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Influence of Biochar and Animal Manures Application on Ammonia and Nitrate Concentrations in the Root and Shoot of Three Varieties of Turnips

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Abstract: Many investigators have focused on the impact of fertilizers on crop yield and ignored fertilizers impact on the plants composition. The impact of seven types of soil treatments (sewage sludge, horse manure, chicken manure, vermicompost, elemental organic fertilizer, inorganic fertilizer, and native soil) and similar seven treatments amended with biochar on the concentrations of NH_3 and NO_3 in the roots and shoots of three commercial varieties of turnips, *Brassica rapa* was investigated. The three varieties (Purple Top White Globe PTWG, Scarlet Queen Red SQR, and Tokyo Cross TC) varied in concentrations of NH_3 and NO_3 levels. High levels of NO_3 in edible plants is associated with harmful effects on human health, due to the risk of creation of carcinogenic N-nitroso compounds. NO_3 in SQR roots and shoots (edible greens) was greater than varieties PTWG and TC. The concentration of NH_3 averaged 20.2, 12.8, and 8.9 μg g^{-1} fresh turnip roots, whereas NO_3 values averaged 107.6, 64.1, and 62.9 μg g^{-1} fresh turnip roots in varieties SQR, PTWG, and TC, respectively. Regardless of soil amendment type, the concentration of NH_3 in the shoots (44.0 μg g^{-1}) was greater than the roots (15 μg g^{-1}). On the contrary, NO_3 was higher in the roots (89.4 μg g^{-1}) compared to the shoots (67.6 μg g^{-1} fresh tissue). Overall, biochar added to vermicompost amended soil increased NH_3 by 73% compared to vermicompost not amended with biochar. Regarding acceptable daily intake (ADI) for NO_3, none of the three varieties analyzed constitute any NO_3 adverse effects on normal human intake. Similarly, consuming turnips grown in any of the animal manures tested do not represent any hazardous issues.

Keywords: vermicompost; chicken dung compost; horse dung compost; municipal sewage sludge; mineral inorganic fertilizer; organic fertilizer

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1. Introduction

Nitrogen (N) supplied to plant roots as nitrate (NO_3) and ammonium ions (NH_4^+) is required in large amounts due to its greatest impact on plant growth [1], plant morphology, and nutrient composition [2]. Accordingly, N plays an important role in the yield and quality of growing plants. Most plants favor NO_3^-N since high concentration of NH_3-N is toxic during plant metabolism [3] and often recommended for application in small amounts after transplantation, due to irreversible alteration of the structure of the plant thylakoid membrane (the site of photochemical and electron transport reaction of oxygenic photosynthesis) [4]. Research results have indicated that the form of N supply has impact on photosynthesis, stomatal conductance and intercellular carbon dioxide (CO_2), but these results found not consistent among different plant species [5]. NH_3 in animal manures reacts with water to form ammonium ions (NH_4^+) that quickly binds to the negatively charged soil organic matter and clays. Nitrification in soil by *Nitrosomonas* and *Nitrobacter* bacteria oxidize NH_3 to NO_2 and NO_3. Plants uptake N from the soil in the form of NO_3, regardless of the form of N fertilizer applied, including animal manures. In humans, 5–10% of NO_3 is converted into the more toxic nitrite (NO_2) by salivary or

gastrointestinal reduction. NO_2 can react with proteins in the body to form carcinogenic N-nitroso compounds, such as nitrosamines [6]. There are legal limits of NO_3 and NO_2 in food. They are hazardous chemicals that can accumulate in vegetables and fruits from application of fertilizers. Vegetables receive relatively high rates of N fertilizers, which adds to the problem of NO_3 poisoning due to vegetables ability to accumulate NO_3 at high levels [7]. Large-scale animal operating production systems yields huge amounts of manure rich in NO_3, which seeps into groundwater and accumulate in edible plants grown in animal manures amended soils. Therefore, keeping NO_3 concentrations below legal limits is a challenge for farmers and health authorities.

NO_3 acceptable daily intake (ADI) values of 0–3.7 mg NO_3 kg^{-1} body weight (BW) established by the Joint Expert Committee of the Food and Agriculture (JECFA) of the United Nations/World Health Organization (WHO) and the European Commissions of Scientific Committee on Food (SCF) [8]. Mensinga et al. [9] estimated 5–8% of the NO_3 from daily diets reduced to NO_2 by the microflora in the oral cavity. Assuming a drinking water consumption of 2 L per day and a daily consumption of 100 g of vegetables, the overall daily NO_3 consumption may easily range from 200 to 400 mg. The ADI of NO_3 estimated to be from 0–3.7 mg kg^{-1} BW, expressed as NO_3 or 277 mg NO_3 per person of 75 kg BW has been established [6].

Vegetables contain NO_3 at varying levels, ranging from 1 to 10,000 mg kg^{-1} [10]. Vegetables can be classified according to their NO_3 content into very low (<200), low (200 to <500), middle (500 to <1000), high (1000 to <2500), and very high, (>2500 mg 100^{-1} g fresh weight), in which turnip has a middle range of 500 to <1000 mg 100^{-1} g fresh weight [11]. NO_3 accumulates in the mesophyll cells of the plant, since they exclusively transported among the plant tissue parts through the xylem [12]. In fact, the primary variables for NO_3 human intake includes the type of vegetables consumed, NO_3 levels in the type of vegetable consumed, and the amounts of vegetables consumed daily. The mean total NO_3 daily intake per person in Europe ranges between 50 and 140 mg and in the USA about 40–100 mg [13]. Toxic doses (with met hemoglobin formation as a criterion for toxicity) ranged from 33–350 mg NO_3- ion kg^{-1} BW [14] and human lethal doses of 67–833 mg NO_3^- ion kg^{-1} body weight (BW) reported. Consumption of one serving of a NO_3 rich food or supplement can exceed the World Health Organization acceptable daily intake for NO_3 (0–3.7 mg/kg body weight per day or 222 mg day^{-1} for a 60-kg adult).

