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# Soil Amendments Enhanced Summer Squash Yield, Fruit Composition, Quality, and Soil Enzymes Activity

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**Submitted:** 08 March 2022

**Accepted:** 01 April 2022

**Published:** 04 April 2022

**ISSN:** 2333-7141



**Keywords**

- Sewage sludge; Horse manure; Chicken manure; Vitamin C, Urease, Invertase

**Abstract**

Summer squash, *Cucurbita pepo* was field grown under fourteen soil treatments: Sewage Sludge (SS); Horse Manure (HM); Chicken Manure (CM); vermicompost; inorganic fertilizer (Inorg); commercial organic fertilizer (Org); and no-mulch (NM) control treatment. Soil treatments were also mixed with biochar to make a total of 14 treatments to assess the impact on 1) squash fruit yield and quality, 2) fruit vitamin C, total phenols, and soluble sugars content, and 3) soil microbial activity expressed as urease and invertase secretions. Results revealed that SS treatments increased squash yield and fruit number by 114 and 116 %, respectively compared to NM control treatment. Fruits of plants grown in Inorg mixed with biochar (InorgBio) increased fruits vitamin C, total phenols, and soluble sugars by 73%, 52%, and 7%, respectively compared to Inorg with no-biochar treatment. However, biochar was not consistent in increasing soil urease and invertase activities. The use of animal manure is an affordable way to reduce dependence on mineral fertilizers. Results revealed that the addition of biochar to Org fertilizer increased squash fruit weight and numbers of fruits compared to Org not treated with biochar. No single amendment increased all fruit composition and soil urease and invertase activities.

## INTRODUCTION

Currently, the world generates 1.3 billion tons year$^{-1}$ of municipal sewage sludge (SS), a by-product of sewage treatment plants, also known as biosolids. By 2025, the world could generate 2.2 billion tons of biosolids per year [1]. The use of SS and other animal manures as organic amendments in agricultural production systems would reduce the need of synthetic fertilizers and improve soil nutrient status at affordable or no cost to limited-resource farmers. SS compost promotes soil health and microbiological activity [2]. Manures intensify soil organic matter, improve soil physical structure, enhance soil fungal and bacterial activity, reduce eutrophication(excess N and P in natural water resources), provide low-cost adsorbents that bind with agricultural contaminants and prevent natural water contamination by pesticide residues and inorganic fertilizers, reducing the impact of agricultural chemicals on natural surface and ground water quality [3].

Microorganisms in animal manures breakdown complex forms of organic nutrients and facilitate the slow release of N, P, and K from soil organic matter for plants uptake. In addition, soil enzymes, such as urease, invertase, dehydrogenase, cellulase, amylase, and phosphatase secreted by microorganisms (Bacteria, fungi, protozoa, and algae) in animal manure are primary means of mineralization and are sensitive indicators of soil health [4]. The literature review on the use of organic amendments in agriculture has been clearly demonstrated [5]. Animal manures contain humus substances, macro- and micro-nutrients important for plant growth. The use of SS as a soil conditioner to enhance soil physical, chemical, and microbial conditions might also enhance soil bioremediation [6]. Agricultural use of SS has been successful in production of vegetables [7, 8]. SS improves soil physical properties, nutrient and water holding capacity, total pore space, aggregate stability, soil erosion, and decreases soil density, and increases soil organic matter, such as humic acid and fulvic acid [9-11] that improve soil aeration and moisture retention. Chicken manure (CM) also enhance soil biological activity and fertility, nutrients status and growth of several groups of microorganisms, such as bacteria, fungi, and actinomycetes [12]. CM contains several essential plant nutrients (N, P, K, S, Ca, Mg, B, Cu, Fe, Mn, Mo, and Zn), and has been documented as an excellent fertilizer [13]. Because of the rapid growth in poultry industry, CM has become available in increasing quantities. Compared to other livestock species, poultry has relatively high dietary requirements for the sulfuric amino acids (methionine and cysteine). Commercial diets for laying hens, broilers and turkeys are usually supplemented with synthetic methionine. Consequently, the sulfur content tends to be higher in poultry manure than in manures from other farm animals. Investigators reported that the concentration of dipropyl disulfide and dipropyl trisulfide that are useful in preventing cardiovascular diseases were greatest in onion plants grown in soil amended with CM [14].

The use of Horse Manure (HM) as organic fertilizer revealed that a ton of HM contains 11N: 2P: 8K [15]. HM nutrient value is relatively small and depends largely on the type of bedding material, the food source and type, age and condition of the animal. Vermicompost (Vermi) is a product of the interaction of earthworms (*Eisenia foetida*) with microorganisms and other fauna within a decomposer, designed for earthworm incubation. The NPK essential plant nutrients and C/N ratio of Vermi revealed its agronomic value as organic soil conditioner. Researchers found that Vermi used in crop production as organic amendment promotes plant nutrient availability. According to Ramnarain et al. [16], Vermi can be of significant value to farmers as replacement of inorganic fertilizers that secure better prices of organic produce.

