Hydrolyzed Organic Fish Fertilizer and Poultry Litter Influence Yield and Rhizosphere Ecology of Sweetpotato

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Additional index words. amendments, carbon, nitrogen, enzyme activity, bacterial ecology

Abstract. Organic fertilization techniques have become an attractive alternative to conventional techniques, but there remains interest in their impact on rhizosphere ecology. This study was aimed at assessing the impacts of various organic fertilizer amendments on storage root yield, chemical, biochemical, and microbial factors in the rhizosphere ecosystem and the bacterial community composition in the rhizosphere ecosystem. Four sweetpotato cultivars (J6/66, NCC-58, TU Purple, and Whatley/Loretan) and four organic fertilizer treatments [poultry litter, Megabloom (fish protein), NPK, and an untreated control] were used in the study. The experiments were conducted as a randomized complete block design with a 4 × 4 factorial treatment arrangement and three replications. Fertilizer treatments were split-applied at the rate of 134–67–67 kg·ha⁻¹ NPK equivalent based on soil test recommendations 1 and 4 weeks after planting as single bands 15 cm from the plants and organic amendments were calculated based on total N content. Rhizosphere soil samples were collected at harvest and analyzed for soil pH, soil organic carbon (SOC), bacterial 16S rDNA, and selected soil enzymes. Organic amendments did not affect storage root yield or percent dry matter but enhanced both the mass and number of US#1 storage roots. Rhizosphere pH varied depending on cultivar and cultivar response varied with pH and ranged from 6.1 to 6.8, whereas SOC was similar regardless of the amendment. The impact of fertilizers was evident as Megabloom (fish protein) treatment suppressed the relative abundance (RA) of nitrifiers (*Nitrosococcus* and *Nitrosomonadaceae*). Also, the rhizosphere of ‘Whatley/Loretan’ seemed to have been a beneficial habitat for populations of common nitrogen-fixing bacteria *Bradyrhizobium elkanii*, and *Rhodospirillaceae* sp. as their RA increased significantly in the rhizosphere. That bacteria associated with carbon and nitrogen cycling under aerobic conditions were found to be ubiquitous in the rhizosphere of sweetpotato, suggesting that certain amendments positively impacted the populations of nitrogen-cycling bacteria, thus making them a viable alternative to NPK when considering increasing or sustaining yield while promoting long-term soil health.

Sweetpotato [*Ipomea batatas* (L.) Lam.] is a tropical root crop that belongs to the Convolvulaceae or morning glory family. It is a creeping dicotyledonous plant and an important crop, widely grown in tropical, subtropical, and warm temperate regions. It ranks the world’s seventh most important crop, with an estimated annual production

of ≈122 million metric tons (Collado et al., 1999; FAO, 2005). Sweetpotato is especially valued because of its agronomic advantages for marginal lands, it is highly adaptable, and tolerates high temperatures, low fertility soil, and drought (FAO, 2010). Sweetpotato is also a nutritious vegetable with a high content of fiber, carbohydrates, and moderate iron (leaves), protein, vitamins A, B6, and C, and calcium, second only to potato (CSPI, 1992). Depending on the variety, sweetpotato has varying amounts of beta-carotene with orange-fleshed types containing more than white or cream-fleshed varieties. Purple-fleshed varieties are high in anthocyanins which have important antioxidant and anti-inflammatory properties, thus its cultivation is encouraged in developing countries where malnutrition associated health issues are high (Mortley et al., 2017; Chang et al., 2010; Choi et al., 2009; Failla et al., 2009).

Several researchers have demonstrated a wide range of effects of fertilizer, including nitrogen on storage root yield of sweetpotatoes. For example, Jet and Mulkey (1996) reported that the applying N at 50.4 kg·ha⁻¹

21 d after transplanting produced greater yields of US#1 storage roots compared with a higher rate. Villordan et al. (2013) reported that lateral root length of storage roots increased substantially as N rates increased from 0 to 50 kg·ha⁻¹, whereas increasing the N rates to either 100 or 200 kg·ha⁻¹ did not result in any further increases in root growth.

