 2.2 ± 0.1 | | +8.7[2] | |
| C | 91 | | 0 | | | 1.2 ± 0.05 | | +4.7[3] ± 0.2 | Bol *et al.* (2005) |
| AM | | | 42–98 | | | 1.3 ± 0.04 | | +5.8[3] ± 0.2 | |
| C | 30 | | 0 | | 0.9 | 1.0 | +2.6 | +2.1 | Nishida *et al.* (2007) |
| AS | | 212 | 60–192 | –0.9 - –1.4[4] | 1.6 | 1.6 | +3.2 | +1.4 | |
| LMC | | 4.0 ± 1.6<br>-6.0 ± 0.9 | 81–127[5] | +6.4 ± 1.0<br>- +17.4 ± 0.7 | 1.0 | 2.6 | +3.7 | +6.9 | |

[1] C — control; AM — animal manure; AS — ammonium sulphate; LMC — livestock manure compost
[2] Values are not significantly different (*p < 0.05)
[3] The archived 1985 crop of sugarbeet (the experiment was started in 1894) had $\delta^{15}$N values of +2.0 and +6.5‰ in the tops in control and manure treatments, respectively
[4] Values for 2002 and 2003 samples
[5] Calculated assuming the tabulated moisture content (0.703–0.811 g/g) was on a dry weight basis
[6] Six samples from individual yr in the 1980s–1990s
[7] Soil depth was 0–20 cm except for Nishida *et al.* (2007) where it was 0–15 cm

ever, Koerner *et al.* (1999) found that soil $\delta^{15}$N signatures could be used to identify past agricultural land use where fields had reverted to forests during the past 70–100 yr. Previous garden soils with a history of manure inputs had significantly higher $\delta^{15}$N signatures on average (+3.8‰) than ancient forests (0.0‰) or previous pastures (+1.4‰).

### *Soil biota*

Dijkstra *et al.* (2006a) found that on average the soil microbial biomass had a consistently higher $\delta^{15}$N value (+9.7‰) compared with the total soil N (+6.6‰) across a broad range of soil types, vegetation and climates. Dijkstra *et al.* (2006b) similarly found consistently higher $\delta^{15}$N values of microbial N compared with total soil $\delta^{15}$N across a cattle manure gradient extending 100 m from a reservoir in a semi-arid, high-desert grassland, and whereas the total N increased closer to the reservoir the $\delta^{15}$N value of the total N remained relatively constant (~+10‰), while the microbial $\delta^{15}$N increased closer to the reservoir from ~+14 to ~+18‰.

Schmidt and Ostle (1999) similarly found that soil invertebrates (earthworms) had higher $\delta^{15}$N signatures compared with total soil N in an experiment where cattle slurry ($\delta^{15}$N = +13.2 to +17.0‰) and $NH_4NO_3$ ($\delta^{15}$N = –2.0 to +0.5‰) were individually applied to plots for three consecutive yr. Soil total N (0–10 cm) in organic- and synthetic-fertilized plots had $\delta^{15}$N signatures of +5.9 ± 0.6‰ and +3.9 ± 0.1‰, respectively, while earthworms had $\delta^{15}$N signatures of +6.5 ± 0.2‰ and +5.2 ± 0.3‰, respectively.

### *Leachates*

Choi, Lee and Ro (2003) highlighted the difficulties of identifying sources of $NO_3^-$ contamination of groundwater using $\delta^{15}$N signatures, since the $\delta^{15}$N value is not only a function of source but also of fractionation during formation or consumption. For example, denitrification enriches $^{15}$N in nitrate, and if this process is significant it could mask predicted differences between sources such as synthetic fertilizers which are relatively more depleted in $^{15}$N compared with animal excreta and composts. In a survey of wells monitored over a 3–y period within defined agricultural management systems, Choi *et al.* (2007) were able to correlate the nitrate concentrations and $\delta^{15}$N signatures of well water with applications of compost, compost + urea fertilizer or no soil amendment.

