Nitrogen Isotope Composition, Nitrogen Amount, and Fruit Yield of Tomato Plants Affected by the Soil–Fertilizer Types

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ABSTRACT: Tomatoes (Solanum lycopersicum) are heavy nutrient feeding crops and require high amounts of nitrogen to maximize fruit production. The type of nitrogen applied and timing of fertilizer applications are important to reduce losses due to volatilization and leaching. Previous research suggested that nitrogen stable isotopes are a useful fingerprinting system for indicating if a crop has been grown with synthetic or organic nitrogen applications. To study the effects of fertilization systems on nitrogen isotopic patterns, "Better Bush" tomatoes were grown in a 2 year greenhouse experiment to analyze nitrogen isotopic composition, nitrogen content, and fruit yield. Three main soil fertility treatments were evaluated, and the results were compared to those obtained on plants grown in unfertilized soil: conventional inorganic (synthetic Miracle Grow (MG)), organic (bonemeal and bloodmeal (BB), BB with liquid Earth Juice (BBL), BB with 25% vermicompost (VC), BBL with 25% VC, and 25% VC), and mixed (MG with 25% VC). The soil fertilizers, treated and untreated soil, immature and mature leaflets tomato fruit peels, and fruit juices were analyzed for both nitrogen isotope ratios and nitrogen concentrations. Plant δ15Nair decreased in the order organic treatment-no fertilizer-mixed treatment-conventional treatment. The average δ15Nair values in leaves, fruit peels, and juice from plants grown with organic treatments ranged from 4.5 to 11.9, 5.4 to 10.1, and 6.1 to 11.1‰, respectively, whereas in the case of the inorganic treatment, the average δ15Nair values varied between −3.0 and 0.4, −1.1 and 0.4, and −0.9 and 1.9‰, respectively. Plant nitrogen concentrations in tomato decreased in the following order (from highest to lowest): inorganic soil fertility treatment, mixed treatments, and organic and control (no fertilizer) treatment. The average weight %N values in leaves and fruit peels from plants grown with organic treatments ranged from 1.3 to 4.2 and 1.1 to 2.3‰, respectively, whereas in the case of the inorganic treatment, the average weight %N values varied between 3.7 and 5 and 1.3 and 2.8‰, respectively. Plants grown under organic treatments have higher δ15Niso, lower weight %N, and are enriched in 15N compared with the original soil than plants grown with inorganic fertilizer, suggesting that the synthetic nitrogen sources are more readily available for plant uptake than the organic ones. The addition of vermicompost increases both δ15Niso and weight %N in plants. Tomato fruit yields did not differ between cluster 1 and cluster 2 harvest, however, total tomato fruit yields differed indicating that synthetically fertilized plants produced the highest total yields (g) (P ≤ 0.05). However, all treatments with VC soil applications indicated an increase in the amount of plant nitrogen, fruit yield, soil cation exchange capacity, soil organic matter content, and released soil nitrogen. Nitrogen isotope ratios of tomatoes can be used to distinguish among various soil fertility treatments, therefore fingerprinting the organic fertilizer applications.

INTRODUCTION

Many aspects of vegetable production are similar among “organic” or “conventional” systems, yet there are several important differences, which are mostly related to fertility and pesticide management.1 Conventional agricultural systems rely on synthetic chemical solutions, whereas sustainable or organic methods tend to rely more on natural fertilizers and longer-term systems that are considered more preventative than reactive.2 Nutrient management is crucial to both conventional and organic crop production with nitrogen being the element that most limits plant growth and causes yield reductions.3,4 Moreover, the nitrogen fertilizer source and timing of applications are both important to maximize crop yields and reduce nitrogen losses that occur through volatilization and soil leaching.

One specific type of technique for assessing nitrogen dynamics includes measuring nitrogen isotope ratios in plants and other ecosystem pools (i.e., the soil profile). Nitrogen isotope ratios

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have previously proved useful because they reflect the total sum of fertilization inputs, nitrogen outputs, and fractionation processes within the plant. However, previous literature suggested gaps when studying the soil processes that control many key nitrogen transformations. The $^{15}$N/$^{14}$N ratios in soil ecosystems and corresponding plant parts provide a useful tool to differentiate between plants grown with various nitrogen fertilization methods.

Plant isotope fractionation is a process that affects the relative abundance of isotopes of the same element due to the preferential uptake of the lighter and more abundant element, such as $^{14}$N, and it occurs during plant conversion of nitrogen compounds to reduced forms. Mycorrhizal fungi are key mediators of N flux and movement in the plant–soil system strongly influencing isotopic patterns. These fungal interfaces provide heavy feeding crops, like tomatoes, access to organic N forms. Assimilation of N onto carbon skeletons has significant effects on plant productivity, biomass, and resulting crop yields as nitrogen deficiency decreases the photosynthetic capacity of the plant. Chemical and biological processes often discriminate against $^{15}$N in agricultural systems. However, mycorrhizal fungi preferentially retain $^{15}$N and, as a consequence, transfer $^{15}$N-depleted forms to plant hosts, allowing for easier assimilation. These fungi also lower N losses in terrestrial ecosystems during denitrification. Generally, organic nitrogen sources have higher $\delta^{15}$N$_{air}$ values, between 3.5 and 16.2‰, than those grown with inorganic or synthetic nitrogen sources with values ranging between ~4 and 4.6‰. Minimal information is available with respect to integrated production systems and their corresponding plant parts (i.e., the soils, plant leaves, fruit peels, and juices). This information can be used to better understand plant nitrogen usage, the denitrification process, plant–microbe interactions, and enrichments or depletions form the soil system. Nitrogen stable isotopes can also determine if a vegetable or fruit product is the result of natural or synthetic fertilization inputs, nitrogen outputs, and fractionation processes within the plant. Previous literature suggested gaps when studying the soil processes that control many key nitrogen transformations. The $^{15}$N/$^{14}$N ratios in soil ecosystems and corresponding plant parts provide a useful tool to differentiate between plants grown with various nitrogen fertilization methods.