Organic amendments were the primary soil amendments until the innovations and techniques of the Green Revolution (Horne, 2008). Inorganic fertilizers began to replace organic fertilizers as producers have relied on them to maintain production demands to keep pace with population growth. In addition, most N-based fertilizers are produced from fossil fuels, which have increased in price exponentially over the past decade (Wen-yuan, 2007). With increasing concerns over fertilizer pricing and reports of negative effects attributed to its use, organic fertilizers and organic practices have received renewed interest.

Organic production in the United States has grown since the inception of national organic production and labeling standards in 2002 (Baker, 2005). Several studies have shown that organic production is comparable to conventional systems in terms of yield; however, organic systems had enhanced microbial biomass and activity, increased soil organic matter, increased nutrient pools, and other soil properties (Delate et al., 2003; Klonsky, 2000). A study comparing compost with or without cover crops and tillage on organic vs. conventional sweetpotato production showed that leaf N, P, and K concentrations were greatest in the compost/cover crop combination compared with the other systems (Treadwell et al., 2008). Other studies found greater P, Mg, and Fe levels in organic systems and those mineral nutrients were on average higher in organically grown crops though results were somewhat inconclusive (Worthington, 2001).

The soil–microbial–plant system holds the capacity to sustain nutrient demands ecologically and efficiently due to the high amounts of nutrients that pass through the microbial biomass (Kennedy, 1995). Soil microorganisms drive most soil processes, such as nutrient cycling, availability, and retention; decomposition of organic materials; and soil organic matter accumulation primarily through the utilization of enzymes (Coleman et al., 2004). Alterations in the physical and chemical nature of soils may lead to shifts in the composition of the microbial community and changes in microbial function. Rhizosphere contains beneficial microorganisms that displace harmful microorganisms through aggressive colonization or through the production of plant growth hormones such as auxins and kiritin (Herman et al., 2008). The rhizosphere has a large impact on plant performance in several ways, as it not only supports the plant in the acquisition of water and nutrients, but also helps modulates the plant’s ability to cope with pathogens as well as supports high diversity of microorganisms. Studies have

Received for publication 8 Nov. 2018. Accepted for publication 6 Feb. 2019.

Contribution of the George Washington Carver Agricultural Experiment Station, Tuskegee University. This research was supported by funds from U.S. Department of Agriculture/National Institute of Food and Agriculture Evans-Allen Grant No. ALX-FV. The authors extend deep gratitude toward all the staff at Tuskegee University George Washington Carver Agricultural Experiment Station.

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shown that different growth stages of plants can influence the rhizosphere microbes, due to changes in rhizodeposition. For example, young roots can supply more energy to soil microorganisms, as they produce more exudates than older roots (Bowen and Rovira, 1976; Hawes et al., 2012; Lynch and Whipps, 1990). Plant growth-promoting rhizobacteria are free-living, soil-borne bacteria that colonize the rhizosphere and, when applied to crops, enhance the growth of plants. Plant growth-promoting rhizobacteria may enhance plant growth either by direct or indirect mechanisms including nitrogen fixation (Vessey, 2003). In the past, understanding the complex diversity of soil microorganisms was limited because only a small portion of soil microbial populations can be cultured and identified using standard approaches. For instance, several studies have used soil enzymes to assess changes in soil, as soil enzymes are an essential part of microbial communities in soil systems and are strongly influenced by changes in microbial community composition (Ndiaye et al., 2000; Sun et al., 2004). The β -glucosidase (β -GLU) and N-acetyl- β -glucosaminidase (β -NAG), and phosphatase enzymes [acid phosphatase (ACP) and alkaline phosphatase (ALKP)] were used for this study because they are known to be sensitive to soil management and have been proposed as soil quality indicators because their ability to provide early indications of changes in organic matter status (Ekenler and Tabatabai, 2003; Moore-Kucera and Dick, 2008). However, use of high-throughput sequencing techniques, such as 454 pyrosequencing of 16S ribosomal RNA (rRNA) gene has allowed for the exploration of microbial communities' structure and taxonomic composition at greater depth and increased the current knowledge of soil microbial communities (Bruinsma et al., 2003; Lauber et al., 2009; Shange et al., 2012). Pyrosequencing has also been shown to detect changes in microbial communities due to organic and conventional farming and increasing current knowledge about the influence of organic amendments has on soil microbial communities (Chaudhry et al., 2012; Leff and Fierer, 2013; Li et al., 2012; Sugiyama et al., 2010; Widmer et al., 2006).