Our hypothesis is that the use of soil amendments, such as animal manures that contain high levels of organic matter and nutrients is an inexpensive method to improve crop yield and soil quality. Reprocessing animal manures would reduce need of synthetic inorganic fertilizers and offer amendments useful for improving soil structure and nutrient composition at low-cost to small farmers. However, animal manures is a source of NH_3. NH_3 in animal manures reacts with water to form ammonium ions (NH_4^+) that quickly binds to the negatively charged soil organic matter and clays. In soil, NH_4^+ is transferred into nitrates (NO_3) that can also be absorbed by plants roots. NO_3 becomes a problem only if exceeded the allowable limits in food. We investigated the impact of animal manures used as organic fertilizers in agricultural production systems on the concentrations of NH_3 and NO_3 in three varieties of turnips, *Brassica rapa* grown in soil amended with animal manures and elemental organic and inorganic amendments on fresh weight basis. Quantification of NO_3 on a fresh-weight basis enables a better comparison of the NO_3 content of vegetables since most vegetables consumed fresh. Following food ingestion, bacteria in the mouth and gut convert NO_3 to NO_2 by salivary and gastrointestinal reduction, then NO_2 reacts with hemoglobin to produce met hemoglobin, which makes hemoglobin no longer able to carry oxygen [15]. Salehzadeh et al. [16] also reported that during various processes in the body, NO_3 usually converted to NO_2, which causes various diseases, such as blue baby syndrome and cancer. In fact, vegetable types and N fertilization influence NO_3 content in vegetables [8,17].

Because turnip can be grown in most locations and has short growing season (60 to 70 days) as a fall, winter, and early spring crop, turnips has a wide adaptation as a cash crop for

limited-resource farmers. Vegetables are major source of NO_3 and NO_2 since they can reach values of 85% of the total intake in the human diet [18]. Monitoring the NO_3 content becomes an important indicator of the quality of plant products.

The literature review bare little information concerning the effect of animal manures and inorganic soil amendments on NO_3 concentrations in vegetable species and varieties in species. Researchers have focused on the crop yield and soil fertility after the incorporation of fertilizers with little attention to the plant internal composition. This study delivered indication of the low impact of animal manures on NO_3 levels in three commercial varieties of turnips and explained the danger of NO_3 accumulation in turnips and other edible plants.

Accordingly, the intend of this study was to identify turnip varieties and/or animal manures mixed and not mixed with biochar (a carbon-rich material produced during pyrolysis process of biomass) on the accumulation of NH_3 and NO_3 in turnip roots and edible shoots (turnip greens). The objectives were: (1)-assess the overall impact of six soil amendments: sewage sludge SS, horse manure HM, chicken manure CM, vermicompost Vermi, commercial organic fertilizer Nature Safe 10N-2P-8K) (Org), inorganic fertilizer (20N-20P-20K) (Inorg), and unamended native soil (UA native soil) on NH_3 and NO_3 concentrations in turnip roots and shoots. (2)-screen three varieties of turnips, *Brassica rapa* (Purple Top White Globe, Scarlet Queen Red and Tokyo Cross) for their accumulation of NH_3 and NO_3. (3)-investigate the impact of adding biochar to soil amendments on the concentration of NH_3 and NO_3 in fresh root and shoot of turnips grown under field conditions.

2. Material and Methods

The field study at the university of Kentucky Research Farm (Fayette County Lexington, Kentucky, USA Latitude: 37.976262, Longitude: −84.533334) included a randomized complete block design (RCBD) of 63 plots (3 turnip varieties × 7 treatments × 3 replicates) not treated with biochar and 63 plots treated with biochar. Native soil pre-experimental properties are: an average of 56% silt, 38% clay, and 6% sand, pH 6.8, CEC 14.7 meq 100 g^{-1}, OM 2.2%, Total N 0.18%, N-NO_3 20.7 mg L^{-1}, N-NH_4-N 5.7 mg L^{-1}, P 95.8 mg L^{-1}, K 336.2 mg L^{-1}, C 1091 mg L^{-1}, Cd 0.04 mg L^{-1}, Cu 1.9 mg L^{-1}, Zn, 1.98 mg L^{-1}, Pb 2.15 mg/L^{-1}, and Ni 0.66 mg L^{-1}.

Each of the 126 plots was 4 ft. (1.22 m) length and 3 ft. (0.91 m) width. The soil treatments included six soil amendments and unamended (UA) native soil used as control treatment. The six soil amendments were sewage sludge SS, horse manure HM, chicken manure CM, vermicomposting Vermi, commercial organic fertilizer (10N-2P-8K) Org, inorganic fertilizer (20N-20P-20K) Inorg. Each of the soil amendments used in this investigation was mixed with the native soil at 5% nitrogen (N) on dry weight basis to eliminate variations among soil treatments due to their variability in N content, since N fertilization has been identified as the major factor that influence NO_3 content of vegetables [8,17]. SS (5% N) purchased from the Metropolitan Sewer District in Louisville (KY, USA) and applied to native soil at 2241.7 kg hectare $^{-1}$. CM (1.1% N) obtained from the Department of Animal and Food Sciences, University of Kentucky (Lexington, KY, USA) and applied at 6592. 8 kg hectare^{-1}. HM (0.7% N) obtained from the Kentucky horse park (Lexington, KY, USA) was applied at 16,011.4 kg hectare^{-1}. Vermi (1.5% N) obtained from Worm Power (Montpelier, Vermont, USA) and applied at 9340.1 kg hectare^{-1}. Org (10% N) and Inorg (5% N) commercial fertilizers obtained from the Southern States Cooperative Stores (Lexington, KY, USA) and applied at 1120.9 and 560.4 kg hectare^{-1}, respectively (Table 1).

The three varieties of turnips, *Brassica rapa* were var. Purple Top White Globe (PTWG), var. Scarlet Queen Red (SQR), and var. Tokyo Cross (TC) (Figure 1). Prior to planting, each amendment added to native soil and rototilled to a depth of 15 cm (~0.5 ft.) top soil. Biochar (a carbon-rich material produced during pyrolysis and thermochemical decomposition of biomass), obtained from Wakefield Agricultural Carbon (Columbia, MO) was added at the rate of 10% (*w/w*). Properties of biochar used in this investigation were: total organic carbon 88%, total inorganic carbon 0.34%, surface area 366 m^2 g $^{-1}$ dry, moisture 54%,

temperature 200 °C, bulk density 480.6 kg m^{-3}, N 0.27%, P 2.06 mg kg^{-1}, K 280 mg kg^{-1}, Ca 1881 mg kg^{-1}, Cu 2.45 mg kg^{-1}, Mg 558 mg kg^{-1}, and Zn 2.09 mg kg^{-1}.

Table 1. Concentrations of NPK in animal manures, organic commercial fertilizer, and inorganic mineral fertilizer used for growing turnip, *Brassica rapa* (Fayette County, Kentucky, USA).