### RESULTS AND DISCUSSION

#### Soil Properties
The main characteristics of the soil mixtures used for tomato plants growth are listed in Table 1. For the soil prepared in 2014, the cation exchange capacity, pH, organic matter content, and nitrogen release varied between 5.8 and 15.5 mequiv/100 g, 6.2 and 7.5, 2.0 and 6.4%, and 67.2 and 119.8 kg/ha, respectively. For the 2015 soil, the same parameters ranged from 7.0 to 17.2 mequiv/100 mg, 4.9 to 7.7, 2.6 to 8.8%, and 109.8 to 177.0 kg/ha, respectively. The most acidic soils were those treated in 2014 with Miracle Grow (MG) and in 2015 the one treated with bonemeal and bloodmeal (BB). The addition of vermicompost (VC) increased the cation exchange capacity, organic matter content, and nitrogen release for each treatment.

#### Nitrogen Stable Isotopes
In general, the organic soil fertilizers have lower $\delta ^{15}$N$_{air}$ than the inorganic ones (Table 2). Fertilizer $\delta ^{15}$N$_{air}$ varied from 3.7 (bloodmeal) to 18.6‰ (Earth Juice Grow). MG had a $\delta ^{15}$N$_{air}$ of 18.1‰. Nitrogen isotope data of samples collected during both 2014 and 2015 revealed marked differences among the soil–fertilizer treatments listed in Table 3. The nitrogen isotope composition of sampled soil did not reflect that of the fertilizers used because of the plant nitrogen uptake and microbiological activity that had taken place prior to sampling.

### Table 1. Soil Fertility Characteristics of All Treatments Used in the N Isotope Evaluation of Tomato Foliage and Fruit in 2014 and 2015 Prior to Liquid Fertilizer Applications

| soil treatment | exchange capacity (mequiv/100 g) | pH | organic matter (%) | release N (kg/ha) |
|----------------|----------------------------------|----|--------------------|------------------|
| 2014 season    |                                  |    |                    |                  |
| no fertilization | 5.8                       | 7.5 | 2.0                | 67.2             |
| MG             | 8.7                       | 6.2 | 2.4                | 76.0             |
| BB             | 8.1                       | 7.0 | 2.7                | 81.8             |
| BBL            | 6.5                       | 7.4 | 2.3                | 87.0             |
| BB w/VC        | 13.6                      | 7.0 | 5.9                | 115              |
| BBL w/VC       | 12.0                      | 7.0 | 6.4                | 119.8            |
| MG w/VC        | 15.3                      | 6.0 | 5.9                | 109.0            |
| VC only        | --                        | --  | --                 | --               |
| 2015 season    |                                  |    |                    |                  |
| no fertilization | 7.0                       | 4.9 | 3.1                | 118.7            |
| MG             | 7.9                       | 5.3 | 2.6                | 116.5            |
| BB             | 9.6                       | 4.8 | 2.7                | 109.8            |
| BBL            | 7.6                       | 5.3 | 2.8                | 113.1            |
| BB w/VC        | 10.8                      | 7.5 | 6.9                | 157.9            |
| BBL w/VC       | 13.2                      | 7.7 | 6.8                | 162.4            |
| MG w/VC        | 17.2                      | 6.0 | 8.8                | 177.0            |
| VC only        | 9.3                       | 7.6 | 6.3                | 157.9            |

aNo fertilization = no fertility, no form of fertilizers applied to the soil. bMG = synthetic, fertilized with 17 g of 12N-5.2P-10.0K and bi-weekly w/20 g Miracle Grow diluted in 3.8 L of water (500 ppm N). cBB = fertilized with 9 g of bonemeal, 5 g of bloodmeal, and 7 g of potassium sulfate. dBBL = fertilized with 9 g of bonemeal, 5 g of bloodmeal, and 7 g of potassium sulfate and bi-weekly with 36 g of Earth Juice Grow (100 ppm N) and Bloom (300 ppm N) diluted in 3.8 L of water. eVC = 25% vermicompost (1.9 mL VC/5.6 mL 1:1:1 mix). f= missing sample.

### Table 2. $\delta ^{15}$N$_{air}$ Values for the Fertilizers Utilized in the Tomato Isotope Evaluation Experiments

| fertilizers            | $\delta ^{15}$N$_{air}$ |
|------------------------|-------------------------|
| 12-12-12               | 11.8                    |
| bonemeal               | 4.2                     |
| bloodmeal              | 3.7                     |
| vermicompost           | 6.6                     |
| Miracle Grow           | 18.1                    |
| Earth Juice Grow       | 18.6                    |
| Earth Juice Bloom      | 5.0                     |

aSynthetic fertilizers. bOrganic fertilizers.