$\delta^{15}$N signatures have proven to be a valuable tool in identifying leakages of animal wastes stored and treated in anaerobic lagoons as well as from land application of the lagoon effluent by spray irrigation onto adjacent fields (Karr *et al.*, 2001 and Karr, Showers and Jennings, 2003; Israel *et al.*, 2005). Nitrate generated from commercial application of swine waste within a catchment was discharged to surface waters by ground water passing beneath the sprayfields and adjacent riparian buffers (Karr *et al.*, 2001). Median values of $\delta^{15}$N of +15.4 ± 0.2‰ were measured *in situ* in the liquid total N of the waste lagoons, and in nitrate in shallow ground water beneath and adjacent to sprayfields, a stream draining sprayfields and waters up to 1.5 km downstream. Israel *et al.* (2005) pointed out that the study of Karr *et al.* (2001) was carried out on a site that had received lagoon effluent for 20 yr prior to the more stringent regulations on land application of animal waste imposed in 1993, and concluded that the riparian buffer was overwhelmed either due to the nitrate concentration in the ground water moving to the stream or to its rapid flux and shallow flow path.

Karr, Showers and Jennings (2003) further demonstrated the sensitivity of $\delta^{15}$N monitoring where low-level export of nitrate from confined dairy farming could be detected even when stream nitrate concentrations were low and derived predominantly from natural soil sources.

## $\delta^{15}$N signatures in the soil-plant-atmosphere continuum in pastures subject to organic N inputs

Long-term (32–36 yr) measurements were made of $\delta^{15}$N in soils and plants inside and outside enclosures in an area subject to native ungulate grazing (Frank and Evans, 1997). Across six topographically diverse sites, $\delta^{15}$N of soil (0–20 cm) outside enclosures was 0.7‰ higher on average than inside enclosures, while plant N was 0.7‰ less on average under grazing. Soil $\delta^{15}$N of urine and dung patches were significantly higher than control areas. Frank and Evans (1997) concluded that grazing probably led to an increase in soil $\delta^{15}$N by promoting N losses ($NH_3$ volatilization, etc). In a later study, Frank,

Evans and Tracy (2004) measured $\delta^{15}N$ in soil, plants and $NH_3$ volatilized from simulated ungulate urine patches. $\delta^{15}N$ of acid-trapped $NH_3$ increased from –28‰ (day 1) to –0.3 ‰ (day 10) from urine that originally had a $\delta^{15}N$ value of +1.2‰. The isotope data showed that shoots absorbed $^{15}N$-depleted $NH_3$ volatilized from the soil, which confirms the results obtained by Denmead *et al.* (1976) using chemical and micro-meteorological techniques.

The natural $^{15}N$ abundance of plants and soils in medium- to long-term experimental plots (8–50 yr) maintained under different management practices were measured in a montane grassland (Watzka, Buchgraber and Wanek, 2006). Plots differed in the types of N inputs (mineral fertilizer, cattle slurry, stable manure) and rates of application (0–200 $kg{\cdot}N{\cdot}ha^{-1}{\cdot}y^{-1}$). $\delta^{15}N$ of topsoil and plants increased with N fertilizer rate, and the $\delta^{15}N$ signatures reflected the higher values for the organic compared with the mineral inputs. N balances were calculated from N inputs (fertilizer, atmospheric deposition, biological $N_2$ fixation) and outputs in harvested material. Strong positive correlations were found between $\delta^{15}N$ of topsoil and N balance (range –60 to +120 $kg{\cdot}N{\cdot}ha^{-1}{\cdot}y^{-1}$). The authors concluded that the different $\delta^{15}N$ signals in topsoil were due to isotopic fractionation arising from increased N losses (mineral fertilizer induced) and to both increased N losses and preservation of the $\delta^{15}N$ input signal (organic fertilizers).

In another long-term experiment (20 yr), Krizan *et al.* (2009) measured changes in the concentration and $\delta^{15}N$ values of total soil N in a lysimeter experiment where cattle slurry ($\delta^{15}N$ = +8.9 ± 0.5‰) and calcium ammonium nitrate ($\delta^{15}N$ = –1.0 ± 0.2‰) were applied at 0 and 480 $kg{\cdot}N{\cdot}ha^{-1}{\cdot}y^{-11}$ to temperate grassland. Total N remained unchanged during the experiment and leaching losses were small. The $\delta^{15}N$ of topsoil increased on average from +1.8 ± 0.4‰ to +6.0 ± 0.4‰ and that of the plants from –1.2 ± 1.3‰ to +4.8 ± 1.2‰ with increasing N fertilizer rate, with samples from slurry plots being relatively more enriched in $^{15}N$. The results suggested that part of the fertilizer $\delta^{15}N$ signal was preserved in the soil, but that isotope fractionation of up to 1.5‰ added to the $\delta^{15}N$ values in soils and plants, indicative of long-term inefficient N usage and past N management.