Soil Amendment	Nitrogen (% N)	Phosphorus (% P)	Potassium (% K)
Sewage Sludge	5.00	3.00	0.00
Chicken Manure	1.10	0.80	0.50
Horse Manure	0.70	0.30	0.60
Vermicompost	1.50	0.75	1.50
Organic Fertilizer	10.00	2.00	8.00
Inorganic Fertilizer	20.00	20.00	20.00
	Amounts of Soil Amendments Added in kg hectare^{-1}		
Soil Amendment	**Nitrogen (N)**	**Phosphorus (P)**	**Potassium (K)**
Sewage Sludge	2241.7	1345.0	0.00
Chicken Manure	6592.8	4794.8	2996.7
Horse Manure	16,011.4	6861.9	13,724.0
Vermicompost	9340.1	4670.0	9340.1
Organic Fertilizer	1120.9	224.2	896.7
Inorganic Fertilizer	560.4	560.4	560.4

Soil amendments were applied to each treatment at 5% N. Determination of NPK was carried out using inductively coupled plasma (ICP) spectrometer.

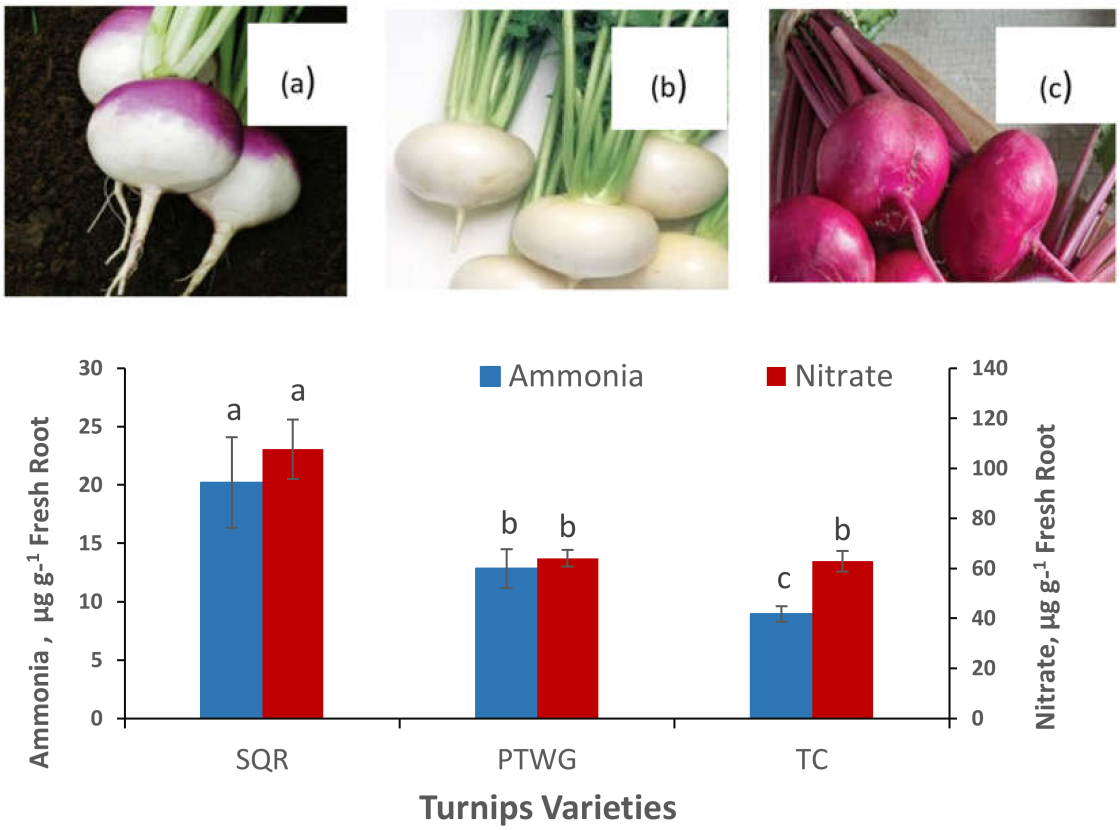


Figure 1. Variability in root morphology of three varieties of turnips (*Brassica rapa)*: (**a**) Purple Top White Globe (PTWG), (**b**) Tokyo Cross (TC), and (**c**) Scarlet Queen Red (SQR) (upper photo) and NH_3 and NO_3 concentrations ± std. error in their roots (lower graph), regardless of soil treatments. Standard errors having different letter indicate significant differences ($p \leq 0.05$). Statistical comparisons carried-out among varieties using Duncan's multiple range test.

Seeds of turnip, *Brassica rapa* were planted in a freshly tilled soil at 45.7 cm in-row spacing, and the plants were drip irrigated as needed. Weeding and other agricultural operations carried out during the growing season regularly as needed. One month after planting, turnip plants were sprayed with the insecticides esfenvalerate (Asana XL) and Baythroide XL (β-cyfluthrin) three times during the growing season at the recommended rate of application [19]. At maturity (70 day old plants), three turnip varieties (PTWG, SQR, and TC) removed from the soil and their shoots (edible greens) and roots were separated using a sharp knife. Turnip greens are the dark leafy green tops that are edible and utilized in many cuisines. Five turnip plants randomly collected from each replicate (15 turnip plants from each treatment), and washed with deionized water for chemical analysis. Roots were cut vertically using a sharped knife into four quarters, one quarter from each root was cut into small cubes and a representative 100 g were selected for sample analysis. Similarly, the shoots (leaves and stems) were chopped using a kitchen shopper, extracted using 80% ethanol, and filtered using Whatman No. 1 filter paper. Quantification of NH_3 and NO_3 was carried out using a Fisher brand XL500 Benchtop Meter equipped with Orion High-Performance ammonia and nitrate electrodes (Fisher brand XL500 Benchtop Orion High-Performance ammonia and nitrate Electrodes) using the methods described by APHA [20].

Turnips roots, shoots, and plant weight were recorded. Concentrations of NH_3 and NO_3 in turnips roots and shoots were analyzed in each of the three turnip varieties) grown under the fourteen soil treatment. Data containing NH_3 and NO_3 in turnips root, shoot, and plant weight of each variety were statistically analyzed using one-way analysis of variance (ANOVA) (SAS Institute, 2016) [21] and the means were compared using Duncan's multiple range test.

