JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Nitrogen Isotope Relationships between Crops and Fertilizer: Implications for Using Nitrogen Isotope Analysis as an Indicator of Agricultural Regime

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The effect of fertilizer type, amount of fertilizer applied, growing medium, and water type on the nitrogen-15 content of carrots, tomatoes, and lettuces has been investigated. Crops grown using synthetic nitrogen fertilizer were isotopically lighter than those grown using pelleted chicken manure. For example, for equivalent amounts of nitrogen applied, carrots grown with ammonium nitrate fertilizer had δ^{15}N values between 3‰ and 4‰ lower than those grown using pelleted chicken manure. Plants grown in peat-based compost were generally found to be isotopically lighter than those grown in composted bark based compost. Results suggest that nitrate content and the δ^{15}N of the nitrate in irrigation water may also influence crop δ^{15}N. Wider implications of using crop δ^{15}N more generally as an indicator of whether synthetic nitrogen fertilizers have been applied to a crop and the possible application and limitations of using crop δ^{15}N as an indicator of agricultural regime (organic/conventional) are discussed.

KEYWORDS: Nitrogen; isotope; fertilizer; organic; conventional; δ^{15}N

INTRODUCTION

Across the European Union, to be labeled as "organic", food must be grown and processed according to European Community Council Regulation (EEC) 2092/91 (*1*). This regulation describes the inputs and practices that may be used in organic farming and growing, and the inspection system which must be in place to ensure that these conditions are met. The regulations apply both to food grown and processed within the European Union and to imported produce. Similar systems exist elsewhere, e.g., the United States of America, where the United States Department of Agriculture has set up a National Organic Program and instigated a set of regulations governing organic production and a certification system.

The organic sector has experienced rapid growth over recent years. The global market is currently worth around £14 billion with the largest markets in the United States of America (£5.9 billion), followed by Germany (£1.6 billion) and the United Kingdom (£1 billion) (*2*). Organic produce tends to retail at a higher price than its conventionally grown equivalent mainly because of higher production costs. Synthetic nitrogen fertilizers are not permitted in organic farming. Instead, soil fertility is maintained through the use of crop rotations that include green manures and also by the application of selected fertilizers which may be permitted where the need is recognized by an inspecting authority. Fertilizers that may be permitted include animal manures, composts, and other products of plant and animal origin, e.g. rapemeal, bloodmeal, fishmeal, and seaweed products. It has been suggested that inputs of chemically synthesized nitrogen fertilizers, used in conventional agricultural regimes, may produce crops that can be differentiated on the basis of their nitrogen isotope composition from crops grown under organic regimes (*3*). Synthetic nitrogen fertilizers tend to have δ^{15}N values within a few per mil of zero (*4*, *5*) since their nitrogen is derived from atmospheric nitrogen ($\delta^{15}N_{atm} = 0‰$) and there tends to be little fractionation during the production process (see subsequent section for an explanation of δ-notation). Animal manures with δ^{15}N values around +5‰ have been reported to produce nitrate with δ^{15}N values in the range of +10‰ to +22‰ (*6*). This enrichment is mainly due to the preferential volatilization of ^{15}N depleted ammonia from the manure. Nitrogen isotope values for other fertilizers which may be permitted under organic regimes are not well documented but are likely to exhibit a much wider range of compositions than synthetic fertilizers due to their more diverse origins.

The possible use of nitrogen isotopes to differentiate between crops grown with or without inputs of synthetic nitrogen is based on the hypothesis that the application of synthetic nitrogen fertilizers with δ^{15}N values close to 0‰ will result in the δ^{15}N of plants grown in conventional regimes being lower than those grown in organic regimes. Previous studies have found that grain crops grown in soils to which synthetic nitrogen fertilizers were added had lower δ^{15}N values than plants grown in the same soil to which manure was added (*7*). Kohl et al. (*8*) found that

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10.1021/jf050374h CCC: $30.25
Published on Web 06/18/2005

Table 1. Summary of Treatments Applied to Each Crop Type Grown in the Pot Experiments[a]

	variables	treatment
carrots	fertilizer type	NH_4NO_3 pelleted chicken manure none
	fertilizer application rate	50 kg of N/ha 100 kg of N/ha
tomatoes	fertilizer type	conventional all-purpose feed pelleted chicken manure none
	growing medium	peat-based compost composted bark based compost
lettuces	fertilizer type	conventional all-purpose feed pelleted chicken manure none
	growing medium	peat-based compost composted bark based compost
	water	deionized nitrogen-free water local tap water

[a] See text for detailed description of the fertilizer type, nitrogen content, and δ^{15}N composition of the fertilizers and growing media.

the δ^{15}N value of a maize crop decreased with increasing applications of a synthetic nitrogen fertilizer.