The objectives of the study were to assess the impacts of various organic fertilizer amendments on storage root yield, and chemical and biochemical factors of the rhizosphere ecosystem, and the bacterial community composition in the rhizosphere ecosystem.

Materials and Methods

The study was conducted as a randomized complete block design with a 4×4 factorial treatment arrangement and three replications. The treatment combinations were conventional NPK fertilizer, poultry litter, Megabloom (hydrolyzed fish protein fertilizer), and an untreated check. The sweetpotato cultivars were J6/66, NCC-58, TU Purple, and Whatley-Loretan. The soil type at the study site was Norfolk sandy loam (fine, siliceous, thermic Typic, Paleudults) with a

pH of ≈ 5.9 and organic matter content of $<1\%$. The field was prepared conventionally and soil samples were collected for elemental analysis at 15-cm depths. The cores were composited and analyzed by the Plant and Soil Testing Laboratory at Auburn University for mineral constituents (Ca, Mg, P, K, pH).

Treatment calculations and planting. Treatments were split-applied at the rate of $134\text{--}67\text{--}67\text{ kg}\cdot\text{ha}^{-1}$ NPK equivalent based on soil test recommendations 1 and 4 weeks after planting as single bands 15 cm from the plants and organic amendments were calculated based on total N content. Poultry litter, unlike commercial fertilizers, is quite variable, and according to Fulhage and Pfof (1994) can vary up to 50% based on animal sources. Available values of litter nutrients using data from Fulhage and Pfof (1994) were total N of $27\text{ kg}\cdot\text{ton}^{-1}$, composed of $24\text{ kg}\cdot\text{ton}^{-1}$ organic N and $3\text{ kg}\cdot\text{ton}^{-1}$ NH_4N , $29.5\text{ kg}\cdot\text{ton}^{-1}$ P_2O_5 , and $19\text{ kg}\cdot\text{ton}^{-1}$ K_2O . In addition, the amount of organic N available was based on days from collection to incorporation, which is 20% beyond 7 days. Calculations were based on the following equation:

$$\frac{\text{crop N} - \text{residual N}}{\text{available NH}_4\text{-N} + \text{available organic N}}$$

Ten stem cuttings of each sweetpotato cultivar were transplanted into 3-row plots $1.2\text{ m} \times 3\text{ m}$ with an in-row spacing of 0.3 m and plants drip irrigated as needed.

Harvest and rhizosphere soil analysis. Plants were harvested from the middle row of each plot by cutting the vines at ground level and fresh weights of foliage and storage roots were recorded. Subsamples of foliage and storage roots (US#1 grade) were dried for 72 h at 70°C . This dry weight information was used to calculate a fresh-to-dry weight conversion factor to determine storage root dry weight. Percent dry matter (DM) [(dry weight/fresh weight) $\times 100$] was calculated for storage roots.

Triplicate rhizosphere soil samples from each plot were taken at harvest, dried and analyzed for pH, soil organic carbon (SOC), and enzyme activity. Soil pH was determined using 1:2.5 soil/water and SOC using the wet oxidation method (Walkley and Black, 1934). Phosphomonoesterase activity was measured by the method of Tabatabai and Dick (2002); β -GLU and β -NAG activity by referenced assays (Eivazi and Tabatabai, 1977; Parham and Deng, 2000).