Fertilizer $\delta ^{15}$N$_{air}$ varied from 3.7 (bloodmeal) to 18.6‰ (Earth Juice Grow). MG had a $\delta ^{15}$N$_{air}$ of 18.1‰. Nitrogen isotope data of samples collected during both 2014 and 2015 revealed marked differences among the soil–fertilizer treatments listed in Table 3. The nitrogen isotope composition of sampled soil did not reflect that of the fertilizers used because of the plant nitrogen uptake and microbiological activity that had taken place prior to sampling.
In the case of the 2014 experiment, the \( ^{15}\text{N}_{\text{air}} \) ranges in plants were 1.0 (MG) to 4.8‰ (BBL w/VC) for soil, 0.4 (MG) to 8.1‰ (VC only) for the immature leaves, −0.3 (MG) to 8.1‰ (VC only) for the mature leaves, 0.4 (MG) to 10.1‰ (VC only) for the cluster 1 skins, 0.3 (MG) to 9.2‰ (BBL w/VC) for the cluster 2 fruit skins, 1.9 (MG) to 11.1‰ (BB w/VC) for cluster 1 tomato juice, and 1.9 (MG) to 9.1‰ (VC only for cluster 2 tomato juice). For 2015, the \( ^{15}\text{N}_{\text{air}} \) ranges were −1.4 (MG) to 4.4‰ (BBL w/VC) for soil, −3.0 (MG) to 11.8‰ (VC only) for the immature leaves, −0.9 (MG) to 8.1‰ (BB w/VC) for the mature leaves, −1.1 (MG) to 9.4‰ (BB w/VC) for the cluster 1 skins, 0.3 (MG) to 8.8‰ (VC only) for the cluster 2 fruit skins, −0.9 (MG) to 9.4‰ (BB w/VC) for cluster 1 tomato juice, and −0.8 (MG) to 8.0‰ (VC only) (Table 3). Generally, over both growing seasons, plants grown using conventional inorganic soil fertilizers displayed lower \( ^{15}\text{N}_{\text{air}} \) than those grown under organic or mixed treatments. Furthermore, the addition of VC resulted in an isotopic enrichment for all treatments and made possible to distinguish the nitrogen isotope composition of the leaves from the soil that was collected together with the immature leaves. This can also explain why no consistent temporal trends of leaf \( ^{15}\text{N}_{\text{air}} \) were present. Furthermore, the addition of VC to the inorganic treatment brought closer the \( ^{15}\text{N}_{\text{air}} \) values of immature and mature leaves.

The tomato fruit skins and juice was enriched in \( ^{15}\text{N} \) compared to leaves when organic fertilizer was used. Overall, plant \( ^{15}\text{N}_{\text{air}} \) decreased in the order organic treatment-no fertilizer-mixed treatment-conventional treatment.

### Table 3. \( ^{15}\text{N}_{\text{air}} \) in Tomato Plants Grown under Conventional and Organic Fertilizer Applications in 2014 and 2015

| Soil treatment | Immature | Mature | C1 | C2 | C1 | C2 |
|----------------|----------|--------|----|----|----|----|
| 2014 season    |          |        |    |    |    |    |
| no fertilization | 1.8 ± 1.1 | 4.9 ± 1.0 | 4.3c ± 1.9 | 4.8c ± 0.3 | 4.6c ± 1.3 | 1.9c ± 1.0 |
| MG             | 1.0d ± 0.5 | 0.4e ± 0.5 | 0.4f ± 0.2 | 0.3d ± 0.4 | 1.9d ± 1.0 | 1.9d ± 0.2 |
| BB             | 2.1c ± 1.0 | 4.5c ± 1.3 | 6.7bc ± 0.5 | 7.7b ± 1.3 | 8.5b ± 1.4 | 7.4b ± 0.2 |
| BBL            | 2.9bc ± 1.0 | 4.6b ± 1.4 | 6.8bc ± 0.3 | 6.9b ± 0.4 | 6.2b ± 1.3 | 7.3b ± 0.2 |
| BB w/VC        | 4.8a ± 0.2 | 6.5a ± 0.4 | 8.1a ± 0.0 | 7.2a ± 0.3 | 11.1a ± 2.9 | 8.5a ± 0.8 |
| BBL w/VC       | 4.8a ± 0.2 | 7.6a ± 0.2 | 9.6a ± 0.6 | 9.2a ± 1.5 | 8.6b ± 2.3 | 8.3a ± 0.9 |
| MG w/VC        | 4.0ab ± 0.4 | 2.9a ± 0.4 | 1.2d ± 0.4 | 1.0d ± 0.9 | 2.6b ± 0.5 | 2.9b ± 0.8 |
| VC only        | 4.6ab ± 0.5 | 8.3a ± 0.0 | 10.1a ± 0.9 | 8.5a ± 2.1 | 10.2a ± 2.4 | 9.1a ± 1.6 |
| P-value        | 0.0001 | 0.0014 | 0.0001 | 0.0001 | 0.009 | 0.0922 |
| 2015 season    |          |        |    |    |    |    |
| no fertilization | 0.9ab ± 2.1 | 6.6b ± 0.8 | 5.7c ± 0.6 | 3.4c ± 2.3 | 5.2c ± 0.8 | 2.3c ± 0.0 |
| MG             | −1.4b ± 1.7 | −3.0c ± 0.6 | −1.1d ± 0.7 | 0.3d ± 2.2 | −0.9e ± 0.8 | −0.8d ± 0.4 |
| BB             | 1.2a ± 0.7 | 6.5b ± 0.5 | 6.7a ± 1.2 | 6.7b ± 1.2 | 6.1b ± 0.9 | 7.5a ± 1.2 |
| BBL            | 0.5ab ± 1.4 | 7.1b ± 0.1 | 6.0b ± 0.1 | 6.5b ± 0.3 | 6.7b ± 0.9 | 6.9b ± 0.4 |
| BB w/VC        | 3.4a ± 0.6 | 11.6a ± 2.0 | 9.4b ± 1.2 | 7.6a ± 1.1 | 9.4a ± 0.7 | 8.0a ± 0.9 |
| BBL w/VC       | 4.4a ± 0.6 | 7.9a ± 0.7 | 6.4a ± 1.3 | 7.6b ± 1.2 | 7.0a ± 0.6 | 6.9b ± 1.0 | 6.8b ± 0.8 |
| MG w/VC        | 3.6a ± 0.9 | 2.8a ± 2.0 | 3.1a ± 2.0 | 0.3d ± 1.2 | 0.6d ± 1.1 | 1.2d ± 0.7 | 1.9d ± 0.5 |
| VC only        | 2.4a ± 1.4 | 11.6a ± 2.0 | 8.7a ± 0.7 | 8.8a ± 1.6 | 8.3a ± 5.0 | 2.3c ± 0.0 |
| P-value        | 0.0049 | 0.0001 | 0.0001 | 0.0001 | 0.005 | 0.005 |