Studies on biochar have indicated that biochar (product of waste incineration) could increase plant nutrient availability, soil Cation Exchange Capacity (CEC), soil organic matter, and soil microbial activities [17, 18] and crop yields [19]. There is a lack of information on the impact of organic amendments on plants nutritional and antioxidant properties. Studies had focused on crop yield and soil physical and chemical properties after the addition of animal manures and other organic amendments with very little information on the nutritional and antioxidant contents of edible plants. Several vegetable species and varieties of the same species have not been completely analyzed for vitamin C and phenolic contents, which have a number of human health benefits. Plant phenols may interfere with stages of the cancer process resulting in a reduction of cancer risk. Phenols prevent oxidative damage to biomolecules, such as DNA, lipids and proteins that play a role in chronic diseases such as cancer and cardiovascular diseases [20]. The role of phenols as antioxidants with properties like vitamins C, E, and ß-carotene have prompted several studies of these phytochemicals.

Shi [21] reported the agricultural and ecological impact of soil enzymes secreted by microorganisms in animal manures on nutrient recycling. Urease (urea amidohydrolase, EC 3.5.1.5) hydrolyzes urea fertilizers into $NH_3$ and $CO_2$, which are associated with rise in soil pH [22], resulting in a rapid N loss to the atmosphere due to $NH_3$ volatilization [23]. Soil enzymes, such as urease and invertase are important for breaking down complex forms of organic matter and release of C and N sources for the growth and multiplication of soil microorganisms. Urease activity in soil has received great attention, due to its vital role in the regulation of N supply to plants after urea fertilization. Soil urease originates mainly from plants [24] and microorganisms [25]. Invertase (β-d-fructofuranosidase) is the enzyme that splits sucrose into glucose and fructose and is available in microorganisms, animals, and plants [26]. Invertase hydrolysis occurs in both acidic and alkaline conditions [27].

Summer squash (*Cucurbita pepo*, variety Raven) is among the most widely grown cucurbits worldwide, it is a seasonal crop that contains beneficial minerals, carotenoids, vitamin C, phenolic compounds [28] that has antioxidant/antiradical, anticarcinogenic, anti-inflammatory, antiviral, and antimicrobial activities [29]. Accordingly, the present objectives were to investigate the impact of soil mixed with animal manures (SS, CM. HM, Vermi), organic and inorganic commercial fertilizers, with and without biochar on: 1) summer squash fruit yield and quality. 2) fruit vitamin C, total phenols, and soluble sugars content. 3) soil urease and invertase activity after the incorporation of soil amendments to native soil, and 4) to answer Kentucky farmers questions, if animal manure could reduce dependence on the costly inorganic mineral fertilizers, increase squash yield, and improve soil quality, and fruit nutritional composition.

## MATERIALS AND METHODS

### Field Study

A field experiment at the University of Kentucky Horticulture Research Farm (Lexington, KY, USA) was established in a Randomized Complete Block Design (RCBD). Each plot was 1.2 m × 3 m (3.6 $m^2$) and the entire study area contained 42 plots (3 replicates × 14 treatments). Seven soil treatments were investigated: 1) sewage sludge (SS), 2) horse manure (HM), 3) chicken manure (CM), 4) vermicompost (Vermi; worm casting), 5) organic (Org) commercial fertilizer (Nature Safe 10:2:8), 6) inorganic (Inorg) commercial fertilizer (Southern State 20:20:20), and 7) control (no-mulch NM untreated soil). The soil in each of the seven treatments also was mixed with 10% (w/w) biochar obtained from Wakefield Agricultural Carbon (Columbia, MO) to make total of 14 treatments. Properties of biochar used in this investigation are: surface area 366 $m^2g^{-1}$ dry, bulk density 480.6 kg $m^{-3}$, total organic carbon 88%, 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 $kg^{-1}$, Zn 2.09 mg $kg^{-1}$, 54% moisture, preparation temperature 200°C, total inorganic carbon 0.34%, particle size (< 0.5 mm), and pH 7.4. The control (no-mulch NM untreated soil) is a Bluegrass-Maury Silty Loam that contains 2.2% organic matter and pH of 6.2 located at the blue grass region (Fayette County, KY) that has 56, 38, and 6% silt, clay, and sand, respectively.

Each soil amendment was applied at the rate of 5% N on dry weight basis to eliminate variations among soil treatments due to N content. SS from the Metropolitan Sewer District, Louisville, KY applied at 0.83 kg $plot^{-1}$. CM (1.1 % N) from the Department of Animal and Food Sciences, University of Kentucky, Lexington, Kentucky applied at 3.78 kg plot $^{-1}$, HM (0.7 % N) from the Kentucky horse park, Lexington, Kentucky and applied at 5.94 kg plot $^{-1}$. Vermi (1.5 % N, worm castings) purchased from Worm Power (Montpelier, Vermont, USA) and applied at 2.78 kg plot $^{-1}$. Organic (Nature Safe 10N:2P:8K) and inorganic (20N:20P:20K) commercial fertilizers (10% and 20% N, respectively), were obtained from the Southern States Cooperative Stores (Lexington, KY, USA) and used at 0.45 and 0.23 kg plot $^{-1}$, respectively. Each soil amendment was mixed with native soil to a depth of 15 cm (area of increased soil microbial activity). Seedlings of Summer squash (*Cucurbita pepo*, cultivar Raven) of 25 d old were planted in raised black plastic mulch (Figure 1) of freshly tilled soil of 42 plots (14 treatments × 3 replicates each) and watered using a drip irrigation system. All agricultural operations were implemented regularly as needed. The plants were sprayed with the insecticide esfenvalerate (Asana XL) and fungicide chlorothalonil (Bravo) three times during the growing season at the recommended rates of application and the plants were grown according to Kentucky Vegetable Production Guide [30], but no other fertilizers were applied.