Whole DNA was extracted from $\approx 0.25\text{ g}$ of soil (oven dry basis of field-moist soil) using the Power Soil Extraction Kit (MO BIO Laboratories, Soloana Beach, CA). Extracted DNA ($2\text{ }\mu\text{L}$) was quantified using Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE). Following extraction and quantification, the samples were then submitted to MRDNA Laboratories (Shallowater, TX) for polymerase chain reaction (PCR) optimization and sequence analysis. The 16S rRNA gene V4 variable region PCR primers 515/806 were used in a

single-step 30 cycle PCR using the HotStar-Taq Plus Master Mix Kit (Qiagen, Valencia, CA). Sequencing was on an Ion Torrent PGM (Roche, Brandford, CT) following the manufacturer's guidelines. The resulting sequence data were processed using an analysis pipeline developed at MR DNA Laboratories [e.g., the removal of barcodes and primers, sequences <150 base pairs (bp) removed, sequences with ambiguous base calls and with homopolymer greater than 6 bp]. Sequences were denoised, operational taxonomic units (OTUs) generated and chimeras removed. OTUs were defined by clustering at 3% divergence (97% similarity). Final OTUs were taxonomically classified using BLASTn against a database derived from RDP II (<http://rdp.cmc.msu.edu>) and National Center for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov). Samples are being submitted to the NCBI Sequence Read Archive. OTUs were then compiled into every taxonomic level into count files that actual number of sequences for each taxonomic classification, and the percentage files that contained the proportion percentage of sequences at each taxonomic classification for each sample.

All data were subjected to analysis of variance (ANOVA) using the general linear model procedure (SAS Institute, 2009). Treatment and cultivar means were separated by Tukey-Kramer's test at the 0.05 level of probability.

Results and Discussion

Biomass production. The main effect of organic amendments on storage root yield and percent DM (Table 1) show that organic amendments had no significant impact on total storage root yield or DM, but did influence the yield of US#1 storage roots. The yield of US#1 storage roots was highest for plants in which poultry litter (PL) was applied relative to that of control plants but not different from plants receiving Megabloom (fish hydrolysate) or NPK. However, plants receiving PL or Megabloom produced a greater number of US#1 storage roots compared with NPK or the control plants. Similarly, cultivar had no significant influence on total storage root yield or on the number of US#1 storage roots produced (Table 2), although 'Whatley/Loretan' produced the highest yield of $26\text{ t}\cdot\text{ha}^{-1}$. Organic amendments had no significant impact on DM (Table 1) and the major influence was based on variety (Table 2), with 'TU Purple' having the highest DM. These DM levels are consistent with these respective varieties when grown in the field.

A contrast of fertilized vs. nonfertilized plants show that on a whole, plants that received nutrient amendments produced greater yields and numbers of US#1 storage roots only while a contrast of organic vs. inorganic showed that organic amendments enhanced yield of US#1 storage roots. These results show that organic amendments exerted greater impact on yield of US#1 storage roots

Table 1. The main effect of organic amendment on total storage and US#1 root yield, number of US#1 and percent dry matter (DM) of four sweetpotato cultivars.

Treatment	Storage root yield			
	Total	US#1	US#1 (no./plot)	DM (%)
	(t·ha ⁻¹)			
Control	12.0 a	7.9 b	7.7 c	28.3 a
Poultry litter	19.7 a	14.2 a	13.3 a	26.4 a
Megabloom	18.1 a	9.9 ab	12.9 a	27.5 a
NPK	21.2 a	10.6 a	10.6 b	27.9 a
Significance ^z	NS ^x	0.001	0.001	NS
Contrast				
Fertilizer vs. nonfertilized	NS	0.002	0.001	0.10
Organic vs. inorganic	NS	0.01	0.07	NS
BL vs. Fish	NS	NS	NS	NS

^zMeans within columns accompanied by the same letter are not significantly different according to Tukey-Kramer's test at 0.05 level of probability.

NS = nonsignificant.

Table 2. The main effects of organic amendment on total storage and US#1 root yield, number of US#1 and percent dry matter (DM) of four sweetpotato cultivars.

Cultivar	Storage root yield			
	Total	US#1	US#1 (no./plot)	DM (%)
	(t·ha ⁻¹)			
TU Purple ^c	17.3 a	14.2 a	11.0 a	32.3 a
J6/66	12.5 a	9.0 b	10.2 a	30.4 b
NCC-58	14.9 a	11.6 ab	11.3 a	23.8 c
Whatley/Loretan	26.3 a	10.4 ab	11.1 a	22.7 c
Significance ^z	NS	0.03	NS	0.0001
Contrast				
Fertilizer vs. nonfertilized	NS	0.002	0.001	0.10
Organic vs. inorganic	NS	0.01	0.07	NS
Poultry Litter vs. Megabloom	NS	NS	NS	NS

^zMeans within columns accompanied by the same letter are not significantly different according to Tukey-Kramer's test at 0.05 level of probability.