Fertilizer treatments had mean separations (e.g., a or b) by Fisher’s least significance differences (LSD) at P ≤ 0.05. Values with the same letter are not significantly different at P > 0.05 according to LSD. Immature = first leaflet harvest (9 Sept 2014 and 5 Sept 2015). Mature = second leaflet harvest (13 Oct 2014 and 7 Oct 2015). C1 and C2 = fruit cluster 1 and cluster 2 tomatoes. 3 replicates per treatment. Standard deviation calc. on intra treatment variation.
The use of conventional inorganic fertilizers led to the lowest enrichment and even depletion in plants. This suggests that nitrogen was easily available from the soil source. Ammonium and nitrate are nitrogen forms found in Miracle Grow, and this synthetic fertilizer is readily soluble in water and easier for plant uptake compared with organic nitrogen sources. In comparison, the addition of VC resulted in depletion only for the inorganic treatments. Orgaically fertilized tomatoes had higher δ^{15}N values than the soil. This suggests that both mineralization and biological transformations were occurring in the soil system and were the main reasons for an increase in the nitrogen isotopic values.\(^{11}\)

The organic treatments indicated differences in isotopic abundance between the various fertilization treatments and their corresponding plant growth stages. The organic and mixed fertilizer applications with VC had the highest δ^{15}N values in the leaf, peel, and juice samples. In contrast, the organic treatments with only rock fertilizers (i.e., bonemeal and bloodmeal) indicated slightly lower δ^{15}N values. Since VC applications to the soil increase organic matter content, they also increase microbial activity. However, VC also enriches the 15N isotopic abundance in the soil profile. Due to this increase, it is harder for living microbes to breakdown organic matter into ammonium and nitrate because this form of nitrogen has a slower vibrational energy.\(^{8}\) As the plants continued to mature into fruit production, nitrogen uptake was maximized in the root and shoot assimilation. Thus, there is a slight decrease in δ^{15}N abundance in all organic treatments because neither 14N nor 15N were preferentially assimilated and the plants utilized all forms of nitrogen that were available in the soil.\(^3\)

The nitrogen isotope patterns in the plants grown under the conventional inorganic, mixed, and organic soil fertility treatments are explained by the type and availability of nitrogen, also by the changes in the physicochemical characteristics that occur when organic fertilizers and especially vermicompost are added. The synthetic and readily available MG-containing NH4 is immediately assimilated in the roots through a single assimilation

### Table 4. Plant—Soil δ^{15}N Air Difference

| soil treatment | tomato leaf δ^{15}N<sub>air</sub> immature | mature | tomato fruit peel δ^{15}N<sub>air</sub> | tomato fruit juice δ^{15}N<sub>air</sub> |
|---------------|------------------------------------------|--------|---------------------------------------|---------------------------------------|
| 2014 season   |                                          |        |                                       |                                       |
| no fertilization | 3.3 ± 0.2 | 2.6 ± 0.3 | 3.2 ± 0.4 | 3.1 ± 0.5 |
| MG            | −0.5 ± 0.1 | −0.7 ± 0.8 | 4.2 ± 0.5 | 5.2 ± 0.6 |
| BB            | 2.3 ± 0.4 | 5.1 ± 0.8 | 5.8 ± 0.4 | 2.4 ± 0.5 |
| BBL           | 2.7 ± 0.4 | 3.7 ± 0.3 | 4.3 ± 0.4 | 3.8 ± 0.4 |
| BB w/VC       | 1.5 ± 0.3 | 3.5 ± 0.3 | 4.3 ± 0.4 | 5.1 ± 0.4 |
| BBL w/VC      | 3.4 ± 0.2 | 4.8 ± 0.4 | 4.4 ± 0.3 | 1.2 ± 0.3 |
| MG w/VC       | −1.8 ± 0.9 | −3.0 ± 1.4 | −1.1 ± 1.0 | −1.0 ± 0.5 |
| VC only       | 4.0 ± 0.3 | 5.5 ± 0.7 | 5.7 ± 0.3 | 3.2 ± 0.2 |
| 2015 season   |                                          |        |                                       |                                       |
| no fertilization | 9.5 ± 0.8 | 8.5 ± 0.6 | 6.3 ± 0.4 | 1.5 ± 0.6 |
| MG            | −0.7 ± 0.2 | 0.9 ± 0.2 | 2.2 ± 0.6 | 0.6 ± 0.6 |
| BB            | 5.0 ± 5.4 | 4.0 ± 5.2 | 4.7 ± 6.3 | 1.0 ± 6.0 |
| BBL           | 6.1 ± 4.7 | 5.1 ± 5.7 | 6.0 ± 6.4 | 6.0 ± 4.5 |
| BB w/VC       | 8.6 ± 4.5 | 5.7 ± 4.1 | 6.0 ± 4.5 | 4.5 ± 4.5 |
| BBL w/VC      | 5.3 ± 2.1 | 2.3 ± 2.4 | 2.3 ± 2.2 | 2.2 ± 2.2 |
| MG w/VC       | −0.8 ± 0.3 | −1.0 ± 0.3 | −2.8 ± 0.5 | −0.5 ± 0.5 |
| VC only       | 9.3 ± 5.7 | 6.2 ± 6.4 | 5.9 ± 5.7 | 5.7 ± 5.7 |