**Figure 1** Laying black plastic mulch and growing summer squash (zucchini), *Cucurbita pepo* variety Raven at the University of Kentucky Horticulture Research Farm (Fayette County, KY).

## Squash Yield and Fruit Quality Characteristics

At harvest (50 d after planting) and before bolting (flowering) when fruit skin had a glossy appearance, fruits were collected, counted, weighed, and graded into U.S. No.1, U.S. No. 2, and unclassified according to the USDA Standards for Grades of Summer Squash (2016) [31]. U.S. No. 1 consists of squash fruits with stems or portions of stems attached, fairly young, tender and well formed, firm, free from decay and breakdown, free from damage caused by discoloration, cuts, bruises, and scars (wounds), freezing, dirt, disease, insects, mechanical or other means. U.S.

No. 2 consists of fruits which are not old and tough, but firm, free from decay and breakdown, free from damage caused by freezing, discoloration, cuts, bruises, scars, dirt, disease, insects, mechanical or other means. Accordingly, squash pickers should use plastic containers and wear soft gloves to avoid fruit bruises, scratches, and fingernail punctures. Ungraded soft-shell squash fruits are considered "unclassified".

## Quantification of Total Phenols, Vitamin C, and Soluble Sugars

Fruits were cleaned with tape water, cut into small cubes, and a representative 20 g samples from each plot were blended with 150 mL of ethanol to extract phenols. The homogenates were filtered through Whatman No. 1 filter paper and 1 mL aliquots of filtrate were used for determination of total phenols colorimetrically using the Folin-Ciocalteu method [32] using a standard calibration curve of 10- 80 µg $mL^{-1}$ of chlorogenic acid (Fisher Scientific Company, Pittsburg, PA, USA). Ascorbic acid (vitamin C) was extracted by blending representative 20 g of fruits with 100 mL of 0.4 % (w/v) oxalic acid solution [33] and determined colorimetrically using the potassium ferricyanide method [34] and a standard curve of ascorbic acid in the rage of 90-300 µg $mL^{-1}$. Soluble sugars in 20 g fruits were extracted with 80% ethanol and quantified using a calibration curve in the range of 100-800 µg $mL^{-1}$ of glucose [35]. Concentrations of ascorbic acid, total phenols, and soluble sugars in squash fruit extracts were calculated using linear regression equations (y = a + b ×) established for each parameter, where y is the absorbance of the color formed, x is the concentration (ppm), a is the intercept of the regression line, and b is the slope of the line.

## Collection and Preparation of Soil Samples

Soil samples (n=3) were collected from the rhizosphere (a zone where soil and plant root make contact) of growing squash plants to a depth of 15 cm (a zone of increased microbial and enzyme activity). Soil samples collected using a core sampler (Clements Associates, Newton, IA) equipped with plastic liner tubes of 2.5 cm i.d. were air-dried at room temperature, passed through a 2 mm sieve, and kept in plastic bags at 4 °C up to 24 h before use.

## Soil Enzymes Analysis

For determination of soil urease activity, five-g of soil collected from each treatment and 10 mL of 0.1 M phosphate buffer (pH 6.7) in 50 mL volumetric flasks were kept in an incubator at 37°C for 24 h, and the procedure was completed as described by Tabatabi and Bremner [36]. The method was developed by measuring the concentrations of $NH_4^+$ ions released in the soil solutions by the selective electrode method [37]. A series of standard solutions of $NH_4Cl$ covering the concentrations of 0.1-100 µg $NH_4$-N $mL^{-1}$ of water was used for calibration. Urease activity was expressed as µg $NH_4$-N released $g^{-1}$ dry soil during the incubation time.

Invertase activity in soil was measured by the method described by Balasubramanian et al. [38]. A standard calibration curve was obtained with each group of samples using analytical grade glucose in the range of 10-50 µg $mL^{-1}$ glucose (Sigma Chemical Company, St. Louis, MO, USA).

## Statistical Analysis

Data containing squash yield, fruit numbers and quality, fruit composition (phenols, vitamin C, and soluble sugars), soil urease, and invertase activity were statistically analyzed using analysis of variance (ANOVA) and the means were compared using Duncan's multiple range test [39].