Yamulki *et al.* (2000) found that cattle excreta patches were an important source of atmospheric $N_2O$, with emissions significantly greater from urine than from dung. Flux patterns showed a marked diurnal variation with maxima in early morning or late afternoon, which were not in phase with soil temperature changes. $\delta^{15}N$ and $\delta^{18}O$ of the $N_2O$ emitted from soil indicated that denitrification was the major loss pathway, and after a heavy rainfall the larger $\delta^{15}N$ and $\delta^{18}O$ values suggested a consumption of $N_2O$ by reduction to $N_2$. In contrast, Tilsner *et al.* (2003) found no unequivocal evidence of the source of $N_2O$ emitted in an extensively managed grassland involving an unfertilized control, a synthetic fertilizer treatment and a slurry treatment based on the $^{15}N$ and $^{18}O$ natural abundance data for emitted $N_2O$. They concluded that the $\delta^{15}N$ values of emitted $N_2O$ were not only influenced by source processes but also by the microbial reduction of $N_2O$ to $N_2$ and that a minimum a flux of 3.4 mmol $N_2O$ $m^{-2}{\cdot}h^{-1}$ was required to obtain accurate isotope data.

Yamulki *et al.* (2001) showed that the temporal intra-molecular distribution of $\delta^{15}N$ and $\delta^{18}O$ in $N_2O$ emitted from urine patches, together with the site preference values for $N_2O$ using the isotopomer methodology, were indicative of a process shift during the measurement period. Using the same methodology, Köster *et al.* (2011) found a rapid shift from denitrification-derived $N_2O$ to $N_2O$ production via nitrification after three weeks following the exhaustion of labile C compounds originating from the addition of biogas fermentation residue to a grassland soil. On the other hand, Cardenas *et al.* (2007) found that the isotopomer signatures for $N_2O$ in control and sheep slurry treatments (lucerne diet) indicated that denitrification was the main process responsible for $N_2O$ emissions from a grassland soil. Based on the majority of the above-mentioned results, it appears that bulk $\delta^{15}N$, $\delta^{18}O$ and isotopomer signatures are promising tools for separating pathways of $N_2O$ production in grassland soils.

**TABLE 8. Range of $\delta^{15}N$ values of plant products according to production system (Inácio *et al.*, 2013)**

| Production system | $\delta^{15}N$ (‰) |
|---|---|
| Conventional[1] | –2.5 to +8.7 |
| Organic[2] | +0.3 to +14.6 |

[1]Synthetic and organic N fertilizers permitted
[2]Synthetic N fertilizers excluded

## Differentiating conventional and organic plant products using $\delta^{15}N$

Because organic and synthetic fertilizer sources often differ markedly in $\delta^{15}N$ composition, it would appear to be a promising marker to distinguish organically- and conventionally-fertilized plant products. The greater the difference between organic and synthetic fertilizer the more robust will be the differentiation. However, different crops show greater or smaller differences between $\delta^{15}N$ values of organic and conventional products, and a certain degree of overlap can occur (Table 8). For example, organic tomatoes showed greater differences in $\delta^{15}N$ values compared with conventional production (+8.1 ± 3.2‰ vs. –0.1 ± 2.1‰, respectively), whereas differences between lettuce were smaller (+7.6 ± 4.1‰ vs. +2.9 ± 4.3‰, respectively), although still statistically significant (Bateman, Keely and Woolfe, 2007). However, $\delta^{15}N$ values of organic and conventional carrots (+5.7 ± 3.5‰ vs. +4.1 ± 2.6‰) were not significantly different. Perennial crops tend to show smaller but significant differences in $\delta^{15}N$ between mode of production, such as orange fruit (+7.3 to +7.9‰ for organic vs. +5.1 to +6.1‰ for conventional) (Camin *et al.*, 2011).

Nevertheless, many production or external factors may confound product designation. e.g. (i) legume products or the use of legume cover crops on organic farms, (ii) crop species with a low N requirement, (iii) annual vs. perennial growth habit, (iv) use of organic fertilizers by conventional farmers, and (v) marketing of organic products as conventional products (Inácio *et al.*, 2013).