### Table 5. Weight Percent Nitrogen (Nitrogen Content) over Two Growing Seasons in Soil, Tomato Leaf, and Fruit Peels

| soil treatment | soil wt %N (mean ± std (%)) | tomato leaflet wt %N (mean ± std (%)) immature | mature | tomato peel wt %N (mean ± std (%)) C1 | C2 |
|---------------|-----------------------------|------------------------------------------------|--------|--------------------------------------|----|
| 2014 season   |                             |                                                |        |                                      |    |
| no fertilization | 0.1c ± 0.0 | 1.1c ± 0.2 | 1.1c ± 0.2 | 1.5c ± 0.1 | 1.5a ± 0.0 |
| MG            | 0.1bc ± 0.0 | 3.8a ± 0.2 | 3.7a ± 0.2 | 1.9a ± 0.2 | 1.3a ± 0.3 |
| BB            | 0.1c ± 0.0 | 1.8c ± 0.2 | 1.9bc ± 0.3 | 2.0a ± 0.0 | 1.9a ± 0.0 |
| BBL           | 0.1c ± 0.0 | 1.3cd ± 0.2 | 1.5bc ± 0.2 | 1.6ab ± 0.2 | 1.8a ± 0.1 |
| BB w/VC       | 0.3a ± 0.0 | 3.5ab ± 0.3 | 2.5b ± 0.3 | 1.5ab ± 0.0 | 1.4a ± 0.2 |
| BBL w/VC      | 0.4± 0.1 | 1.9c ± 0.4 | 1.9bc ± 0.4 | 1.6ab ± 0.1 | 1.4a ± 0.3 |
| MG w/VC       | 0.3a ± 0.1 | 4.2a ± 0.3 | 4.2a ± 0.3 | 1.9a ± 0.1 | 1.8a ± 0.2 |
| VC only       | 0.2ab ± 0.1 | 2.9b ± 0.3 | 2.9b ± 0.3 | 1.5b ± 0.3 | 1.1a ± 0.2 |
| P-value       | 0.001 | 0.00001 | 0.0001 | 0.0038 | 0.0951 |
| 2015 season   |                             |                                                |        |                                      |    |
| no fertilization | <0.1b ± 0.0 | 2.1b ± 1.3 | 1.9c ± 0.7 | 1.5b ± 0.1 | 1.7b ± 0.4 |
| MG            | <0.1b ± 0.0 | 5.0a ± 0.8 | 4.8a ± 0.9 | 2.8a ± 0.3 | 2.4a ± 0.8 |
| BB            | 0.1b ± 0.1 | 2.0b ± 0.3 | 1.8c ± 0.6 | 1.5b ± 0.5 | 1.7b ± 0.4 |
| BBL           | 0.1b ± 0.1 | 2.3b ± 0.4 | 2.4b ± 0.7 | 1.6b ± 0.2 | 1.6b ± 0.2 |
| BB w/VC       | 0.2ab ± 0.2 | 2.6b ± 0.3 | 2.6b ± 0.3 | 1.8b ± 0.1 | 1.6b ± 0.4 |
| BBL w/VC      | 0.3b ± 0.2 | 2.5b ± 0.9 | 2.6b ± 0.5 | 1.7b ± 0.3 | 1.8b ± 0.3 |
| MG w/VC       | 0.5a ± 0.3 | 4.2a ± 0.9 | 4.0a ± 0.9 | 2.2a ± 0.3 | 2.3a ± 0.2 |
| VC only       | 0.1ab ± 0.7 | 1.5c ± 2.1 | 1.8c ± 0.5 | 1.7b ± 0.4 | 1.2b ± 0.2 |
| P-value       | 0.0011 | 0.001 | 0.0001 | 0.0010 | 0.0053 |
event. In contrast, NO$_3^-$ assimilation occurs in the roots and shoots via two-assimilation events.$^{16}$ The average isotopic composition of ammonia fluctuates between $-42.4$ and $7.1\%e$ for nitrate from $-2$ to $3\%e$. $^{15,17-19}$ The availability of both nitrogen sources led to a preferential incorporation of $^{14}$N, which was amplified by the addition of soil-improving VC. This explains why the plants grown under the conventional inorganic soil treatment displayed the lowest $\delta^{15}$N$_{air}$ values. However, in 2014, plants fertilized with MG indicated an increase in $\delta^{15}$N$_{air}$ content only in fruit juices. The majority of beneficial nutrients found in tomatoes are concentrated in the tomato flesh and juice, which may explain the increase in nitrogen isotopic abundance as compared to that in the leaflets and fruit peel. This increase in $^{15}$N is also due to preferential uptake of $^{15}$N during plant growth and maturation. The heavier nitrogen isotope remained in the soil and was assimilated during the fruit ripening stages.$^{8,17}$