# RESULTS AND DISCUSSION

Average weight of squash fruits of plants grown in soil amended with municipal SS, Inorg, Vermi, CM, HM were significantly ($P \leq 0.05$) greater than weight of fruits obtained from plants grown in Org and NM control treatment. Biochar added to SS (SSBio), Vermi (VermiBio), Inorg (InorgBio), CM (CMBio), HM (HMBio) did not impact squash fruit weight. Whereas, Biochar added to Org (OrgBio) and NM (NMBio) significantly increased squash yield (Figure 2A). Average number of squash fruits collected from plants grown in soil amended with SS, Inorg, Vermi, and CM were greater compared to fruits of plants grown in NM control plots (5.5 fruits plan $^{-1}$) (Figure 2B).

Regarding fruit quality, Table 1 revealed that SS increased the number of U.S. No. 1 fruits compared to Org and NM control treatments. Biochar added to SS (SSBio) treatments did not significantly increase number of U.S. No.1 and U.S. No.2 fruits

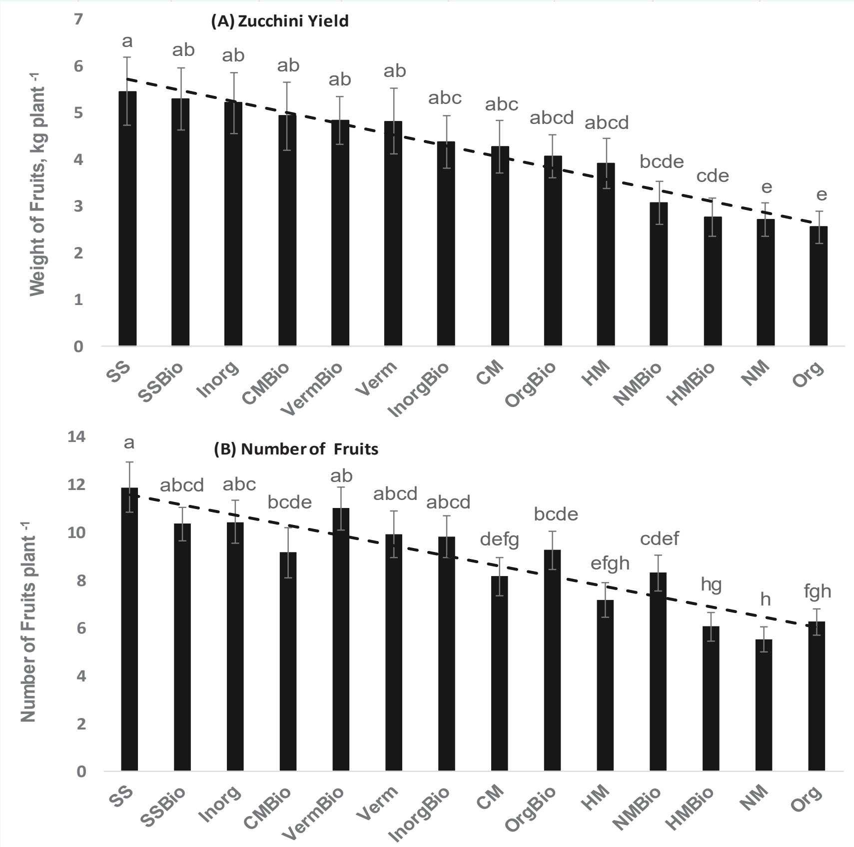


**Figure 2** Yield of summer squash (zucchini) fruits expressed as weight of fruits (A) and number of fruits (B) of plants grown under fourteen soil management practices: sewage sludge (SS), SS mixed with biochar (SSBio), inorganic commercial fertilizer (Inorg), chicken manure mixed with biochar (CMBio), vermicompost mixed with biochar (VermiBio), vermicompost (Vermi), inorganic commercial fertilizer mixed with biochar (InorgBio), chicken manure (CM), organic commercial fertilizer mixed with biochar (OrgBio), horse manure (HM), no-mulch native soil mixed with biochar (NMBio), horse manure mixed with biochar (HMBio), no-mulch soil (NM), and organic commercial fertilizer (Org). Statistical comparisons were carried out among fourteen soil management practices. Bars accompanied by the same letter(s) are not significantly different (P> 0.05) using Duncan's multiple range test.

**Table 1:**

| Soil Amendment | Number of U.S. No. 1 Plant$^{-1}$ | Number of U.S. No. 2 Plant$^{-1}$ | Number of Unclassified Plant$^{-1}$ |
|---|---|---|---|
| SS | 4.48 ± 0.66 a | 6.81 ± 1.27 ab | 0.57 ± 0.21 a |
| CM | 3.52 ± 0.58 ab | 4.43 ± 0.36 ab | 0.19 ± 0.11 a |
| HM | 3.33 ± 0.52 abc | 3.38 ± 0.51 b | 0.43 ± 0.16 a |
| Vermi | 3.62 ± 0.49 ab | 5.76 ± 0.73 a | 0.53 ± 0.22 a |
| Organic | 2.09 ± 0.36 d | 3.86 ± 0.39 b | 0.29 ± 0.12 a |
| Inorganic | 4.05 ± 0.53 a | 6.0 ± 0.86 ab | 0.38 ± 0.17 a |
| No-Mulch | 2.10 ± 0.38 d | 3.1 ± 0.29 b | 0.3 ± 0.16 a |
| SSBio | 4.19 ± 0.51 a | 5.76 ± 0.55 ab | 0.38 ± 0.12 a |
| CMBio | 3.43 ± 0.53 ab | 5.28 ± 0.25 ab | 0.43 ± 0.11 a |
| HMBio | 2.19 ± 0.28 cd | 3.62 ± 0.36 ab | 0.24 ± 0.11 a |
| VermiBio | 4.19 ± 0.67 a | 6.21 ± 0.59 ab | 0.62 ± 0.22 a |
| OrgBio | 3.43 ± 0.23 ab | 5.57 ± 0.50 ab | 0.24 ± 0.09 a |
| InorgBio | 3.57 ± 0.49 ab | 5.75 ± 0.28 ab | 0.27 ± 0.17 a |
| No-Mulch Bio | 2.62 ± 0.44 abc | 5.04 ± 0.33 ab | 0.62 ± 0.22 a |