For animal products, $\delta^{13}C$ might be the most promising marker for mode of production in temperate regions because of its relationship to diet, i.e. differences between C4 maize grain used in intensive animal feeding operations and C3 pasture grasses and legumes available under free-range conditions. However, $\delta^{13}C$ seems unlikely to be useful in tropical regions for grazing animals due to abundant C4 pasture grasses, e.g. *Brachiaria spp.* (Inácio *et al.*, 2013).

## CONCLUSIONS

The $^{15}N$ natural abundance of animal excreta and composts is a useful tool for following the fate of organic N sources in the soil-plant-atmosphere continuum. The alternative approach of using organic fertilizers artificially-enriched in $^{15}N$ is time-consuming, expensive and inefficient, with the attendant risks of non-uniform labelling and perturbation of the system under study. However, fewer studies have been conducted at the level of natural $^{15}N$ abundance compared with artificially-enriched materials, and δ values have generally been

used as qualitative rather than quantitative measures of N processes at the system level.

Nevertheless, recent results showing significant positive and negative linear relationships during storage of animal excreta, between the bulk $\delta^{15}N$ signature and cumulative $NH_3$ loss and between bulk $\delta^{15}N$ of composts and N concentration, respectively, suggest that $\delta^{15}N$ may have wider applications in estimating the efficiency of N conservation during storage or composting. The combined use of bulk $\delta^{15}N$ and $\delta^{18}O$ signatures of $N_2O$ evolved during storage and composting, together with the isotopomer-derived site preference of $N_2O$, are powerful tools for identifying the processes of $N_2O$ formation in order to enable formulation of mitigation strategies.

Finally, it has been demonstrated that $\delta^{15}N$ signatures could play a role in differentiating organic and conventional plant products provided each of the two populations for each product has been adequately described by a frequency distribution that enables a statistical analysis of a sample. The potential use of stable isotopes in differentiating conventional and organic animal products remains to be addressed. In addition, a review and analysis of the post-1998 literature on animal excreta artificially enriched in $^{15}$N needs to be undertaken to complement and extend the results obtained with $^{15}$N natural abundance reported here.

## REFERENCES


**Bateman, A.S., Kelly, S.D. & Woolfe, M.** 2007. Nitrogen isotope composition of organically and conventionally grown crops. *J. Agric. Food Chem.,* 55: 2664–2670.

**Bol, R., Eriksen J., Smith, P., Garnett, M.H., Coleman K. & Christensen B.T.** 2005. The natural abundance of $^{13}$C, $^{15}$N, $^{34}$S and $^{14}$C in archived (1923–2000) plant and soil samples from the Askov long-term experiments on animal manure and mineral fertilizer. *Rapid Commun. Mass Spectrom.,* 19: 3216–3226.

**Bol, R., Toyoda, S., Yamulki, S., Hawkins, J.M.B., Cardenas, L.M. & Yoshida, N.** 2003. Dual isotope and isotopomer ratios of $N_2O$ emitted from a temperate grassland soil after fertiliser application. *Rapid Commun. Mass Spectrom.,* 17: 2550–2556.

**Camin, F., Perini, M., Bontempo, L., Fabroni, S., Faedi, W., Magnani, S., Baruzzi, G., Bonoli, M., Tabilio, M.R., Musmeci, S., Rossmann, A., Kelly, S.D. & Rapisarda, P.** 2011. Potential isotopic and chemical markers for characterizing organic fruits. *Food Chem.,* 125: 1072–1082.

**Cardenas, L.M., Chadwick, D., Scholefield, D., Fychan, R., Marley, C.L., Jones, R., Bol, R., Well, R. & Vallejo, A.** 2007. The effect of diet manipulation on nitrous oxide and methane emissions from manure applied to incubated grassland soils. *Atmos. Environ.,* 41: 7096–7107.

**Chalk, P.M.** 1995. Isotope ratios. *In* A. Townshend & P. Worsfold, eds. *Encyclopedia of analytical science,* pp. 2409–2415. London, Academic Press Limited.

**Chalk, P.M.** 1997. *The dynamics of soil, fertilizer and biologically-fixed nitrogen in soil-plant systems.* The University of Melbourne. (D. Agr. Sc. dissertation). 618 pp.