3. Results

There were significant differences in NH_3 and NO_3 content among the three turnip varieties tested. Figure 1 shows that the concentrations of NH_3 averaged 20.2, 12.8, and 8.9 μg g^{-1} fresh turnip roots, whereas NO_3 values averaged 107.6, 64.1, and 62.9 μg g^{-1} fresh turnip roots in the three varieties (SQR, PTWG, and TC), respectively. These data revealed that variety SQR had significantly ($p \leq 0.05$) greater concentrations of NH_3 and NO_3 content compared to varieties PTWG and TC. Table 2 revealed a significant ($p \leq 0.001$) positive correlation (r = 0.57) between NH_3 and NO_3 content in variety PTWG grown in soil amended with biochar, while NH_3 and NO_3 were not significantly correlated in varieties TC and SQR. In addition, when biochar not added to soil, a significant negative correlation observed in variety TC. This negative correlation indicates that increasing the concentration of NH_3/NH_4^+ in variety TC is followed by low accumulation of NO_3, a needed attribute for increasing agricultural products human safety.

Table 2. Overall Pearson's correlation coefficients (r) and probability of significance (*P*) between ammonia and nitrates concentrations in turnip plants (root and shoot) grown in soil treated with biochar (A) and soil not treated with biochar (B).

(A)	PTWG	TC	SQR
Ammonia	r = 0.57	r = −0.17	r = −0.165
Nitrate	($p \leq 0.001$) *	(p = −0.2644)	(p = 0.2956)
(B)	PTWG	TC	SQR
Ammonia	r = −0.24	r = −0.51	r = −0.217
Nitrate	(p = 0.1397)	(p = 0.006) *	(p = 0.1723)

Purple Top White Globe (PTWG), Tokyo Cross (TC), and Scarlet Queen Red (SQR); * indicates significant correlation ($p \leq 0.05$).

Regardless of turnip variety, results also revealed that Vermi, Inorg, CM, HM, and Org amended soil significantly increased NO_3 concentrations in turnip roots compared to the roots of plants grown in the unamended (UA) control treatment (Figure 2). In addition, soil amended with Vermi and SS increased NH_3 concentrations in turnip roots compared to

other soil amendments and the control treatment (UA treatment). Although N content in the six amendments was applied at 5% N, Vermi and SS were superior in elevating NH_3 concentrations in turnip roots.

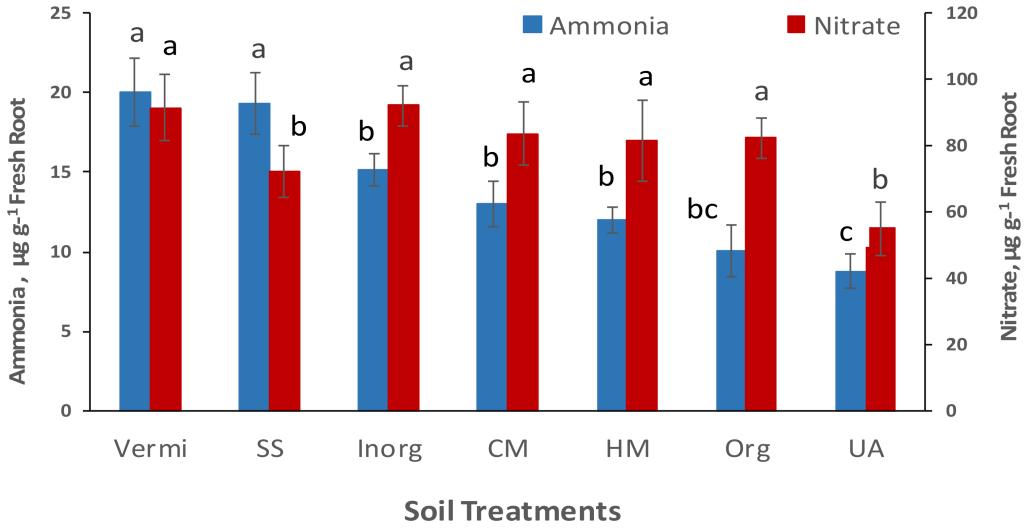


Figure 2. Concentrations of ammonia and nitrates ± std. error in turnips roots of plants grown under seven soil treatments, regardless of turnips variety. Vermi vermicompost, SS sewage sludge, Inorg inorganic fertilizer, CM chicken manure, HM horse manure, Org elemental organic fertilizer, and unamended (UA) native soil. Statistical analysis was carried-out using analysis of variance (ANOVA). Standard errors having different letter(s) indicate significant differences ($p \leq 0.05$) using Duncan's multiple range test.

As described earlier, the use of animal manures and mineral N fertilizers in agricultural production systems is a major source of ammonia (NH_3/ NH_4^+) emission [22], NH_3 emissions from animal manure used in agricultural production systems is generated by several physical, chemical, and biological processes [23]. Loss of NH_3/ NH_4^+ from manure are destructive, because they decrease the amount of manure N available for the crop and increase N contamination in groundwater through soil seepage (infiltration). Accordingly, variability among soil amendments, such as particle size, compaction, infiltration rate, moisture holding capacity, microbial activity, enzymes secretion, texture, pH, and other animal manures properties and composition are the main factors that control NH_3 emissions and NO_3 formation. However, regardless of amendment type used in this investigation, NO_3 in turnip greens of variety SQR had the highest concentration compared to PTWG and TC (Figure 3).

Regardless of turnip varieties, Figure 4 revealed that concentrations of NH_3 was greater in Vermi, SS, and Inorg treatments compared to CM, HM, Org, and UA control treatment, indicating that the addition of CM, HM, and Org fertilizer did not add NH_3 in turnips shoots of plants grown in unamended soil. In addition, all soil treatments increased the NO_3 content compared to the control (UA treatment). Results in Figure 5 revealed significant variability in NH_3 and NO_3 concentrations between turnip root and shoot. Concentrations of NH_3 averaged 44.2 and 14.9 µg g^{-1} fresh shoot and root tissue, respectively, whereas the concentrations of NO_3 averaged 67.6 and 89.4 µg g^{-1} fresh shoot and root tissue, respectively. These results of greater concentration of NO_3 in turnip roots compared to the shoot (edible greens) represents about 32% increase.