In this paper, we present results from experiments in which tomatoes, lettuces, and carrots were grown under greenhouse conditions with controlled inputs of nitrogen. The effects of fertilizer type (synthetic chemical fertilizer or pelleted chicken manure), amount of fertilizer applied, water type (deionized nitrogen-free water or nitrate-containing local tap water), and growing medium were examined. The variables examined for each crop type are summarized in **Table 1** and described in more detail in the following section. The potential for using crop δ^{15}N more generally as an indicator of whether synthetic nitrogen fertilizers have been applied to a crop during cultivation is discussed.

MATERIALS AND METHODS

Analytical Procedures. Samples for nitrogen isotope analysis were freeze-dried and homogenized during grinding to a fine powder using a ball mill. Nitrogen content (% N) and δ^{15}N were determined using a PDZ Europa ANCA-GSL elemental analyzer connected to a 20:20 continuous flow isotope ratio mass spectrometer. Samples were analyzed in triplicate and values accepted when precision (σ^{n-1}, $n = 3$) was <0.3‰. Dried samples were weighed into tin capsules, and standards and samples matched to give 0.1 mg (±20%) of N per analysis. The total-N content of standards and that of samples were closely matched in order to minimize errors associated with source-linearity effects (*9*). Nitrogen isotope data are reported in conventional δ-notation in units of per mil (‰) with respect to atmospheric nitrogen (air) according to eq 1,

$$\delta^{15}\mathrm{N}_{\mathrm{sample}}(‰) = \left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}} - 1\right) \times 1000 \qquad (1)$$

where $R = {}^{15}\mathrm{N}/{}^{14}\mathrm{N}$ and the standard is atmospheric nitrogen with a ^{15}N/^{14}N ratio of 0.00368 and a δ^{15}N value of 0‰. Inorganic fertilizer samples were referenced against IAEA-N1 (ammonium sulfate reference material certified by the International Atomic Energy Agency) with a δ^{15}N composition of +0.4‰. Organic samples were referenced against a casein in-house standard with an accepted value of +6.3‰ (previously calibrated against IAEA reference materials during an interlaboratory comparison exercise involving 6 European Food Control laboratories as part of European Union Project SMT4-CT98-2236 to develop methods to determine the origin of milk, butter, and cheese). Long-term performance of the mass spectrometer was monitored by analysis of a secondary reference material in every batch. The secondary reference materials for inorganic and organic samples were IAEA-N2 (ammonium sulfate) and L-alanine (in-house reference) with accepted δ^{15}N values of +20.3‰ and +8.7‰, respectively. The long-term standard deviation of the values obtained from measurements of the secondary laboratory standards was 0.16‰.

Soil, Compost, and Fertilizer. Carrots (*Daucus carota* L. cv. Early Nantes 2) were cultivated from seed in 20 cm diameter pots in soil from a plot that had been uncultivated for the previous 5 years. The δ^{15}N of the soil (total soil nitrogen) determined at the start of the experiment was +8.4‰, and N content was 0.3%. Pots were subsequently fertilized either with pelleted chicken manure (δ^{15}N = +5.4‰, % N = 6 wt %, dry matter) or with ammonium nitrate (NH_4NO_3, δ^{15}N = −1.3‰, % N = 34.5 wt %, dry matter) or were given no additional fertilizer. Fertilizer applications equivalent to 50 kg of N/ha and 100 kg of N/ha were made for each fertilizer type based on the surface area of the pots and the % nitrogen content of each fertilizer. These levels of nitrogen application are typical of those used in carrot cultivation although the amount of nitrogen applied is dependent on local conditions. The pots were kept in an unheated greenhouse and watered with nitrogen-free deionized water. One application of fertilizer, either the pelleted chicken manure or ammonium nitrate as appropriate, was applied one month after sowing. Three carrots were harvested from each of the pots, and the δ^{15}N of each individual was determined separately.