**Chalk, P.M., Magalhães, A.M.T. & Inácio, C.T.** 2013. Towards an understanding of the dynamics of compost N in the soil-plant-atmosphere system using $^{15}$N tracer. *Plant Soil,* 362: 373–388.

**Cheng, L., Kim, E.J., Merry, R.J. & Dewhurst, R.J.** 2011. Nitrogen partitioning and isotopic fractionation in dairy cows consuming diets based on a range of contrasting forages. *J. Dairy Sci.,* 94: 2031–2041.

**Choi, W.J., Arshad, M.A., Chang, S.X. & Kim, T.H.** 2006. Grain $^{15}$N of crops applied with organic and chemical fertilizers in a four-year rotation. *Plant Soil,* 284: 165–174.

**Choi, W-J., Han, G-H., Lee, S-M., Lee, G-T., Yoon, K-S., Choi, S-M. & Ro, H-M.** 2007. Impact of land-use types on nitrate concentration and $\delta^{15}N$ in unconfined groundwater in rural areas of Korea. *Agric. Ecosyst. Environ.,* 120: 259–268.

**Choi, W-J., Lee, S-M. & Ro, H-M.** 2003. Evaluation of contamination sources of groundwater $NO_3^-$ using nitrogen isotope data: A review. *Geosci. J.,* 7: 81–87.

**Choi, W-J., Lee, S-M., Ro, H-M., Kim, K-C. & Yoo, S-H.** 2002. Natural $^{15}$N abundances of maize and soil amended with urea and composted pig manure. *Plant Soil,* 245: 223–232.

**Clough, T.J., Ledgard, S.F., Sprosen, M.S. & Kear, M.J.** 1998. Fate of $^{15}$N labelled urine in four soil types. *Plant Soil,* 199: 195–203.

**Denmead, O.T., Freney, J.R. & Simpson, J.R.** 1976. A closed ammonia cycle within a plant canopy. *Soil Biol. Biochem.,* 8: 161–164.

**Dijkstra, P., Ishizu, A., Doucett, R., Hart, S.C., Schwartz, E., Menyailo, O.V. & Hungate, B.A.** 2006a. $^{13}$C and $^{15}$N natural abundance of the soil microbial biomass. *Soil Biol. Biochem.,* 38: 3257–3266.

**Dijkstra, P., Menyailo, O.V., Doucett, R.R., Hart, S.C., Schwartz, E. & Hungate, B.A.** 2006b. C and N availability affects the $^{15}$N natural abundance of the soil microbial biomass across a cattle manure gradient. *Eur. J. Soil Sci.,* 57: 468–475.

**Dittert, K., Georges, T. & Sattelmacher, B.** 1998. Nitrogen turnover in soil after application of animal manure and slurry as studied by the stable isotope $^{15}$N: A review. *J. Soil Sci. Plant Nutr.,* 161: 453–463.

**Frank, D.A. & Evans, R.D.** 1997. Effects of native grazers on grassland N cycling in Yellowstone National Park. *Ecol.,* 78: 2238–2248.

**Frank, D.A., Evans, R.D. & Tracy, B.F.** 2004. The role of ammonia volatilization in controlling the natural $^{15}$N abundance of a grazed grassland. *Biogeochem.,* 68: 169–178.

**Gerzabek, M.H., Haberhauer, G. & Kirchmann, H.** 2001. Nitrogen distribution and $^{15}$N natural abundance in particle size fractions of a long-term agricultural field experiment. *J. Plant Nutr. Soil Sci.,* 164: 475–481.

**Högberg, P.** 1997. Tansley Review No. 95. $^{15}$N natural abundance in soil-plant systems. *New Phytol.,* 137: 179-203.

**Hristov, A.N., Zaman S., Vander Pol, M., Ndegwa, P., Campbell, L. & Silva, S.** 2009. Nitrogen losses from dairy manure estimated through nitrogen mass balance and chemical markers. *J. Environ. Qual.,* 38: 2438-2448.

**Inácio, C.T., Chalk, P.M. & Magalhães, A.M.T.** 2013. Principles and limitations of stable isotopes in differentiating organic and conventional foodstuffs: 1. Plant products. *Crit. Rev. Food Sci. Nutr.,* (in press)

**Israel, D.W., Showers, W.J., Fountain, M. & Fountain, J.** 2005. Nitrate movement in shallow groundwater from swine-lagoon-effluent spray fields managed under current application regulations. *J. Environ. Qual.,* 34: 1828-1842.