Regarding the impact of soil amendments, overall concentration of NH_3 in turnips root and shoot of plants grown in Vermi compost amended with biochar (VermiBio) was significantly ($p \leq 0.05$) greater (39.9 µg g^{-1} fresh tissue) compared to Vermi compost (Vermi) not amended with biochar (23.1 µg g^{-1} fresh tissue) (Figure 6). This significant increase revealed the positive impact of biochar (73.3% increase) on NH_3 concentration when biochar added to Vermi compost amended soil (VermiBio). Other than VermiBio, there was no

impact of biochar addition on NH_3 concentrations in turnip plants before and after biochar addition. Figure 7 revealed that soil amended with inorganic fertilizer treated with biochar (InorgBio) significantly increased the concentration of NO_3 compared to biochar added to unamended control treatment (UABio). Other than InorgBio, no significant differences found in NO_3 concentrations among turnip plants grown in soil amendments treated with biochar and soil amendments not treated with biochar, regardless of turnip varieties.

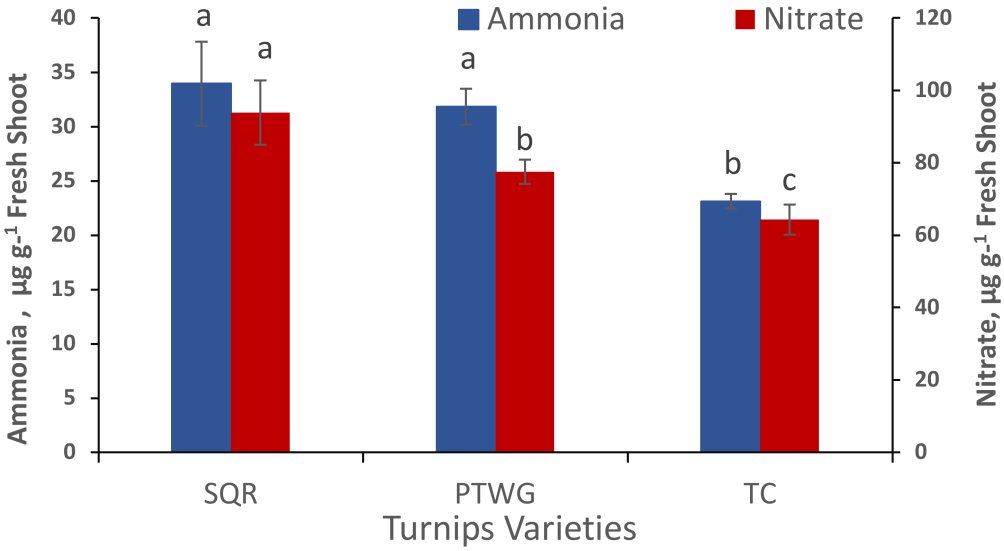


Figure 3. Variability in the concentrations of ammonia and nitrates ± std. error in the shoots of three varieties of turnips: Scarlet Queen Red (SQR), Purple Top White Globe (PTWG), and Tokyo Cross (TC), regardless of soil treatments. Statistical comparisons carried-out among varieties using analysis of variance (ANOVA). Standard errors having different letter indicate significant differences ($p \leq 0.05$) using Duncan's multiple range test for means comparison.

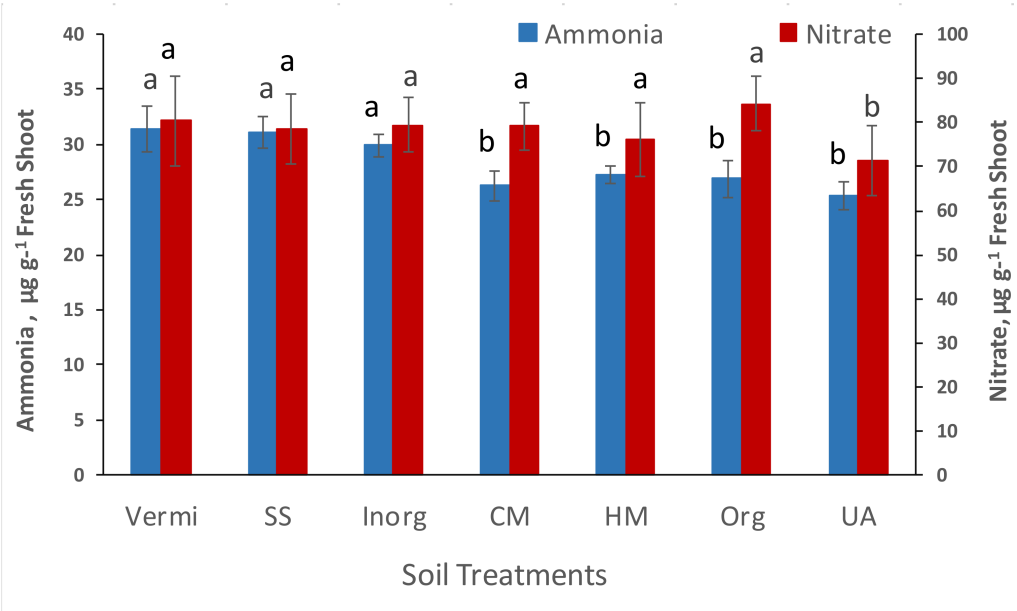


Figure 4. Concentrations of ammonia and nitrates ± std. error in turnips shoot of plants grown under seven soil treatments, regardless of turnips variety. Vermi vermicompost, SS sewage sludge, Inorg inorganic fertilizer, CM chicken manure, HM horse manure, Org elemental organic fertilizer, and unamended (UA) native soil. Statistical analysis was carried_out using analysis of variance (ANOVA). Standard errors having different letter indicate significant differences ($p \leq 0.05$) using Duncan's multiple range test for means comparison.