Tomato plants (*Lycopersicon esculentum* Mill. cv. Moneymaker) were grown from seed in a temperature- and light-controlled greenhouse. All plants received identical treatment until transplanting after 4 weeks. At this stage, half were transplanted into a commercial peat-based compost enriched with plant nutrients ($\delta^{15}\mathrm{N}_{\mathrm{peat}}$ = −0.3‰, % N = 1.6 wt %, dry matter) and half were transplanted into a compost made from composted bark and enriched with plant nutrients ($\delta^{15}\mathrm{N}_{\mathrm{composted\ bark}}$ = −0.1‰, % N = 1.3 wt %, dry matter), a commercially available compost endorsed by the Henry Doubleday Research Association, a U.K. based organic organization. Three replicates transplanted into each compost type were subsequently fertilized with a commercial conventional all-purpose water soluble plant food ("Miracle Gro", δ^{15}N = −0.4‰, % N = 15 wt %, dry matter), three were fertilized with pelleted chicken manure (δ^{15}N = +5.4‰, % N = 6 wt %, dry matter), and three had no fertilizer addition. The plants to which fertilizer was applied received 0.06 g of nitrogen in each of five applications. All pots were watered with nitrogen-free deionized water.

Lettuce plants (*Lactuca sativa* L. cv. All the Year Round) were grown from seed in a temperature- and light-controlled greenhouse. Half were cultivated in the commercial peat-based compost and half in the compost derived from composted bark, as described above. Three replicates grown in each compost type received either the conventional all-purpose plant food, the pelleted chicken manure, or no fertilizer. The plants to which fertilizer was applied received 0.06 g of nitrogen in each of three applications. All these plants were watered with nitrogen-free deionized water. Additionally in this experiment, a second set of plants were grown and treated as described above except that the plants were watered with local tap water that contains nitrate. Three replicates for each treatment were analyzed except for the lettuces cultivated in the compost made from composted bark and watered with nitrogen-free deionized water. Lettuces receiving this treatment germinated successfully but did not continue to develop, presumably due to lack of a nutrient/s. Only 3 of these plants transplanted successfully 4 weeks after sowing, and therefore the three subsequent fertilizer treatments (conventional/pelleted chicken manure/none) were applied to one plant only, not in triplicate as for all other treatments.

Statistical analysis was performed using the SPSS 11.0.1 package for Windows (SPSS Inc., Chicago). Factorial analysis of variance (ANOVA) has been used to ascertain if there is any statistically significant difference between the δ^{15}N values of crops grown under different conditions (fertilizer, compost, water) and to identify any interaction between these factors.

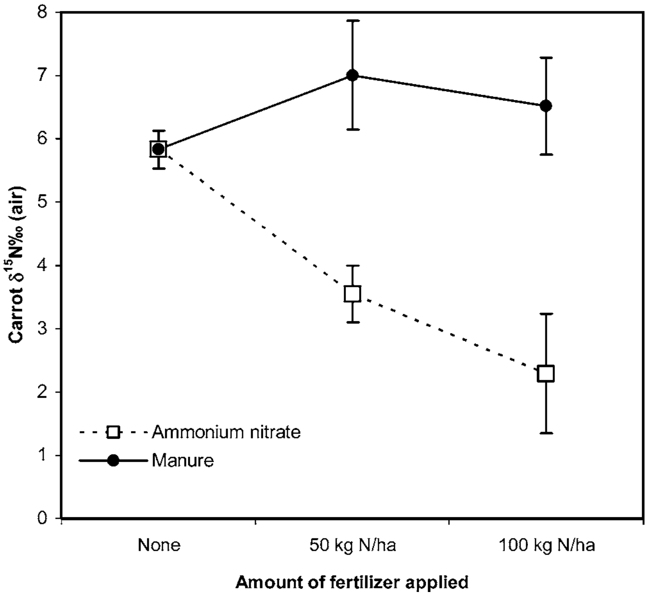

Figure 1. $\delta^{15}N$(‰)$_{air}$ of carrots grown with different amounts and types of fertilizer. Values are mean and standard deviation (σ^{n-1}) of 3 samples. $\delta^{15}N(NH_4NO_3)$ = –1.3‰. $\delta^{15}N$(manure) = +5.4‰.