NS = nonsignificant.

and number but did not influence total yield or storage root DM.

Nutrients in organic fertilizer are released through mineralization by the activity of soil microorganisms. Depending on existing soil conditions including pH and moisture status, mineralization rates can be impacted, and it is probable that the lack of total yield response to organic amendments in this study could be related in part to slower mineralization rates resulting in fewer nutrients available for plant uptake (Boyhan et al., 2010). As mineralization rates of PL have been reported as slow (Whitmore, 2007), researchers in the region have suggested applying organic fertilizer 14 to 20 d earlier than normal to compensate for slow mineralization rates. These results suggest that plant responses would be more positive in subsequent years.

Soil pH and organic carbon. There were significant interactions between fertilizer amendments and cultivar for rhizosphere pH (Table 3). Rhizosphere pH varied depending on cultivar and cultivar response varied with pH and ranged from 6.1 to 6.8. The results suggest that the organic amendments lowered rhizosphere pH values in 'Whatley/Loretan' and 'NCC-58' treated with Megabloom and 'TU Purple' plots receiving PL having the lowest pH values. Cultivar influenced SOC (Table 3) in the rhizosphere and

ranged from 0.63% for 'Whatley/Loretan' to 1.1% for 'TU Purple'. The significantly lower SOC content in the rhizosphere of 'Whatley/Loretan' compared with that of the other three cultivars suggest that there might have been higher organic matter content in the rhizosphere of 'TU Purple', 'NCC-58', and 'J6/66' in general, as well as from decaying plant materials, slough off root hairs, and root exudates.

Selected soil enzymes. Generally, the fertilizer amendments influenced rhizosphere enzyme activity regardless of cultivar (Table 4). The activity of ACP increased substantially with all three fertilizer amendments relative to the control plots with the highest activity obtained in the rhizosphere of plants receiving Megabloom. On average, ACP activity increased 44% compared with those of the control plots. Results for ALKP show that there was a 3-fold increase in rhizosphere activity in plots receiving fertilizer amendments compared with the control plots. Thus, all three fertilizer amendments increased ALKP activity with NPK-treated plots having the highest. The activity of β -glucosaminidase (β -NAG) also increased in plots receiving PL, Megabloom, and NPK in relation to the control plots. The magnitude of increase in β -NAG activity ranged from 40% to 89% with PL and NPK plots having

the highest activity. Even though plots treated with Megabloom had marginally lower activity relative to the other treated plots, it was still 40% greater than in control plots. Similarly, as with ALKP, all three amendments increased the activity of β -GLU 3-fold, with NPK-treated plots having the highest activity followed by Megabloom and PL-treated plots. Thus the control plots had lower enzyme activity, whereas those receiving fertilizer amendments had greater activity, suggesting that the organic amendments did not adversely affect enzyme activity relative to NPK-treated plots (Table 4).

The addition of organic amendments increased soil enzyme and microbial activity, which is consistent with what others have reported (Edmeades, 2003; Gutierrez-Miceli et al., 2007). Further, activities of most soil enzymes increase as soil substrate increases, reflecting larger microbial communities, and increased stabilization of enzymes by humic materials (Burns, 1983). Provocation of enzymatic activity can also be a result of the release of exudates in the rhizosphere ecosystem. These exudates can thus activate the microbial synthesis of intracellular and extracellular enzymes. In contrast, the C-rich exudates can also serve as an energy source for microbial production of extracellular enzymes that increase decomposition activities (Gianfreda, 2015). In addition to exudate release and pathogen control, one of the most important functions in the bacterial community of the rhizosphere is phosphate solubilization. As this process is stewarded by bacteria and the extracellular enzymes that they produce (ACP, ALK, and more), the results showing increased activity of phosphatase enzymes substantiate the literature that soil amendments (inorganic and organic) have a stimulating impact on rhizosphere phosphatase (Ai et al., 2012).