**Junk, G. & Svec, H.J.** 1958. The absolute abundance of the nitrogen isotopes in the atmosphere and compressed gas from various sources. *Geochim. Cosmochim. Acta,* 14: 234–243.

**Karr, J.D., Showers, W.J., Gilliam, J.W. & Andres, A.S.** 2001. Tracing nitrate transport and environmental impact from intensive swine farming using delta nitrogen-15. *J. Environ. Qual.,* 30: 1163–1175.

**Karr, J.D., Showers, W.J. & Jennings, J.D.** 2003. Low-level nitrate export from confined dairy farming detected in North Carolina streams using $\delta^{15}N$. *Agric. Ecosyst. Environ.,* 95: 103–110.

**Kim, Y-J., Choi, W-J., Lim, S-S., Kwak, J-H., Chang, S.X., Kim, H-Y., Yoon, K-S. & Ro, H-M.** 2008. Changes in nitrogen isotopic compositions during composting of cattle feedlot manure: effects of bedding material type. *Bioresour. Technol.,* 99: 5452–5458.

**Koerner, W., Dambrine, E., Dupouey, J.L. & Benoît, M.** 1999. $\delta^{15}N$ of forest soil and understory vegetation reflect the former agricultural land use. *Oecologia,* 121: 421–425.

**Köster, J.R., Cárdenas, L., Senbayram, M., Bol, R., Well, R., Butler, M., Mühling, K.H. & Dittert, K.** 2011. Rapid shift from denitrification to nitrification in soil after biogas residue application as indicated by nitrous oxide isotopomers. *Soil Biol. Biochem.,* 43: 1671–1677.

**Kriszan, M., Amelung, W., Schellberg, J., Gebbing, T. & Kühbauch, W.** 2009. Long-term changes in the $\delta^{15}N$ natural abundance of plants and soil in a temperate grassland. *Plant Soil,* 325: 157–169.

**Lee, C., Hristov, A.N., Cassidy, T. & Heyler K.** 2011. Nitrogen isotope fractionation and origin of ammonia nitrogen volatilized from cattle manure in simulated storage. *Atmosphere,* 2: 256-270.

**Lim, S-S., Lee, S-M., Lee, S-H. & Choi, W-J.** 2010. Nitrogen isotope compositions of synthetic fertilizer, raw livestock manure slurry, and compost livestock manure. *Korean J. Soil Sci. Fert.,* 43: 453–457.

**Lynch, D.H., Voroney, R.P. & Warman, P.R.** 2006. Use of $^{13}C$ and $^{15}N$ natural abundance techniques to characterize carbon and nitrogen dynamics in composting and in compost-amended soils. *Soil Biol. Biochem.,* 38: 103–114.

**Maeda, K., Toyoda, S., Shimojima, R., Osada, T., Hanajima, D., Morioka, R. & Yoshida, N.** 2010. Source of nitrous oxide emissions during the cow manure composting process as revealed by isotopomer analysis of and *amoA* abundance in betaproteobacterial ammonia-oxidizing bacteria. *Appl. Environ. Microbiol.,* 76: 1555–1562.

**Mariappan, S., Exner, M.E., Martin, G.E. & Spalding, R.F.** 2009. Variability of anaerobic animal waste lagoon delta $^{15}N$ source signatures. *Environ. Forensics,* 10: 19–26.

**Nicholson, R.J. & Brewer, A.J.** 1997. Estimates of volumes and exposed surface areas of stored animal manures and slurries in England and Wales. *J. Agric. Eng. Res.,* 66: 239–250.

**Nishida, M., Iwaya, K., Sumida, H. & Kato, N.** 2007. Changes in natural $^{15}N$ abundance in paddy soils under different, long-term soil management regimes in the Tohoku region of Japan. *Soil Sci. Plant Nutr.,* 53: 310–317.

**Robinson, D.** 2001. $\delta^{15}N$ as an integrator of the nitrogen cycle. *Trends Ecol. Evolut.,* 16: 153–162.

**Sasaki, H., Kitazume, O., Nonaka, J., Hikosaka, K., Otawa, K., Itoh, K., & Nakai, Y.** 2006. Effect of a commercial microbiological additive on beef manure compost in the composting process. *Anim. Sci. J.,* 77: 545–548.