RESULTS AND DISCUSSION

Figure 1 shows the nitrogen isotope composition of carrots grown in pots to which different amounts of nitrogen were added either as synthetic NH_4NO_3 fertilizer or as pelleted chicken manure. Also shown is the mean nitrogen isotope composition of carrots grown without any fertilizer addition. The carrots grown in the soil amended with NH_4NO_3 fertilizer have lower $\delta^{15}N$ values than carrots grown using pelleted chicken manure and the carrots grown without any fertilizer addition. For equivalent amounts of nitrogen applied, carrots grown with NH_4NO_3 have $\delta^{15}N$ values that are between 3.2‰ and 4.0‰ lower than the $\delta^{15}N$ values of carrots grown using pelleted chicken manure. Plant $\delta^{15}N$ is a function of the $\delta^{15}N$ of the utilizable nitrogen pools, the proportion the plant derives from each pool and any isotopic fractionation associated with uptake and assimilation. The $\delta^{15}N$ values of the carrots grown with the NH_4NO_3 fertilizer suggest that these plants used a mixture of nitrogen from the applied fertilizer ($\delta^{15}N = -1.3$‰) and nitrogen that was naturally present in the soil. The $\delta^{15}N$ of the carrots is lower when the amount of NH_4NO_3 applied was higher, suggesting that a greater proportion of the assimilated nitrogen was derived from the NH_4NO_3 when there was more available, although this is not a strong signal. There is no clear difference in the $\delta^{15}N$ signature of the carrots grown using pelleted chicken manure and those grown without added fertilizer. Carrots grown without any additional fertilizer had $\delta^{15}N$ values approximately 2.5‰ lower than the soil total nitrogen $\delta^{15}N$ value (+8.4‰). There is thought to be negligible fractionation during plant uptake in most nitrogen limited systems (*10*). The nitrogen available for plant growth is a small component of the total amount of soil nitrogen, most of which is bound in forms that are unavailable to plants. The $\delta^{15}N$ of the carrots grown without additional fertilizer therefore probably provides a good indication of the $\delta^{15}N$ of the plant-available nitrogen component of the soil.

These observations are supported by factorial analysis of variance (ANOVA) with fertilizer type and fertilizer amount specified as fixed factors and $\delta^{15}N$ as the dependent variable and applying a univariate general linear model (GLM). Factorial ANOVA identifies a significant main effect of fertilizer type

Table 2. Summary of Factorial ANOVA[a]

	degrees of freedom	F	signif
Carrots			
fertilizer type	1	73.7	0.000
fertilizer amount	1	3.79	0.088
fertilizer type * fertilizer amount	1	0.74	0.415
Tomatoes			
compost type	1	15.2	0.002
fertilizer	2	30.4	0.000
compost type * fertilizer	2	14.7	0.000
Lettuces			
compost type	1	394	0.000
fertilizer type	2	50.2	0.000
water type	1	0.325	0.576
compost type * fertilizer	2	43.0	0.000
water type * fertilizer	2	0.129	0.880
water type * compost type	1	27.5	0.000
compost type * fertilizer * water type	2	6.19	0.009

[a] An effect is considered significant if the significance is <0.05. Asterisk (*) signifies interaction between the factors.

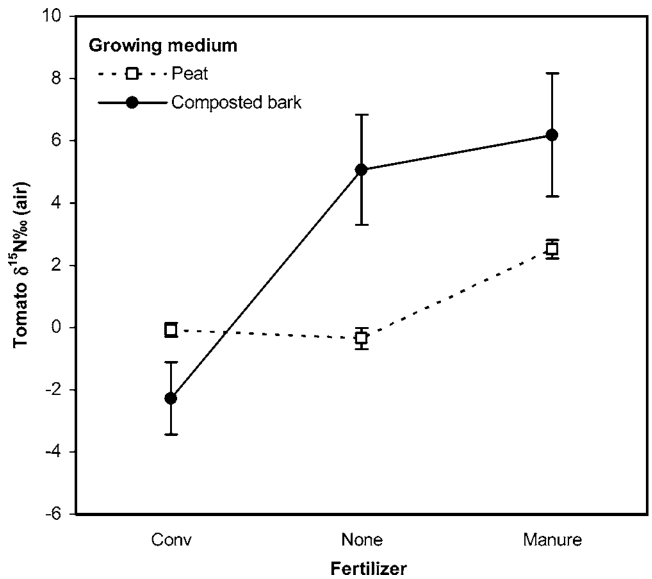

Figure 2. $\delta^{15}N$(‰)$_{air}$ of tomatoes grown in different growing media and with different types of fertilizer. Values are mean and standard deviation (σ^{n-1}) of 3 tomatoes (1 from each of 3 plants grown using the same treatment); see text for details. $\delta^{15}N$(conventional fertilizer) = –0.4‰. $\delta^{15}N$(manure) = +5.4‰.

on carrot $\delta^{15}N$, but the effect of fertilizer amount is not significant (see **Table 2**). The GLM has an R^2 of 0.902 and an adjusted R^2 of 0.872. An adjusted R^2 value takes into account the number of variables used in a model and will plateau below 1 as additional unimportant terms are added unlike an R^2 value that would rise asymptotically toward 1 regardless of whether variables added are significant or not. The adjusted R^2 value indicates that 87.2% of the variability in carrot $\delta^{15}N$ is explained by the GLM that is based on the measured variables.