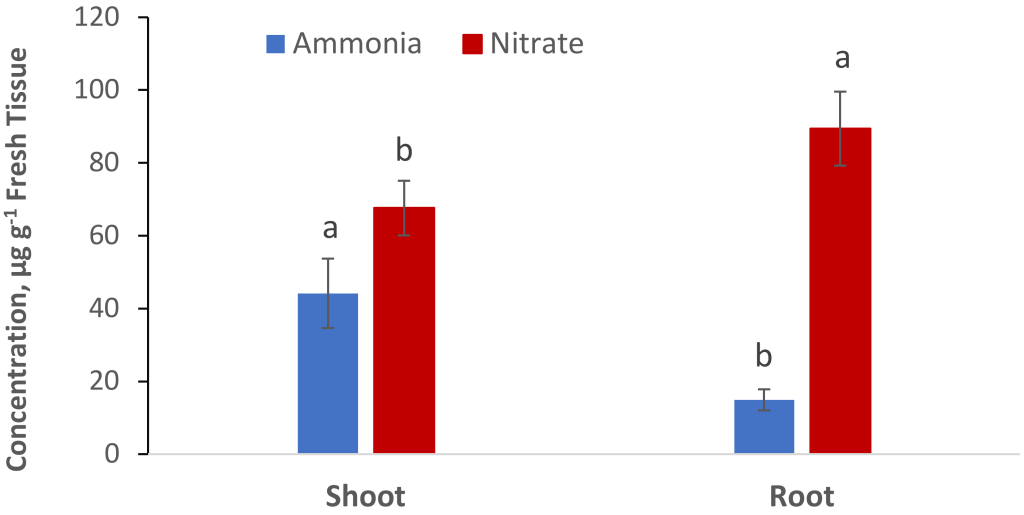


Figure 5. Overall concentrations of ammonia and nitrates in turnips shoot and root plants, regardless of turnips variety. Statistical comparisons were carried- out between the shoot and root using analysis of variance (ANOVA). Standard errors having different letter indicate significant differences ($p \leq 0.05$) using Duncan's multiple range test for mean comparisons.

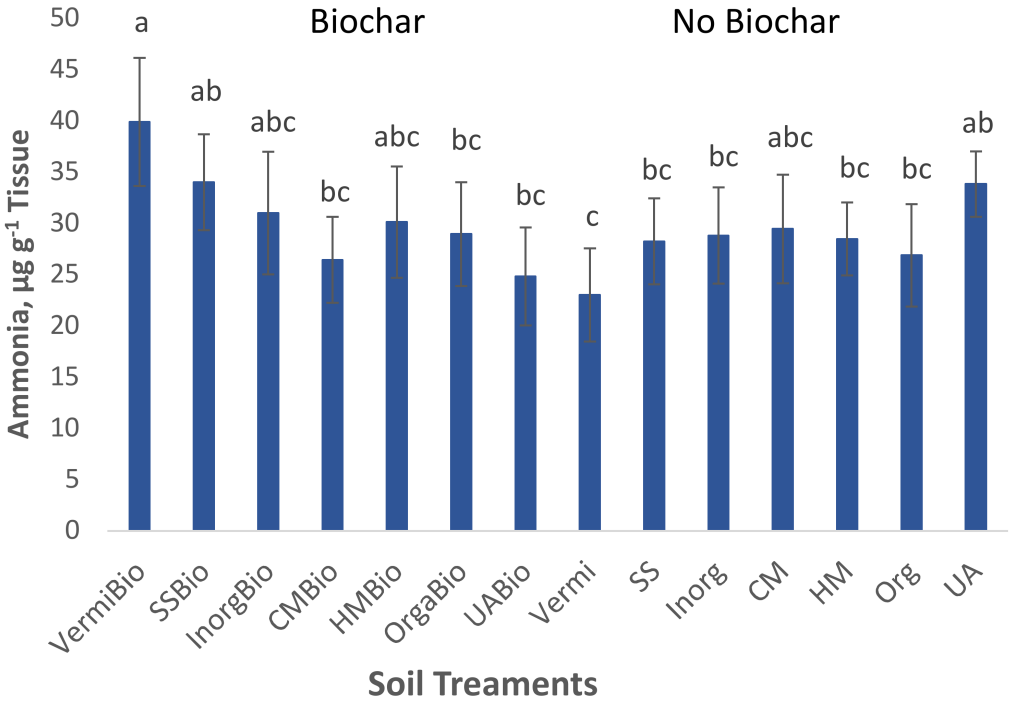


Figure 6. Concentrations of ammonia ± std. err in turnip plants grown under seven soil treatments not amended with biochar (vermicompost Vermi, sewage sludge SS, inorganic fertilizer Inorg, chicken manure, CM, horse manure HM, organic fertilizer Org, and unamended UA control), and seven soil treatments amended with biochar (VermiBio, SSBio, InorgBio, CMBio, HMBio, OrgBio, UABio), regardless of turnip varieties. Statistical analysis was carried out among 14 soil treatments using analysis of variance (ANOVA). Standard errors having different letter(s) indicate significant differences ($p \leq 0.05$) using Duncan's multiple range test for means comparison.

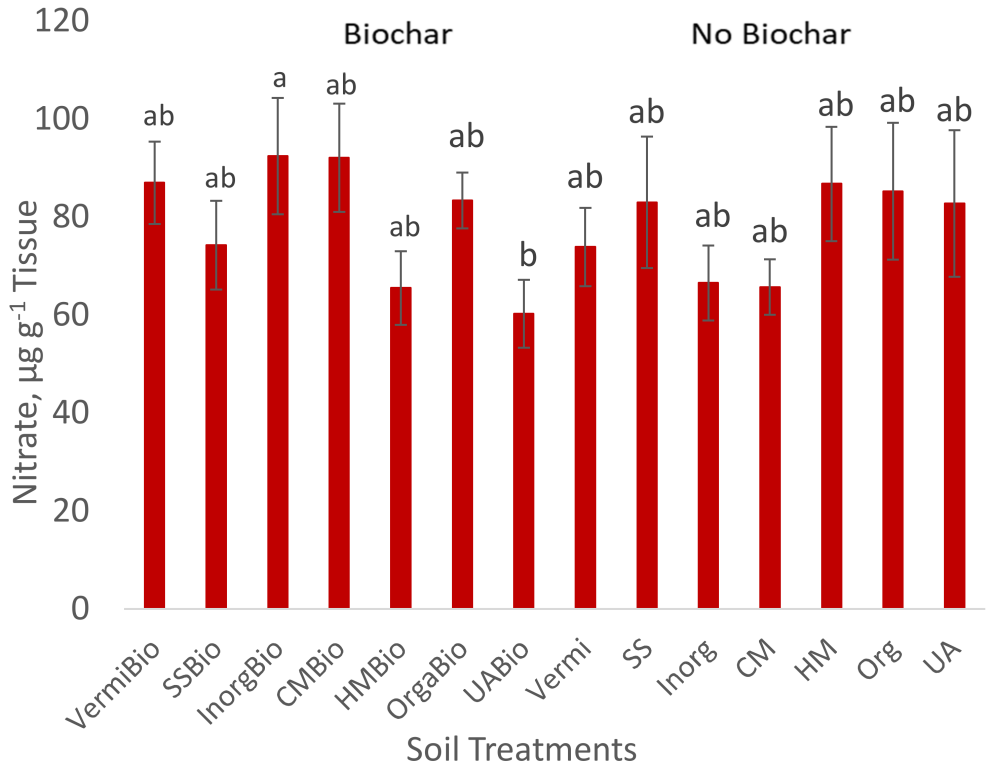


Figure 7. Concentrations of nitrate in turnip plants grown in seven soil treatments not amended with biochar (vermicompost Vermi, sewage sludge SS, inorganic fertilizer Inorg, chicken manure, CM, horse manure HM, organic fertilizer Org, and unamended UA control soil), and seven soil treatments amended with biochar (VermiBio, SSBio, InorgBio, CMBio, HMBio, OrgBio, UABio), regardless of turnip varieties. Statistical analysis was carried out among 14 soil treatments using analysis of variance (ANOVA). Standard errors having different letter(s) indicate significant differences ($p \leq 0.05$) using Duncan's multiple range test for means comparison.