**Schmidt, O. & Ostle, N.J.** 1999. Tracing nitrogen derived from slurry in earthworms using $^{15}N/^{14}N$ stable isotope ratios at natural abundance. *Appl. Soil Ecol.,* 12: 7–13.

**Selles, F. & Karamanos, R.E.** 1986. Variations in natural N-15 abundance as an aid in manure- nitrogen studies. *J. Environ. Qual.,* 15: 24–30.

**Sponheimer, M., Robinson, T.F., Roeder, B.L., Passey, B.H., Ayliffe, L.K., Cerling, T.E., Dearing, M.D. & Ehleringer, J.R.** 2003. An experimental study of nitrogen flux in llamas: Is $^{14}N$ preferentially excreted? *J. Archaeol. Sci.,* 30: 1649–1655.

**Steele, K.W. & Daniel, R.M.** 1978. Fractionation of nitrogen isotopes by animals: a further complication to the use of variation in the natural abundance of $^{15}N$ for tracer studies. *J. Agric. Sci.,* 90: 7–9.

**Sutka, R.L., Ostrom, N.E., Ostrom, P.H., Breznak, J.A, Gandhi, H., Pitt, A.J. & Li, F.** 2006. Distinguishing nitrous oxide production from nitrification and denitrification on the basis of isotopomer abundances. *Appl. Environ. Microbiol.,* 72: 638–644.

**Szpak, P., Millaire, J-F., White, C.D. & Longstaffe, F.J.** 2012. Influence of seabird guano and camelid dung fertilization on the nitrogen isotopic composition of field-grown maize (*Zea mays*). *J. Archaeol. Sci.,* 39: 3721–3740.

**Tilsner, J., Wrage, N., Lauf, J. & Gebauer, G.** 2003. Emission of gaseous nitrogen oxides from an extensively managed grassland in NE Bavaria. Germany. II. Stable isotope natural abundance of $N_2O$. *Biogeochem.,* 63: 249–267.

**Watzka, M., Buchgraber, K. & Wanek, W.** 2006. Natural $^{15}N$ abundance of plants and soils under different management practices in a montane grassland. *Soil Biol. Biochem.,* 38: 1564–1576.

**Wrage, N., Lauf, J., del Prado, A., Pinto, M., Pietrzak, S., Yamulki, S., Oenema, O. & Gebauer, G.** 2004. Distinguishing sources of $N_2O$ in European grasslands by stable isotope analysis. *Rapid Commun. Mass Spectrom.,* 18: 1201–1207.

**Yamulki, S., Toyoda, S., Yoshida, N., Veldkamp, E., Grant, B. & Bol, R.** 2001. Diurnal fluxes and the isotopomer ratios of $N_2O$ in a temperate grassland following urine amendment. *Rapid Commun. Mass Spectrom.,* 15: 1263–1269.

**Yamulki, S., Wolf, I., Bol, R., Grant, B., Brumme, R., Veldkamp, E. & Jarvis, S.C.** 2000. Effect of dung and urine amendments on the isotopic content of $N_2O$ released from grasslands. *Rapid Commun. Mass Spectrom.,* 14: 1356–1360.

**Yoh, M., Akita, Y., Toyoda, S. & Yoshida, N.** 2003. Nitrogen stable isotope abundance of $N_2O$ from composting of livestock waste: Its variation and mechanics. *Geochim. Cosmochim. Acta Suppl.,* 67 Issue 18: 565.

**Yun, S-I., Lim, S-S., Lee, G-S., Lee, S-M., Kim, H-Y., Ro, H-M. & Choi, W-J.** 2011. Natural $^{15}N$ abundance of paddy rice (*Oryza sativa* L.) grown with synthetic fertilizer, livestock manure compost, and hairy vetch. *Biol. Fertil. Soils,* 47: 607–617.

**Yun, S-I., & Ro, H-M.** 2009. Natural $^{15}N$ abundance of plant and soil inorganic-N as evidence for over-fertilization with compost. *Soil Biol. Biochem.,* 41: 1541–1547.

**Zhao, B., Maeda, M. & Ozaki, Y.** 2002. Natural $^{15}N$ and $^{13}C$ abundance in Andisols influenced by long-term fertilization management in Japan. *Soil Sci. Plant Nutr.,* 48: 555–562.