Figure 2 shows the nitrogen isotope composition of tomatoes from plants grown in the different compost types and to which different fertilizers (or no fertilizer) were applied. Comparison of the $\delta^{15}N$ values of tomatoes grown in the peat-based compost and the composted bark without any subsequent addition of fertilizer shows that compost type affected the $\delta^{15}N$ value of the tomatoes. Tomatoes grown in the peat-based compost have $\delta^{15}N$ values that are on average 5.0‰ lower than the $\delta^{15}N$ values of tomatoes grown in the composted bark, when no additional fertilizer was applied. Given that the $\delta^{15}N$ values of the total nitrogen in each of the composts were similar (−0.3‰ and −0.1‰ for the peat-based compost and composted bark, respectively), this suggests that the $\delta^{15}N$ of the readily available nitrogen in the peat-based compost is lower than that available from the composted bark. Both commercially available composts were described as being "enriched in plant nutrients". In the case of the peat-based compost, these plant nutrients probably include synthetic nitrogen with a $\delta^{15}N$ value typical of chemically produced nitrogen fertilizer (∼0‰) that is in a highly soluble form, e.g. nitrate, and readily available to plants.

Figure 2 also illustrates that the type of fertilizer applied affects the $\delta^{15}N$ of the tomatoes. The mean $\delta^{15}N$ value for tomatoes from plants grown in the peat-based compost and subsequently amended with the conventional fertilizer is 0‰ (σ^{n-1} = 0.2‰, n = 3). This compares with a mean $\delta^{15}N$ value for those subsequently amended with the pelleted chicken manure of +2.5‰ (σ^{n-1} = 0.3‰, n = 3). Similarly, tomatoes from plants grown in the composted bark and subsequently amended with conventional fertilizer have a lower mean $\delta^{15}N$ value ($\bar{x}$ = −2.3‰, σ^{n-1} = 1.2‰, n = 3) than those subsequently amended with the pelleted chicken manure ($\bar{x}$ = +6.2‰, σ^{n-1} = 2.0‰, n = 3). Factorial ANOVA identifies a significant effect of both compost type and fertilizer type on tomato $\delta^{15}N$ and also a significant interaction between compost type and fertilizer type. **Figure 2** shows that the effect of conventional fertilizer is more pronounced when composted bark has been used as the growing medium compared to when the peat-based compost has been used, and this may be the interaction identified by the factorial ANOVA. This may be partly because the readily available nitrogen in the peat (from the added nutrients) and from the conventional fertilizer is synthetically derived in both cases, and so they have similar $\delta^{15}N$ compositions, resulting in a relatively small effect of the conventional fertilizer on crop $\delta^{15}N$ when peat has been used as the growing medium. It may also be due to a relatively small amount of plant-available nitrogen in the composted bark, meaning that plants grown in the composted bark are quick to take up nitrogen from the subsequently applied conventional fertilizer lowering the $\delta^{15}N$ values of tomatoes for these plants. It is evident from **Figure 2** that there is greater variability in the $\delta^{15}N$ composition of the tomatoes analyzed from the plants grown in the composted bark compared to those grown in the peat-based compost (from comparison of the size of the error bars). A Levene's test for equality of variances confirms that the variances are not equal across groups. There is no obvious explanation for why this should be the case. The GLM has an R^2 of 0.896 and an adjusted R^2 of 0.856.