SS= sewage sludge, CM= chicken manure HM- horse manure, Vermi= vermicompost, Organic= commercial organic fertilizer, Inorganic=inorganic commercial ertilizer, No-Mulch= no-mulch native soil, SSBio= ss mixed with biochar, CMBio= chicken manure mixed with biochar, HMBio= horse manure mixed with biochar, VermiBio=vermicompost mixed with biochar, OrgBio= organic commercial fertilizer mixed with biochar, InorgBio= inorganic commercial fertilizer mixed with biochar, and No-Mulch Bio=no-mulch native soil mixed with biochar. Each value in the table is an average of 21 replicates ± standard deviation.
Values in each column accompanied by the same letter(s) are not significantly different (P> 0.05) using Duncan's multiple range test (SAS Institute 2016.

compared to SS treatment not amended with biochar, whereas biochar added to each of the soil amendments did not affect number of unclassified fruits. Plants grown in soil amended with Vermi had greater number of U.S. No. 2 fruits compared to plants grown in HM, Org, and NM treatments. Other than that, no significant differences ($P \geq 0.05$) were found in U.S. No. 2 fruit grades among soil amendments tested. Overall, the number of unclassified fruits plant $^{-1}$ were not significantly different among soil treatments. Accordingly, other than some impact on U.S. No. 1 fruits, soil amendments impacted squash yield, but did not impact fruit grades.

Regarding fruit composition, it is recognized that that diet constituents may contain cancer-causing substances as well as many cancer-preventive agents [40]. Antioxidants, such as vitamin C and phenols in plants tend to give their electrons to free radicals to neutralize them, preventing the cells from potential damage, which in turn cure numerous human diseases. Squash fruits of plants grown in Inorg fertilizer mixed with biochar (InorgBio) as well as organic fertilizer (Org) exhibited a higher concentration of vitamin C (ascorbic acid) by 11 and 12% compared to plants grown in NM treatments (Figure 3A). InorgBio increased vitamin C in squash fruits by 73% compared to squash fruits of plants grown in soil amended with Inorg commercial fertilizer (Inorg) not mixed with biochat, indicating the role of biochar in elevating the concentration of vitamin C. On the contrary, biochar added to SS (SSBio) reduced vitamin C concentration by 22% compared to SS with no biochar addition. In addition, plants grown in soil amended with Inorg fertilizer mixed with biochar (InorgBio) increased total phenols in squash fruits from 118 to 179 μg g $^{-1}$ fresh fruits indicating 52% increase due to biochar addition to Inorg fertilizer (Figure 3B). Figure 4B also revealed that plants grown in CMBio, CM, and InorgBio significantly increased total phenols compare to all other treatments tested, whereas plants grown in soil amended with SS did not increase total phenols content in fruits of plants grown in NM treatment. Figure 4 revealed that plants grown in SS amended with biochar (SSBio) increased soluble sugars in squash fruits compared to all other 13 treatments tested, whereas addition of biochar to Vermi, CM, and Inorg (VermiBio, CMBio, and InorgBio, respectively) did not increase soluble sugars concentrations after biochar addition.

Vitamin C and total phenols have antioxidant properties and are thus important quality attributes in edible plants. Concentrations of these two phytochemicals in squash fruits varied significantly among soil treatments. One can investigate whether the higher content of vitamin C, phenols, and soluble sugars in some treatments is due to higher synthesis of these water-soluble compounds by squash plants, or due to increased absorption from soil by the plant roots. Alternatively, these