Antonious et al. [24] reported that NO_3 concentrations in Vermi was significantly greater compared to other animal manures. In addition, urease activity (the enzyme that breakdown urea forming NH_4^+ and CO_2) also was greater in Vermi. NO_3 toxic doses due to methaemoglobin formation (exposure of hemoglobin to a variety of highly reactive oxygen free radicals produced during normal cell metabolism), ranged from 33–350 mg NO_3^- ion kg^{-1} body weight (BW) have been reported by Speijers [14]. The oral lethal dose to humans was estimated to vary from 33 to 250 mg NO_2^- ion kg^{-1} BW. Doses of 1 to 8.3 mg NO_2^- ion kg^{-1} BW, gave rise to induction of methemoglobinemia in which the hemoglobin iron (Fe) oxidized and cannot reversibly bind oxygen [14]. Salehzadeh et al. [16] reported that a person with an average weight of 70 kg should not consume more than 255.5 mg of NO_3 daily. Boink and Speijers [6] reported that the acceptable daily intake (ADI) for NO_3 is assigned as 0–3.7 mg kg^{-1} body weight (BW) or 277 mg NO_3 per person of 75 kg average weight.

Table 3 revealed a significant ($p \leq -0.69$) negative correlation (r = 0.0014) between NH_3 and NO_3 content in turnip plants grown in soil amended with municipal SS mixed with biochar. A significant ($p \leq 0.0014$) negative correlation (r = −0.69) between NH_3 and NO_3 content in turnip plants grown in soil amended with vermicompost not mixed with biochar was also obtained, while correlations between NH_3 and NO_3 in turnips grown in other soil amendments were not significantly correlated. This negative correlation indicates that increasing the concentration of NH_3/NH_4^+ is followed by low accumulation of NO_3, which is a needed attribute for increasing human safety.

Table 3. Overall Pearson's correlation coefficients (r) and probability of significance (*P*) between ammonia and nitrates concentrations in turnip plants (root and shoot) grown in soil treatments treated with biochar (A) and soil treatments not treated with biochar (B).

(A)	Vermi	SS	Inorg	CM	HM	UA
Ammonia Nitrate	r= −0.15 (p = 0.54)	r = −0.69 (p = 0.0014) *	r = 0.18 (p = 0.47)	r = 34 (p = 0.158)	r = 0.08 (p = 0.74	r = 0.14 (p = 0.57)
(B)	Vermi	SS	Inorg	CM	HM	UA
Ammonia Nitrate	r= −0.694 (p = 0.0014) *	r = 0.43 (p = 0.069)	r = 0.0015 (p = −0.69)	r = −0.31) (p = 0.214)	r = 0.05 (p = 0.84)	r = 0.13 (p = 0.58)

Vermi vermicompost, SS sewage sludge, Inorg inorganic fertilizer, CM chicken manure. HM horse manure, Org elemental organic fertilizer, and UA unamended control treatment. * indicates significant correlation ($p < 0.05$).

4. Discussion

Vegetables and animal manures contain ammonia (NH_4^+) and nitrate (NO_3^-) ions that constitute a potential health hazard to consumers. NH_4^+ and NO_3^- are natural constituents of vegetables and fruits. The toxic effects of NO_3 are due to its endogenous conversion to nitrite (NO_2^- in saliva and human gastrointestinal tract. NO_3 toxicity among vegetable consumers and growers interested in growing turnips in animal manures amended soils have received increased attention, which resulted in several investigations on the dietary exposure to these compounds. Investigators reported that about 5% of the dietary NO_3^- reduced to NO_2^- in saliva and the gastrointestinal tract and this number might reach 20% for individuals with a high rate of conversion [25].

Hmelak and Cencic [11] reported that NO_3 extensively distributed in nature and different concentrations of NO_3 are detected in soil, water, and food, but ingestion and exposure to NO_3 is mainly from vegetables and water. A moderate reduction in plants yield caused by NH_4 + stress could be prevented by the application of nitrification inhibitors, such as 2-chloro-6-(trichloromethyl) pyridine (nitrapyrin), dicyandiamide (DCD), and 3,4-dimethylepyrazole phosphate (DMPP) with NH_4+ fertilizers or organic fertilizers which makes high concentrations of NH_4 + stable in the soil for several weeks. On the other hand, the plant cell has several strategies to keep NH_4+ levels under control either by NH_4+ efflux to the plant rhizosphere area, or by storing NH_4+ in the cell vacuole, or by NH_4+ incorporation into organic compounds [26]. The positive effects of low concentrations of NO_3 and NO_2 presented by Parvizishad et al. [27] could have a protective effect on the cardiovascular system, blood pressure regulation, and maintaining homeostasis (stability) of vessels. The authors [27] discussed the different opinions about the allowable concentrations of NO_3 and NO_2 in food and water. They concluded that these compounds have beneficial and adverse effects on human health, and encouraged the need of more research to make proper judgments about setting the standards concentration in food and drinking water.