Bacterial community composition. In terms of the bacterial ecology, the results for the phyla and class level (Fig. 1) indicated that *Proteobacteria* was the most dominant phylum and class identified. When considering the class level of taxonomy (of which certain groups become more functionally evident) (Shange et al., 2012), four classes of *Proteobacteria* (*Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria*, *Gammaproteobacteria*), as well as the class *Actinobacteria* were the most prevalent in the class groups. 'TU Purple' and 'Whatley/Loretan' significantly influenced *Gemmatimonadetes* at every taxonomic level, suggesting that these cultivars produce root exudates that may have attracted these bacteria.

Taxonomic response to fertilizer treatment. There were 27 genera present in the rhizosphere samples. Significant effects were observed for amendments on the populations of *Flexibacter*, *Mycobacterium*, *Nitrosococcus*, *Nitrosomonadaceae*, and *Roseiflexus* (Table 5). Of the 1896 species observed, 30 were in great quantities in the rhizosphere samples. The ANOVA showed that organic amendments had the greatest effect on species composition. Megabloom significantly decreased the bacterial relative abundance (RA) of *Nitrosococcus*,

Nitrosomonadaceae, and *Flexibacter* genera compared with the control and the other amendments except PL, where the RA was not different for *Nitrosococcus* and *Nitrosomonadaceae* (Table 5). In contrast, Megabloom increased the populations of *Mycobacterium* and *Rubrobacter* compared with the control (Table 5). NPK increased *Nitrosomonadaceae*, whereas Megabloom increased the RA of *Gemmatimonas*, *Mycobacterium*, and *Rubrobacter* genera, and decreased the RA of *Flexibacter*, *Nitrosococcus*, *Nitrosomonadaceae*, and *Roseiflexus* genera. Thus, Megabloom continued to impact bacterial populations at the species level as it had at the genus level compared with the control. For example, *Novosphingobium* sp. decreased significantly compared with the control and other amendments, whereas the RA of *Rhodospirillaceae* sp. increased in plots receiving NPK and organic amendments compared with the control plots (Table 5). The addition of fertilizer and organic amendments had a significant impact on bacteria at every taxonomic level compared with the control; however, Megabloom and PL organic rhizosphere soils had similar or significantly different effects on RA at the genus and species taxonomy level compared with rhizosphere soils amended with inorganic fertilizers.

These results demonstrate that at the genus and species levels of taxonomy, the type of fertilizer, and the type of organic substrate influence the changes in some of the most abundant groups in the rhizosphere ecosystem. Not only are there changes to the composition of the community that seem to be functionally obtuse, there were changes to taxa that have been identified as playing important functional roles in the soil ecosystem. *Nitrosomonadaceae* is a family that includes *Nitrosomonas*, *Nitrospira*, and *Nitrosovibrio* lithotrophic ammonia oxidizing bacteria (AOB). *Nitrosococcus*, another AOB from the *Gammaproteobacteria* class responded positively to the treatments. Although *Flexibacter* is well known for its fish pathogenicity (Crump et al., 2003), there have been other isolates found in soils that participate in denitrification (Wu et al., 1994). As this process requires net $\text{NO}_3\text{-N}$ availability, there may have been a lower availability of $\text{NO}_3\text{-N}$ due to the slow release of N from Megabloom (Mikkelsen and Hartz, 2008). It would be reasonable to surmise that soil *Flexibacter* would reduce RA in such an environment. Although PL is also a slow-release organic fertilizer, it has been stated that rates of mineralization can be highly variable (Gaskell and Smith, 2007). Further evidence to substantiate this notion is the parallel RA of the nitrifiers (*Nitrosococcus* and *Nitrosomonadaceae*). The statistical significance of RA of the nitrifying bacteria relative to the control might be due to soil environment factors. According to Wang et al. (2015), soil pH value, number of ions, NH_4^+ , soil temperature, and organic matter content have a critical impact on the bacterial population.

Table 3. Statistical significance from analysis of variance of fertilizer amendments, cultivar and fertilizer amendments $\times$ cultivar interaction for soil pH, organic carbon, and enzyme activities.