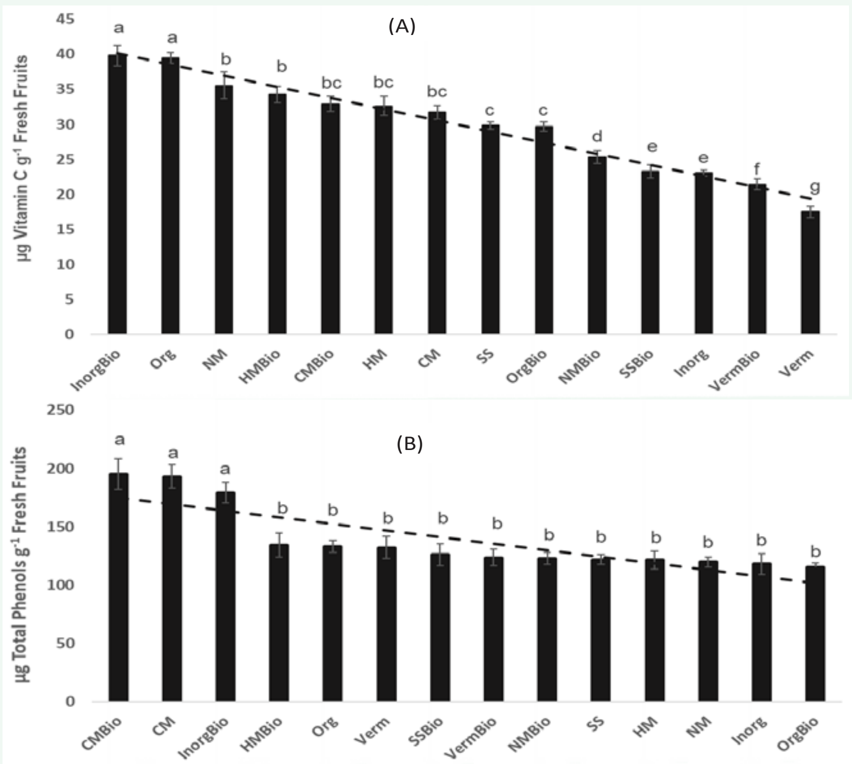


**Figure 3** Average concentrations of ascorbic acid (A) and total phenols (B) ± std. error in fresh summer squash (zucchini) fruits of plants grown under fourteen soil management practices: inorganic commercial fertilizer mixed with biochar (InorgBio), organic commercial fertilizer (Org), no-mulch native soil (NM), horse manure mixed with biochar (HMBio), organic commercial fertilizet (Org), vermicomposrt (Verm), sewage sludged mixed with biochar (SSBio), vermicompost mixed with biochar ((VermiBio), no-mulch native soil mixed with biochar (NMBio), sewage sludge (SS), horse manure (HM), no-mulch native soil (NM), inorganic commercial fertilizer (Inorg), and organic commercial fertilizer mixed with biochar (OrgBio). Statistical comparisons were carried out among soil management practices for each parameter. Bars accompanied by the same letter are not significantly different (P> 0.05) using Duncan's multiple range test.

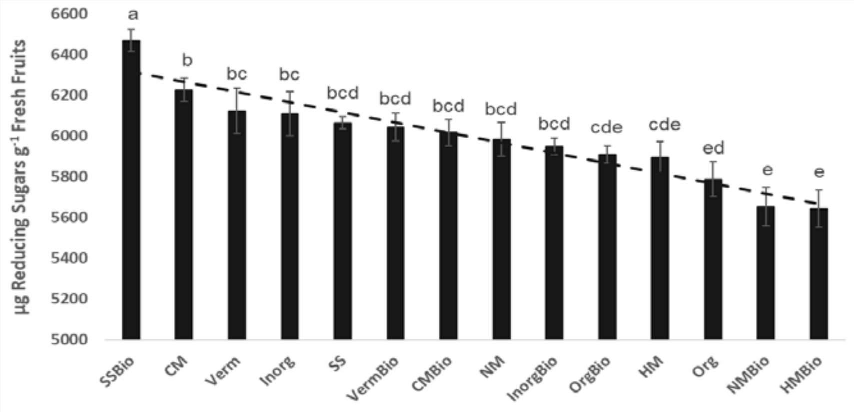


**Figure 4** Average concentrations of soluble sugars ± std. error in fresh summer squash (zucchini) fruits of plants grown under fourteen soil management practices: sewage sludged mixed with biochar (SSBio), chicken manure (CM), vermicomposrt (Verm), inorganic commercial fertilizer (Inorg), sewage sludge (SS), vermicompost mixed with biochar ((VermiBio), chicken manure mixed with biochar (CMBio), no-mulch native soil (NM), inorganic commercial fertilizer mixed with biochar (InorgBio), organic commercial fertilizer mixed with biochar (OrgBio), horse manure (HM), organic commercial fertilizet (Org), no-mulch native soil mixed with biochar (NMBio), and horse manure mixed with biochar (HMBio). Statistical comparisons were carried out among soil management practices. Bars accompanied by the same letter(s) are not significantly different (P> 0.05) using Duncan's multiple range test.

elevated concentrations in the fruits of certain some treatments might be due to increased soil organic matter and microbial activity after addition of certain soil amendments. Based on the results in Figures 3A, plants grown in NM native soil (control plants) contained low concentrations of vitamin C compared to InorgBio and organic commercial fertilizer (Org) treatments. Total phenols concentrations in fruits of plants grown in NM native soil were also lower than fruits of plants grown in CM, CMBio, and InorgBio indicating the role of these treatments in promoting the concentrations of phenols (Figure 3B). Concentration of soluble sugars were also greater in SS mixed with biochar (SSBio) compared to fruits of plants grown in other treatments including the NM control (Figure 4). Animal manures (SS, CM, HM, and Vermi) contain several enzyme substrates, such as urea, sucrose, and orthophosphates and enzymes that secrete soil urease, invertase, and phosphatase, respectively allowing the breakdown of complex forms of organic materials in soil and release nutrients for plant uptake.