Due to the danger of the potential high NO_3 levels in food, several studies in different institutions and countries around the world monitored and established their own regulations for the control of NO_3 contaminations of vegetables [28]. Wu et al. [29] found that inappropriate vegetable cultivation methods, such as excessive use of nitrogen fertilizers could certainly cause extreme NO_3 accumulation in leafy vegetables. In addition, the unsuitable vegetable cooking processes can trouble their NO_3 balance and potential NO_2 safety risk. The authors detected a decrease in NO_3 content and the rapid increase in NO_2 content during 12–24 h of storage due to the effect of microorganisms in the storage environment. Kyriacou et al. [30] also reported that due to the abuse of chemical fertilizers and unreasonable planting methods, the NO_3 content of intensively planted vegetables tend to reach excessively high NO_3 levels. Salehzadeh [16] investigated the impact of cooking vegetables on NO_3 concentrations in relation to health risks of NO_3 in vegetables. They found that NO_3 concentration in leafy vegetables was higher than root and fruit vegetables and these values were higher in autumn than in spring growing seasons. The results of their

study revealed that cooking reduced NO_3 levels and lowers the health risk of eating raw vegetables. Recently Xu et al. [31] studied the effect of N fertilizer rates on NH_3 oxidizing archaea (AOA) and NH_3 oxidizing bacteria (AOB) community. They found that the major phyla of AOA and AOB were *Thaumarchaeota* and *Proteobacteria*, respectively. They also conducted a correlation analysis between AOA and AOB abundance and found that AOA abundance showed significantly positive correlations with soil pH, and negative correlation with soil NH_4-N, NO_3-N, whereas AOB abundance positively correlated with soil NO_3-N, but negatively correlated with soil pH. Li et al. [32] applied a model to clarify the factors affecting loss of NH_3 and NO_3 from greenhouse vegetables. They found that drip irrigation amplified NH_3 volatilization and reduced NO_3 leaching by 20 kg N ha^{-1} and 75 kg N ha^{-1}, respectively, whereas combining drip irrigation with lessening N application by 50%, significantly decreased greenhouse gas emission without any sacrifice in vegetable yield.

Investigators have found an amplified risk of thyroid cancers with developed NO_3/NO_2 intake [33,34] and high NO_3 absorption is associated with bigger risk of cancers in urinary bladder [35]. High levels of NO_3 may also decrease the nutritional value of consumed vegetables as it affects carotenoid, vitamins A and B degradation [36].

We monitored the concentration of NH_4^+ and NO_3^- in roots and shoots of three field grown varieties of turnips, *Brassica rapa* (Purple Top White Globe PTWG, Scarlet Queen Red SQR, and Tokyo Cross TC) grown under soil mixed with six types of soil amendments mixed and not mixed with biochar. In this study, we found greater NO_3^-level in turnip roots (89.4 μg g^{-1} fresh tissue) compared to the shoot (67.6 μg g^{-1} fresh tissue). On the contrary, NH_4^+ level was greater in the shoot (44.2 fresh tissue) compared to the roots (15 μg g^{-1} fresh tissue), regardless of turnips variety. Several factors, such as consumption of other vegetables and amount consumed per person and per day might contribute to NO_3 toxicity in human diet. Overall, there was a significant increase (73%) in NH_3 concentration in turnips plants (root and shoot) grown in Vermi compost amended with biochar compared to Vermi not amended with biochar. Soil amended with inorganic fertilizer treated with biochar significantly increased the concentration of NO_3 by 35%, compared to biochar control treatment. The observed variability among turnip varieties and soil amendments applied in this investigation might be attributed to variability within turnip varieties and activity of amendments' hydrolyzing enzymes, such as nitrate reductase, urease, as well as the type of fertilizer applied. In fact, consuming turnips is not the only source of NO_3 intake. Other sources of NO_3 such as drinking water and other foodstuff determine the actual health risk associated with NO_3 ingestion.

Our future objectives will focus on monitoring the impact of animal manures on the activity of nitrate reductase (the enzyme that reduce the conversion of NO_3^- to nitrite (NO_2^-) and urease (the enzyme that hydrolyze urea to NH_4^+ and CO_2 in field-grown vegetables and fruit species in relation to the allowable NO_3^- intake. We will also monitor the mobility of NH_4^+ and NO_3^- from animal manures amended soil into runoff and seepage water following natural rainfall events under field conditions that influence the quality of natural water resources.

5. Conclusions

The average content of NO_3 detected in each of the three turnip varieties tested in this investigation (Figure 1) indicated that the concentration of NO_3 in variety SQR is greatest (108 μg g^{-1} fresh root tissue) compared to the PTWG and TC varieties (64 and 62 mg kg^{-1} fresh root tissue, respectively). Therefore, a person with an average weight of 75 kg consuming 100 g of variety SQR would have 10.8 mg NO_3 in his diet and 0.14 mg kg^{-1} NO_3 per BW. These values would be 0.09 and 0.09 mg kg^{-1} BW for consuming turnip varieties PTWG and TC, respectively. Biochar increased the concentration of NH_3 in turnip plants grown vermicompost amended soil by 73% compared to vermicompost not amended with biochar. Other than that, there was no impact of biochar addition on NH_3 concentrations in turnip plants before and after biochar addition, regardless of turnip varieties (Figure 6). Addition of biochar to inorganic fertilizer (InorgBio) significantly

increased NO_3 concentration by 35% compared to unamended native soil treated with biochar (Figure 7).

Based on our investigation, the assigned ADI for NO_3 range of 0–3.7 mg kg^{-1} BW is acceptable and none of the three varieties tested could cause any NO_3 adverse effects on average human consumption. Similarly, consuming turnip shoot (edible greens) grown in any of the animal manures amended soil do not represent any hazardous issues. We concluded that the quantity and quality of elemental fertilizers as well as animal manures applied in agricultural production systems are crucial aspects that regulate the concentration of NH_4^+ and NO_3^- absorbed from soil amendments into edible plants.

The average total intake of NO_3^- level per person in USA ranges between 40–100 mg day^{-1} [9]. In this study, we found that NO_3 contents never exceeded the EU limit concentration of 200 mg kg^{-1} BW. The average total intake of NO_3 per person in Europe ranges between 50 and 140 mg day^{-1} and in the USA about 40–100 mg day^{-1} [9]. According to the European Union Legislation [37] on food contaminants, concentrations of NO_3 in the root and shoot of turnips in each of three varieties tested or among the soil amendments treated with biochar or no-biochar, NO_3 concentrations never exceeded the permitted limits in turnips.

The application of animal manure as organic fertilizer has important properties that cannot be obtained from synthetic inorganic fertilizers. Microorganisms in animal manures facilitate the slow release of the three main plant nutrients, N, P, and K from soil organic matter, reducing their offsite mobility to natural water resources and eutrophication. The literature review revealed a lack of information regarding the impact of organic and inorganic amendments on NO_3 concentrations in vegetable species and varieties within species. Investigators have focused on the plant yield and soil physical and chemical characteristics following the incorporation of fertilizers with very little information on the plant internal composition. We provided evidence of the low impact of animal manures (a great source of N) on NO_3 levels in three varieties of turnips that reduce or eliminate the danger of NO_3 accumulation in fresh turnips roots and shoots.

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