Figure 3 shows the lettuce $\delta^{15}N$ values for 12 different combinations of compost type, fertilizer treatment, and water type applied to the lettuces. All the lettuces grown in the conventional peat-based compost have lower $\delta^{15}N$ values than those of the lettuces grown in the composted bark irrespective of the type of fertilizer treatment or whether the plants were watered with deionized water or local tap water. Lettuces that received conventional fertilizer generally have the lowest $\delta^{15}N$ values when compared against otherwise identical treatments. The exception to this is the lettuces grown in the peat-based compost and watered with deionized water. In this case, the lettuces that did not receive any additional fertilizer had slightly lower $\delta^{15}N$ values than those that were amended with the conventional fertilizer. This may be because although a smaller reservoir of nitrogen was available for the lettuces grown without any additional fertilizer, the readily available nitrogen in the

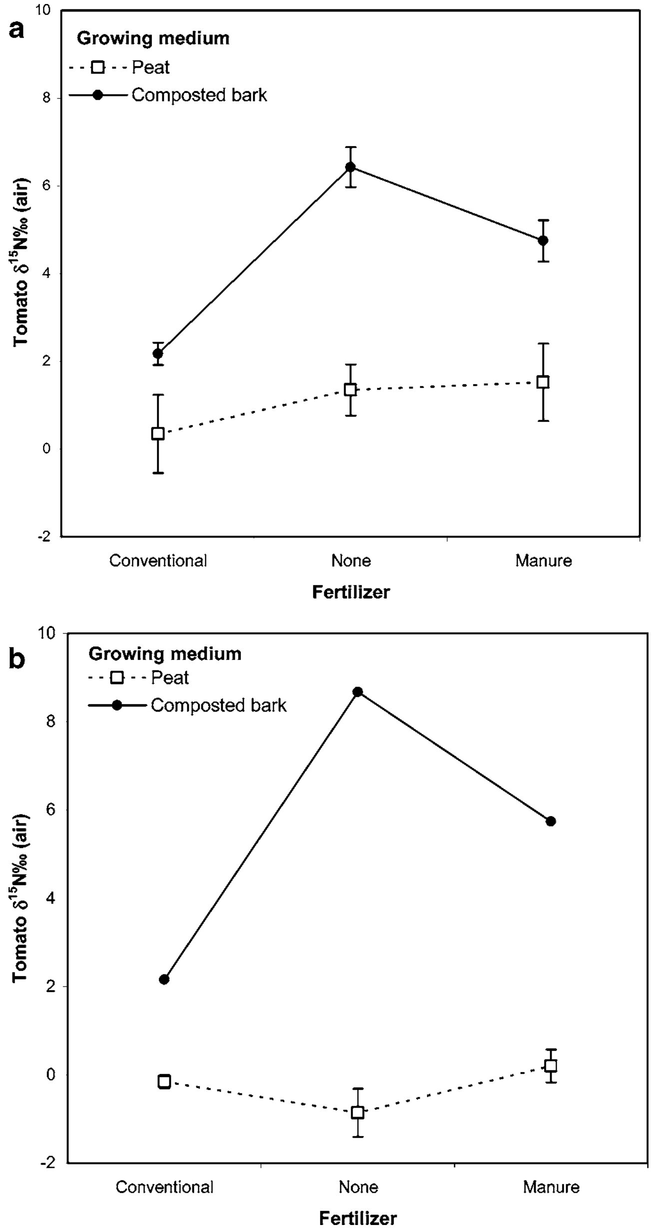

Figure 3. $\delta^{15}N(‰)_{air}$ of lettuces grown in different growing media with different types of fertilizer applied and irrigated with either **(a)** local (Norwich, U.K.) tap water or **(b)** deionized water. Values are mean and standard deviation (σ^{n-1}) of 3 samples, except for the values shown without error bars, for which only one sample was analyzed; see text for details. $\delta^{15}N$-(conventional fertilizer) = –0.4‰. $\delta^{15}N$(manure) = +5.4‰.

peat-based compost may have been isotopically lighter than that available from the conventional fertilizer. It is notable that the lettuces grown in the composted bark that received manure as fertilizer have lower $\delta^{15}N$ values than those to which no subsequent fertilizer was applied. This suggests that the plant-available nitrogen in the composted bark is isotopically heavier than that available from the manure. Where the manure has been applied, the $\delta^{15}N$ values of the tomatoes are close to the $\delta^{15}N$ value of the applied fertilizer (+5.4‰).