Despite the increased cation exchange capacity, organic matter content, and nitrogen release of the soils fertilized with organic mixtures, it is probable that nitrogen was not so readily available to plants. Moreover, there could have been a competition for the many nitrogen sources. Therefore, as the plant matured, different parts become enriched in $^{15}$N compared with the supporting soil. Applying nitrogen fertilizer to soil changes the abundance of $^{15}$N/$^{14}$N ratios in plant parts, as reported previously.$^{11}$

**Nitrogen Content.** The weight percent nitrogen (wt %N) values obtained in soils, tomato plant leaves, and fruit peels were influenced by fertilizer treatments in 2014 and 2015 (Table 5). In 2014, soil was lowest in wt %N compared with plant material and soil values ranged from 0.1 (MG) to 0.4% (BB w/VC). For the immature and mature leaves, nitrogen content ranged from 1.1 (no fertilization) to 4.2% (MG w/VC). For cluster 1 tomato peel, nitrogen content ranged from 1.5 (no fertilization) to 2.0% (BB) and for cluster 2 peel, from 1.5 (no fertilization) to 1.9% (BB). In 2015, the soil wt %N was again lower than all other plant parts and ranged from 0.02 (no fertilization) to 0.66% (VC only). For immature leaves, nitrogen content ranged from 1.5 (VC only) to 4.2% (MG w/VC). Similarly, mature leaf nitrogen content was between 1.9 (no fertilization) and 4.8 wt %N (MG w/VC). Cluster 1 peel had values between 1.5 (no fertilization) and 2.8% (MG only), and similarly cluster 2 peel values were between 1.2 (VC only) and 2.8% (MG only). Over both growing seasons, the soil nitrogen values were lower than plant parts. The synthetically fertilized treatments with MG and MG w/VC had the highest wt %N values.

**Tomato Fruit Yield.** Tomato yield parameters were influenced by the various fertilization treatments listed in Table 6. In 2014, our results indicated that there were no differences in tomato yields for both cluster 1 and cluster 2. However, total tomato yields differed between treatments with the conventionally fertilized tomatoes (MG and MG w/VC) having higher fruit weights compared with all other fertilization treatments. In 2015, tomato fruit parameters were also influenced by fertilizer treatments. Differences in fruit yield were apparent in cluster 1, with mixed nitrogen fertilization sources (BB w/VC, BBL w/VC, and MG w/VC) having the highest tomato fruit weight compared with all other treatments; however, no differences were seen in cluster 2. Total tomato yields were also influenced by fertilization treatment, with conventional inorganic fertilizers (MG and MG w/VC) having highest total yield weights compared with all other treatments.

Tomatoes are considered high-value horticultural crops as they can generate great revenue in comparison to many other vegetables.$^{20}$ Optimizing fruit yields in both organic and conventional growing systems is important in tomato production. Thus, the results of our study compared the differences found in the $^{15}$N/$^{14}$N ratios based upon fertilization treatment and how this translated to plant growth and fruit yields. Few differences were seen in cluster 1 and cluster 2 fruit yields for both 2014 and 2015 (Table 6). During early plant growth and initial mineral uptake, immature plants photosynthesize less and uptake nutrients similarly in both conventionally and organically fertilized soils.$^{21}$ As the plants continued to mature, differences were detected in total fruit yields between the conventionally and organically fertilized plants and generally indicated that tomatoes fertilized with conventional inorganic sources indicated greater fruit yields. Organic production by use of manures (e.g., vermicompost) can be difficult to manage due to variability in nutrient concentrations and mineralization release.$^{20,21}$ Organic fertilizers applied to the soil take time to breakdown and N sources are more difficult for plant uptake due to enrichments of $^{15}$N in the soil, which related to a slight decrease in fruit.$^{22,23}$ In comparison, conventionally fertilized soils with ammonium and nitrate tend to have a more consistent supply of nitrogen throughout the production.$^{24}$

**Nitrogen Assimilation.** The differences in yields and isotopic abundance result from where the various assimilates are moving in the tomato plant. Tomato crop yield and productivity is related to assimilation processes and source to sink capacities. Under normal conditions, assimilate availability (source capacity) is lower than assimilate demand (sink capacity) and fruit growth is source limited.$^{24}$ Leaf photosynthetic capacity can also strongly depend on source to sink balance, as well as the types of nitrogen fertilizers utilized during crop production. Nitrogen availability is an important factor that may limit source-sink assimilates.$^{25}$ For example, when conventional inorganic nitrogen was applied, tomato roots had sufficient nitrogen

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**Table 6. Tomato Fruit Yield Weights (g) Influenced by the Conventional and Organic Fertilizer Treatments for 2014 and 2015**

| soil treatment | tomato yield C1 | tomato yield C2 | tomato yield total |
|----------------|-----------------|-----------------|--------------------|
| 2014 season    |                 |                 |                    |
| no fertilization | 30.4a           | 79.4a           | 109.6b             |
| MG             | 46.9a           | 68.0a           | 1081.5a            |
| BB             | 68.0a           | 79.3a           | 251.5b             |
| BBL w/VC       | 68.0a           | 45.4a           | 438.5b             |
| BB w/VC        | 63.5a           | 61.3a           | 567.0b             |
| BBL w/VC       | 48.3a           | 45.4a           | 570.0b             |
| MG w/VC        | 46.8a           | 45.4a           | 1007.0a            |
| no fertilization w/VC | 45.4a | 60.5a | 241.9b |
| P-value | 0.48 | 0.8112 | 0.001 |
| 2015 season    |                 |                 |                    |
| no fertilization | 63.5a           | 90.7a           | 154.2c             |
| MG             | 78.6a           | 75.6a           | 902.7a             |
| BB             | 93.5a           | 128.5a          | 392.1c             |
| BBL w/VC       | 116.4ab         | 143.6ab         | 384.8b             |
| BB w/VC        | 113.4ab         | 131.5a          | 479.3b             |
| BBL w/VC       | 151.2b          | 170.9b          | 443.0b             |
| MG w/VC        | 105.8b          | 98.3a           | 1058.4a            |
| no fertilization w/VC | 121.0b | 121.0a | 321.3c |
| P-value | 0.0011 | 0.050 | 0.05 |