Accordingly, the pronounced increase in soil urease and invertase activity (Figure 5) could be attributed to increased microbial activity and the enzymes they produce. Recent studies carried out by Antonious et al. [5] revealed that animal manures increased the activities of soil urease and invertase. Soil enzymes secreted by soil microorganisms also promote soil processes such as synthesis of humus substances and breakdown of organic matter present in animal manure and consequently release soil nutrients through mineralization. CM amended soil increased soil urease activity by 119% compared to NM native soil (Figure 5A). This increase in soil urease revealed the transformation of N in CM mixed soil from urea to ammonium ions ($NH_4^+$). On the contrary, CMBio, VermiBio, OrgBio, MBio, InorgBio, and OrgBio did not increase urease activity, indicating that biochar has no role in increasing soil urease activity in these treatments. Figure 5B also indicated that biochar added to SS (SSBio), Vermi (VermiBio), Organic fertilizer (OrgBio), and Inorganic fertilizer (InorgBio) reduced soil invertase activity.

In fact, some animal manures, such as SS, CM, and HM are associated with inorganic and organic toxic compounds, such as trace metals, hormones, antibiotics and pesticides that when incorporated into soil, can cause a pollution problem and consequently toxic effects to soil microorganisms that control nutrients availability to growing plants. The increased demand for animal protein is leading to a great use of antibiotics in agriculture to raise food-producing animals in intensive production systems. More antibiotics are currently used in poultry, horses, swine, and cattle raising to promote growth and prevent diseases (CDDEP) [41]. According to the Lexington Herald-Leader on November 14, 2012, Kentucky is the highest five states in the USA in overuse of antibiotics (Kentucky Health News [42]. Yang et al. [43] studied the influence of CM fertilization on antibiotic-resistant bacteria in soil and the endophytic bacteria of Pakchoi (a type of Chinese cabbage, *Brassica rapa*). They detected these bacterial populations in CM, CM amended soil, and in harvested vegetables grown in manure-amended soil and concluded that they presented a potential threat to human health.

In addition, a considerable area of agricultural land is contaminated with cadmium (Cd) and lead (Pb) due to land application of fertilizers, animal manures, and atmospheric deposition [44]. Cd has a retention time of 150 years in soil [45]. Pb is also estimated to have a long soil retention time [46]. These metals have negative impact of soil hydrolyzing enzymes that breakdown complex forms of organic matter and release of plant nutrients. Accordingly, our results are not surprising since other investigators reported that the increased concentrations of Cd and Pb have negative impacts on soil microbes, and the effect of Cd on soil urease activity is more than that on invertase, while Pb has more effect on invertase activity than Cd [47]. Investigators reported variability of biochar effect on soil enzymes activity that might be due soil type and chemical composition of soil, trace metals in biochar, or variations in biochar properties of absorbing and retaining water molecules that impact microbial activity and enzymes secretions. Park et al. [48] and Kumar et al.