A comparison of otherwise similar treatments of lettuces watered with deionized water and local (nitrate containing) tap water suggests a consistent influence on the $\delta^{15}N$ of the lettuces from nitrate in the tap water. Lettuces watered with deionized water exhibit greater extremes in their nitrogen isotope compositions when they were not given any fertilizer or when manure was used as a fertilizer. An explanation for this might be that the lettuces irrigated with tap water assimilate some nitrogen from nitrate present in the water and that the δ^{15}N of the assimilated nitrogen from the tap water has a δ^{15}N value of between +2‰ and +6‰ which consistently moderates the δ^{15}N of the lettuces away from the more extreme values observed when the plants were irrigated with deionized water. The apparent absence of a similar effect on the lettuces grown with conventional fertilizer may be due to a greater supply of readily available nitrogen to these plants such that the uptake of tap water derived nitrogen has a negligible effect on lettuce δ^{15}N values. High concentrations of nitrate are found in the water used for public supply in the Norwich area: values around 30 mg/L NO_3^- are typical. Norwich tap water is river derived, and the rivers are groundwater fed. Values for the δ^{15}N of groundwater nitrate were found to be generally between +4‰ and +6‰ in a study of groundwater from a similar catchment in Norfolk (*11*). An influence of the water used for irrigation on the δ^{15}N of the plants is a significant finding since the nitrate content and δ^{15}N composition of supply water will vary geographically.

Factorial ANOVA identifies a significant effect of compost type and fertilizer on lettuce δ^{15}N (see **Table 2**). The ANOVA does not identify the type of water that the plants were irrigated with as being a significant factor despite the apparent influence of the tap water as described above. There is significant 2-way interaction between compost and the two other variables and also significant 3-way interaction. The GLM has an R^2 of 0.972 and an adjusted R^2 of 0.954. The interaction between the compost type and fertilizer identified by the ANOVA is probably due to a more pronounced effect of fertilizer when composted bark has been used as the growing medium probably because of the relatively low amount of plant-available nitrogen in the composted bark. There are much smaller differences in lettuce δ^{15}N when the lettuces were grown in the peat-based compost, suggesting that this compost had a greater and more sustained impact on the nitrogen nutrition of the lettuces.

These controlled cultivation experiments have shown that fertilizer type is an important factor influencing the nitrogen isotope compositions of the crops. We have shown that, under controlled conditions, applications of synthetic nitrogen fertilizer result in crops with lower δ^{15}N values when other factors that are also potentially important in determining crop δ^{15}N are not variable. In order for plant δ^{15}N to be used more generally as an indicator of whether synthetic nitrogen fertilizers have been applied to crops, the tendency for applications of synthetic nitrogen fertilizer to result in crops with lower δ^{15}N values must predominate over many other factors which may also influence plant δ^{15}N. Such factors include variability in the δ^{15}N of synthetic nitrogen fertilizers, the form of nitrogen in the applied synthetic fertilizer (NO_3^-/NH_4^+/urea), variability in the δ^{15}N of nonsynthetic nitrogen fertilizers, timing of fertilizer application, the pedoclimatic conditions of the location, and the use of leguminous plants for enhancing the nitrogen fertility of soils. These factors may influence the turnover of nitrogen in the soil through the processes of nitrification, denitrification, mineralization, volatilization, leaching, etc. Isotopic fractionations are associated with these nitrogen turnover processes and could potentially override any variations in plant δ^{15}N due to fertilizer influence.

Total soil δ^{15}N values have been reported showing that variability may occur spatially (*12*) and vertically through a soil profile (*13*). However, as previously discussed, total soil nitrogen is generally not a good indicator of the δ^{15}N of nitrogen that is plant-available because it is dominated by a recalcitrant pool of nitrogen which may have a turnover time of hundreds of years (*14*). The turnover time of the biologically active nitrogen pool, principally the inorganic nitrogen components, is thought to be of the order of days (*10*), and the δ^{15}N value of this pool is likely to vary both spatially and temporally.

Changes in plant δ^{15}N over time during the growth period have been observed after an application of a synthetic nitrogen fertilizer (*15, 16*). In these studies it was observed that, during the early stages of growth, the δ^{15}N values of crops receiving synthetic nitrogen fertilizer were significantly lower than those not receiving the synthetic fertilizer. However, both studies observed increases in the δ^{15}N of the crops during the later stages of growth attributable to a decrease in the availability of the synthetic fertilizer over time due to uptake, losses, and immobilization, and an increasing contribution of natural soil nitrogen to plant total nitrogen.