**Mean weight of tomato fruit yields represented in gram for each of eight soil fertilization treatments.**

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conditions and increased N uptake sufficiently to the shoots. This availability of $^{14}\text{N}$ maximized tomato photosynthetic capacity and increased fruit yields. However, in the organic fertilization treatments heavier in $^{15}\text{N}$, both the roots and shoots expanded photosynthetic energy to uptake N assimilates, decreasing source capacities and lowering fruit yields. 20 Tomato plants also indicated thrips during both growing seasons. This damage may have interfered with tomato production, leading to lower yield weights. Tomatoes also indicated necrosis and chlorosis half-way through fruit production. This discoloration could have been from disease or nutrient deficiency and also may have resulted in lower fruit production and yields. However, these growing discrepancies would not change the nitrogen isotopic composition due to root and shoot assimilation events that have been previously discussed. 16

## CONCLUSIONS

This research indicates that plant nitrogen isotope analyses can provide useful intelligence to detect differences between organically and conventionally fertilized tomatoes. When various forms of nitrogen are applied to the soil, the method of nitrogen uptake differs between conventional and organic production systems and this also leads to differences in plant enrichment and depletion from the soil source. Moreover, the applications of various nitrogen sources change the water holding capacity of the soil system, which is reflected in tomato foliage and fruit isotopic compositions. By utilizing systems that are more sustainable and environmentally friendly, we can help build soil structure and compositions. By utilizing systems that are more sustainable and economic gain while reducing nitrogen fertilizer inputs.

Organic certification agencies are highly interested in whether vegetable products such as tomato have been grown using organic or inorganic origins; plant $\delta^{15}\text{N}$ values have the potential to be utilized in this process. Organic growers producing over U.S. $5000$ gross income a year must go through organic certification processes, and there are several rules that farmers must implement regarding fertilization and pesticide usage. 27 Stable isotope analyses could be utilized during the certification process to determine if crops have been grown using the correct nitrogen fertilizers. We suggest that mature tomato leaflets are best suited for sampling nitrogen isotope analyses because at the mature growth stages, plants are maximizing nutrient uptake and photosynthetic capacity. Thus, on the basis of results of this study, nitrogen stable isotope patterns in mature tomato plants can indicate whether a crop has been fertilized by organic or inorganic methods.

## MATERIALS AND METHODS

A greenhouse experiment was set up in a randomized complete block design with three replications and eight tomatoes per replication. Tomatoes were fertilized with the same fertilizer applications repeated for two growing seasons. The experiment was conducted from 10 July to 15 Dec 2014 and 11 July to 11 Dec 2015 at the Horticulture Research Center Greenhouse at Southern Illinois University in Carbondale, Illinois. “Better Bush” tomatoes (Tomato Growers Supply Company, Fort Meyers, FL) were grown from seed in 8 L plastic pots filled with 1:1:1 steamed-sterilized Belknap silt loam/sand/peat mix. During both growing seasons, the average temperature of the greenhouse fluctuated between 13 and 36 °C and the plants received only natural sunlight. Three main soil fertility treatments were studied, and the results were compared with those obtained on plants grown in unfertilized soil: conventional inorganic, organic, and mixed. The inorganic fertilization treatment applied synthetic Miracle Grow (24.0N-3.5P-13.3K, Scotts, Marysville, OH) (MG) to soil in addition to all-purpose 12-12-12 fertilizer (12.0N-5.2P-10.0K). The average percentage of ammonia found in Miracle Grow is monobasic ammonia phosphate at 11% and nitrate is found at 17%. The organic treatments made use of the following: (1) organic bonemeal (6.0N-3.9P-0.0K, Whitney Farms, Independence, OR) and bloodmeal (12.0N-0.0P-0.0K, Whitney Farms, Independence, OR) (BB), (2) organic BB with liquid Earth Juice Grow (2.0N-0.5P-0.8K, Hydro-Organics, Chico, CA) and liquid Earth Juice Bloom (0.0N-1.4P-0.8K, Hydro-Organics, Chico, CA) (BBL), (3) organic BB with 25% vermicompost (VC), (4) organic BBL with 25% VC, and (5) 25% VC only. The mixed treatment consisted in the application of MG with 25% VC. In addition, potassium sulfate was added to the organic treatments. For each growing season, a new batch of steamed soil was used prior to being mixed with fertilizers.

The soil—fertilizer mixtures were prepared using a cement mixer and placed into plastic pots. The vermicompost was developed using red wiggler earthworms (Eisenia fetida) feeding on coffee grounds and vegetable wastes. The rock fertilizers (e.g., bonemeal, bloodmeal, and potassium sulfate) and vermicompost were added separately prior to tomato planting. Each homogenized soil mixture was analyzed for pH, cation exchange capacity, organic matter content, and nitrogen release (Table 1). The pots were watered for 7–10 days to induce volunteer vegetable seedlings from the vermicompost, which were then hand-weeded prior to sowing of tomato seeds. At seeding, three tomato seeds were covered with about 0.5 cm of soil and watered daily. At the four-leaf stage, tomato plants were thinned to one plant per pot. The pots were watered once or twice daily, and the tomato plants were pruned and topped to one main stem with several side shoots and trellised onto bamboo stakes once the plants were 0.5 m tall.