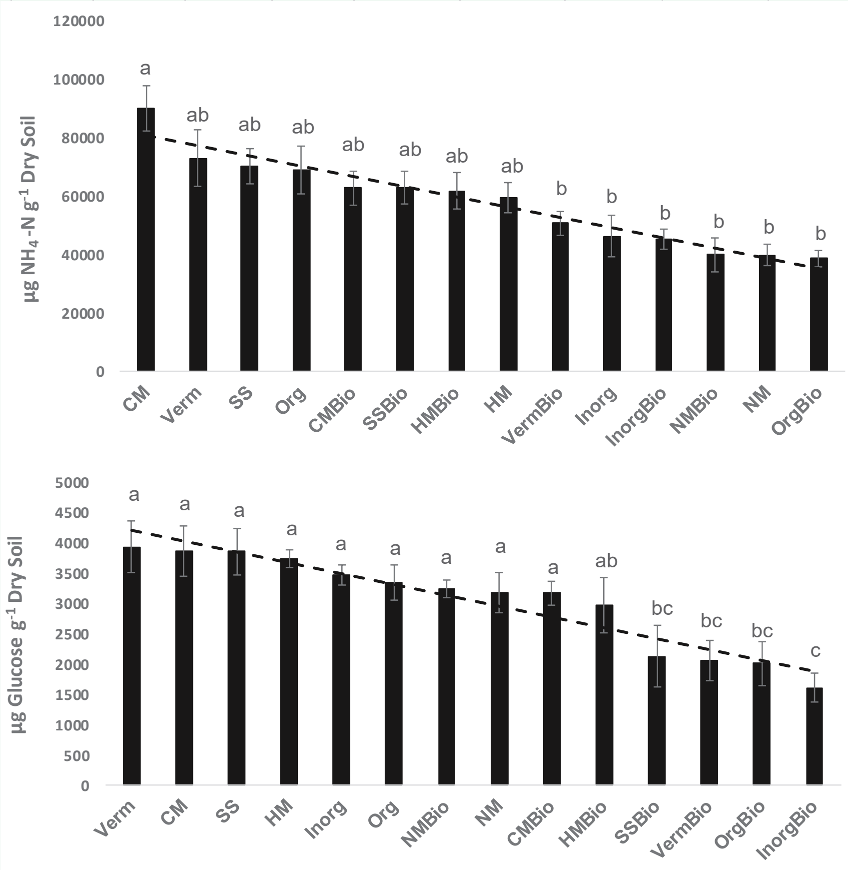


**Figure 5** Urease activity ± std. error expressed as µg NH4-N released g-1 dry soil (A) and invertase activity ± std. error expressed as µg glucose released g-1 dry soil (B) in soil mixed with chicken manure (CM), vermicomposrt (Verm), sewage sludge (SS), organic commercial fertilizet (Org), chicken manure mixed with biochar (CMBio), sewage sludged mixed with biochar (SSBio), horse manure mixed with biochar (HMBio), horse manure (HM), vermi-compost mixed with biochar ((VermiBio), inorganic commercial fertilizer (Inorg), inorganic commercial fertilizer mixed with biochar (InorgBio), no-mulch native soil mixed with biochar (NMBio), no-mulch soil (NM), and organic commercial fertilizer mixed with biochar (OrgBio). Statistical comparisons were carried out among soil management practices. Bars accompanied by the same letter(s) are not significantly different (P> 0.05) using Duncan's multiple range test.

[49] reported positive effect of biochar on soil enzymes, whereas Lehmann et al. [50] reported negative effects, while Wu et al. and Lammirato et al [51, 52] reported a non-biochar effect on soil enzymes. Accordingly, continuous monitoring of soil enzymes secreted by microorganisms in relation to the antioxidant contents of plants grown in animal manure is recommended when using animal manures for growing edible plants. These results revealed that each soil amendment used in this investigation has a unique impact on increasing each of the fruit quality attributes and no single amendment increased squash yield and all nutrients in squash fruits and/or soil urease and invertase activities. Further work will be continued in our future studies to investigate the potential impact of using mixtures of animal manures incorporation with biochar on elevating the nutritional composition of squash fruits as well as soil enzymatic activities.

## CONCLUSION

The present investigation provided new information on the nutritional value of summer squash fruits, along with some beneficial effects in relation to composting. Municipal SS and CM generation is expected to be available in increasing quantities due to increased municipal SS composting facilities and the rapid growth in the poultry industry. Using animal waste as a low-cost organic fertilizer has a positive effect on the growth and crop yield. SS treatments were superior in increasing squash yield and fruit number by 114 and 116 %, respectively compared to NM control treatments. The presence of organic matter in recycled manure often improves soil physical and chemical properties and promotes soil biological activities. Composts improved summer squash yield. Fruits of plants grown in inorganic fertilizer mixed with biochar (InorgBio) as well as organic fertilizer with no biochar (Org) exhibited higher increase of vitamin C (ascorbic acid) by 11 and 12%, respectively compared to plants grown in NM control treatments. Plants grown in soil amended with Inorg fertilizer mixed with biochar (InorgBio) increased total phenols in squash fruits from 118 to 179 µg $g^{-1}$ fresh fruits indicating 52% increase due to biochar addition to Inorg fertilizer. Soluble sugars in plants grown in soils amended with SS mixed with biochar (SSBio) were significantly increased compared to other soil treatments. CM amended soil increased soil urease activity by 119% compared to NM native soil, whereas the addition of biochar to SS, Vermi, Org, and Inorg reduced soil invertase activity. Organic amendments from animal manure can be used as alternative to inorganic fertilizers. The increase of organic matter in soil after addition of animal manure has a great impact on the biological and biochemical properties of soil. However, soil amendments such as SS could be contaminated with inorganic and organic compounds such as heavy metals, hormones, antibiotics, and pesticides that when incorporated into soil might constitute a pollution problem and therefore impact the activity of soil microorganisms and their enzymatic secretions. On the other hand, increasing costs of commercial fertilizers and release of large amounts of SS, CM, and HM worldwide have made cropland application of this waste an attractive disposal option. Results of this investigation revealed a significant increase in total vitamin C, total phenols in squash fruits of plants grown Inorg fertilizer mixed with biochar (InorgBio).

## AUTHOR CONTRIBUTIONS

G.F.A. designed the field study, implemented the laboratory analysis, and wrote the manuscript. M.H.D. conducted the statistical analysis and field work, E.T.T., and T.G.T. organized the field work and collected the plant and soil samples.

## FUNDING

This investigation was funded by a grant # KYX-10-18-P65 Accession # 1017900 from the United States Department of Agriculture, National Institute of Food and Agriculture (USDA/NIFA) to Kentucky State University.

## ACKNOWLEDGMENTS

The authors thank Steven Diver and his farm crew for preparation of the soil amendments and maintaining the field experimental plots.

## CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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**Cite this article**

*Antonious GF, Dawood MH, Turley ET, Trivette TG (2022) Soil Amendments Enhanced Summer Squash Yield, Fruit Composition, Quality, and Soil Enzymes Activity. JSM Environ Sci Ecol 10(1): 1079.*