Minimal isotopic fractionation occurs during N_2 fixation by leguminous plants (*17*), and the δ^{15}N signature of leguminous plant material is therefore usually close to that of atmospheric nitrogen (i.e. close to 0‰). Legume root nitrogen is likely to represent the largest source of nitrogen to soils in rotational farming systems (*18*) although legumes with a large harvest nitrogen index will make only a marginal contribution to the nitrogen status of a soil (*19*). However, even when only a marginal contribution is made to soil total nitrogen, the influence on the δ^{15}N of the available soil nitrogen pool may still be significant. Symbiotically fixed nitrogen is contributed to soil when plant remains of N_2-fixing legumes decay. In one study, a decrease in the δ^{15}N composition of grasses grown over a 4 year period in a pure *Leucaena leucocophala* stand (from +7.3‰ to +0.7‰) was observed, suggesting that the nitrogen fixed by the legume was being cycled to the grass (*20*). Yoneyama (*21*) reports that an observed variation (from +1‰ to +14‰) in the δ^{15}N composition of sorghum plants across a number of fields appeared to be related to the history (frequency) of legume cultivation across the fields but not to the intensity of fertilizer application. It is suggested that this is because fertilizer nitrogen is easily leached from the soil while legume residues reside for longer and consequently their influence on the δ^{15}N of subsequent crops may be longer lasting. Determining the likely impact of the use of leguminous crops on the δ^{15}N of subsequent crops is important since the above studies suggest that the fixation of atmospheric nitrogen in this way may cause a decrease in the δ^{15}N of subsequent crops similar to the effect seen when a synthetic nitrogen fertilizer is applied. However, the impact of the use of leguminous plants on the δ^{15}N composition of subsequent crops when legumes are used in an agricultural rotation warrants further investigation. Although an input of leguminous material may initially lower the δ^{15}N of the soil nitrogen pool, the subsequent decomposition of such material will release volatile nitrogen compounds depleted in ^{15}N with a resultant increase in the δ^{15}N of the remaining fraction.

The preceding discussion relates to whether plant δ^{15}N can be used as a marker of synthetic nitrogen fertilizer application. There are additional considerations if this concept is taken a step further and plant nitrogen isotope composition is considered as a potential discriminator between crops grown under conventional and organic regimes (*3*). Restrictions on the use of synthetic nitrogen fertilizers are only one of a range of conditions that must be met in order to satisfy requirements for organic food production. Additional obligatory conditions are a period of conversion, registration with an appropriate certifying authority, and restrictions on the use of pesticides and other soil improvers and conditioners. More broadly, organic farming includes inter alia the principles of (i) minimizing reliance on external inputs by, for example, building soil fertility using crop rotations that include green manures, (ii) maintaining genetic diversity in agricultural systems, (iii) protecting wildlife habitats, and (iv) seeking to avoid environmental pollution as a result of farming practices. It is therefore important to emphasize that even if crop $\delta^{15}N$ can be used to distinguish satisfactorily between crops that have been grown with and without the application of synthetic nitrogen fertilizer, this is not the same as being able to distinguish between conventionally and organically grown crops because of the additional statutory requirements that apply to organic farming. In summary, if synthetic nitrogen fertilizers have been applied to a crop, then the crop cannot be described as organic. However, if synthetic fertilizers have not been applied to a crop, it does not follow that the crop can be described as "organic" since the crop may not have been grown in conditions which comply with all the other requirements of organic cultivation.

It is also worth noting that conventional growers do not always apply synthetic nitrogen fertilizers to their crops. A conventional farmer may use a synthetic nitrogen fertilizer or any of the natural fertilizers that may be permitted in organic farming and/or they may practice crop rotation. In some circumstances, depending on the crop to be grown, the soil type, rainfall, and the preceding land use, it may not be necessary for conventional farmers to apply any additional nitrogen fertilizer. In these circumstances, the conventionally grown crop would not bear the signature of a synthetic nitrogen fertilizer. For example, carrots have a low nitrogen requirement, and application of a nitrogen-containing fertilizer is often considered unnecessary or only necessary at relatively low levels. In contrast, there are some crop types, specifically those with high nitrogen requirements, e.g. lettuce/spinach, or high-value horticultural crops, to which it is usual for a conventional grower to apply a synthetic nitrogen fertilizer throughout the growth period, e.g. tomatoes. For these crop types, the $\delta^{15}N$ of conventionally grown crops are more likely to be significantly different from the $\delta^{15}N$ values of the same crops grown organically.

ACKNOWLEDGMENT

This work was financed by the UK Food Standards Agency as part of their Food Authenticity and Labelling Programme. The views expressed in this paper are those of the authors and do not necessarily reflect the views of the UK Food Standards Agency.

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Received for review February 17, 2005. Revised manuscript received May 17, 2005. Accepted May 21, 2005.

JF050374H