**Sample Collection.** Tomato leaves were collected at two growth stages (immature leaflets harvested in Sept and mature leaflets harvested in Oct), and fruits were collected from the first two fruit clusters at the red-ripe stage in Nov 2014 and Dec 2015. Five tomato leaflets were harvested from each plant and were collected from the tip, middle, and closest to the stem of the topmost branch (branch 1) and from only the very tip of the next two lower branches (branches two and three). Soil samples were taken concomitant with the immature plant leaves at a depth of 5–7 cm where the average temperature was 22 °C and from an area with no root material.

Tomato harvest began on 12 Oct 2014 and 8 Oct 2015. Tomatoes were harvested at the red-ripe stage with harvest completed on 28 Nov 2014 and 1 Dec 2015. Two marketable tomatoes were harvested from clusters 1 and 2 and were placed into separately labeled paper bags to decrease chances of contamination and brought to the laboratory for sample preparation. The yield weight (g) of each tomato was taken at the time of harvest. However, two treatments did not produce cluster 2 tomatoes due to small amounts of N fertilizer applied to the soil media (e.g., no fertilization and BB).

**Sample Preparation for Nitrogen Stable Isotope Analyses.** The fertilizers utilized in this study were analyzed...
for isotopic composition. The fertilizers and VC were oven dried for 2–3 days at 70 °C, ground to a fine powder, and dried for another 1 day longer (Table 2). All collected leaf samples were oven dried for 1–2 days at 75 °C, ground to a fine powder with mortar and pestle, and further dried for 1–2 days at 75 °C.28 Between 1.5 and 1.9 mg of each leaf sample were packed into a Costech Analytical 3.5 × 5 mm² tin capsule. Soil samples were oven dried for 3–4 days at 70 °C, ground to a powder, and oven dried again for another 1–2 days at 70 °C. The ground soil samples (1–1.5 mg) were packed into tin capsules, as described previously. Tomatoes were prepared by making a circular incision 3–5 cm in diameter about 0.5 cm deep into the tomato peel with scalpel and blade. The tomato peel was removed from each fruit and immediately oven dried for 3–4 days at 75 °C. The samples were ground into a fine powder and oven dried for an additional 1–2 days. For each peel sample, 1.5–2 mg was packed into a tin capsule. The peeled tomatoes were placed into large beakers and ground into juice with a large pestle. After macerating the tomatoes, roughly 35–40 g were placed into large test tubes and centrifuged at 3951 g-force for 5 min to obtain the supernatant (tomato juice) on a Thermo Scientific Sorvall ST 16 centrifuge. The resulting juice was filtered through Sterilitech micropore filters and immediately frozen at roughly −18 °C. Just prior to packing in tin capsules, tomato juice samples were thawed, and approximately 0.4 mL dropped with a syringe on 20 mg of inert Sigma-Aldrich silicon dioxide powder placed into a 5 × 9 mm² tin capsule.

Elemental Combustion-Continuous Flow-Isotope Ratio Mass Spectrometer (IRMS). All packed samples (fertilizer, tomato leaf, fruit peel, fruit juice, and soil) were combusted in a Costech 4010 elemental combustion system. The temperatures of the combustion/oxidation tube, which contained chromium oxide as oxidation catalyst, reduction tube, which contained reduced copper wire for the removal of excess oxygen, and gas chromatography column, which was used to separate the N₂ from CO₂ gas, were 1020, 680, and 57 °C, respectively. The gases were carried in an ultrapure He stream, passed through a magnesium perchlorate water trap, and entered into a DELTA V Plus IRMS, where the nitrogen isotope ratios were measured, via a ConFlo IV unit, both from Thermo Scientific.

The nitrogen isotopic ratios (\(^{15}\text{N}/^{14}\text{N}\) ratio) were expressed as

\[
\delta^{15}\text{N}(\%_{\text{o}} \text{ vs AIR}) = \left[\frac{(^{15}\text{N} / ^{14}\text{N})_{\text{sample}}}{(^{15}\text{N} / ^{14}\text{N})_{\text{standard}}}\right] - 1 \times 1000
\]

\(R_{\text{standard}}\) is the ratio of the heavy isotope to the light isotope in the standard, and \(R_{\text{sample}}\) is the ratio of the heavy isotope to the light isotope in the sample. \(\delta^{15}\text{N}_{\text{AIR}}\) is weight %N were determined. \(\delta^{15}\text{C}_{\text{VPDB}}\) data was collected but is not presented. USGS 40 and 41 (l-glutamic acid) were used as bracketing certified standards for nitrogen isotopes; analytical precision for \(\delta^{15}\text{N}_{\text{us}}\) was ±0.07‰. Acetanilide was used as a certified standard to calculate weight %N; analytical precision was ±0.15% for wt %N.

**Statistical Analysis.** Data were processed statistically and expressed as the isotopic means ± standard deviation (SD), wt % N means ± standard deviation (SD), and tomato yield means (g) ± standard deviation (SD). To study the differences among sample groups, the data was processed using a two-tailed analysis of variance (JMP Statistical Discovery Software, JMP, 2014 and 2015 Cary, NC). The least significant difference (LSD) and Student’s t multiple range tests were used to separate fertilizer treatment means at \(P \leq 0.05\). Orthogonal contrast was used to compare the treatments with vermicompost from those without any (SAS: Analytics, Business Intelligence and Data management, SAS, 2014, Cary NC).

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**Notes**

The authors declare no competing financial interest.

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