Source of variation	df	pH	%C	ACP	ALKP	β -NAG	β -GLU
Amendments	3	NS	NS	0.02	0.005	0.01	0.03
Cultivar	3	NS	0.02	NS	NS	NS	NS
Amendments $\times$ Cultivar	9	0.001	NS	NS	NS	NS	NS

ACP = acid phosphatase; ALKP = alkaline phosphatase; β -GLU = β -glucosidase; β -NAG = β -glucosaminidase; NS = nonsignificant.

Table 4. Main effect of fertilizer amendments on soil enzyme activity.

Fertilizer amendments	ACP	ALKP	β -NAG	β -GLU
	$\mu\text{g p-nitrophenol/g soil/hr}$			
Control	172.9	2.2	16.6	13.1
Poultry litter	287.7	7.1	31.4	45.9
Megabloom	329.9	7.4	23.1	48.3
NPK	308.7	7.7	29.4	49.5
Significance	0.05	0.01	0.002	0.01

ACP = acid phosphatase; ALKP = alkaline phosphatase; β -GLU = β -glucosidase; β -NAG = β -glucosaminidase.

Fig. 1. Bacterial composition at the phylum level for each sample of sweetpotato rhizosphere. 0bh to 0tu = Control 'TU Purple', 'J6/66', 'NCC-58', 'Whatley'; bl = poultry litter; fsh = Megabloom (fish hydrolysate); fert = NPK fertilizer all combined with the same four sweetpotato cultivars.

Table 5. Effect of fertilizer and organic amendments on bacterial relative abundance in sweetpotato rhizosphere at the genus and species taxonomic level.

	Control	Poultry litter	NPK	Megabloom
Genus				
<i>Flexibacter</i>	1.20 a ^z	0.90 ab	0.97 a	0.40 b
<i>Mycobacterium</i>	1.33 b	1.86 a	1.67 ab	1.91 a
<i>Nitrosococcus</i>	1.60 a	2.42 a	1.79 a	0.69 b
<i>Nitrosomonadaceae</i>	1.70 ab	1.95 ab	2.38 a	1.04b
<i>Roseiflexus</i>	1.77 a	1.51 a	1.48 a	0.57 b
<i>Rubrobacter</i>	1.85 b	1.76b	2.29 ab	3.05 a
Species				
<i>Novosphingobium</i>	0.72 a	0.91 a	0.80 a	0.36 b
<i>Rhodospirillaceae</i>	0.52b	0.90 ab	0.85 a	0.97 a

^zMeans within rows accompanied by the same letter are not significantly different according to Tukey-Kramer's test at 0.05 level of probability.

Novosphingobium has the ability to degrade large aromatic rings, leading to a high diversity of environments in which they have been found, including oil-contaminated soil

(Kämpfer et al., 2011), surface water sediments (Balkwill et al., 1997; Liu et al., 2005; Sohn et al., 2004), and wastewater facilities (Fujii et al., 2003; Neef et al., 1999). Only

recently, this group has been discovered in plant-associated habitats such as the internal stem of *Gossypium hirsutum* (Kämpfer et al., 2015), the rhizosphere of *Arabidopsis thaliana* (Gan et al., 2013), and surface of a crown gall tumor on grapevine (Lin et al., 2014). One suggestion for *Novosphingobium* association with plants in the rhizosphere is the presence of xenobiotic aromatic compounds. Root exudates contain various molecules that are structurally related to aromatic xenobiotics (Ledger et al., 2012) that can potentially serve to create an ecosystem favorable to those bacteria with the ability to catabolize such compounds.

Taxonomic response to varieties. Significant effects of cultivar were observed for *Mycobacterium* and *Sinobacter*. The population of *Sinobacter* increased in the rhizosphere of 'Whatley/Loretan' and *Mycobacterium* in the rhizosphere of 'TU Purple' compared with other cultivars (Table 6). 'Whatley/Loretan' significantly influenced the rhizosphere populations of *Bradyrhizobium elkanii*, *Rhodospirillaceae* sp., and *Sinobacter* sp. (Table 6) compared with 'TU Purple' and 'J6/66' but was not different from 'NCC-58'.

A significant interaction between the organic amendment and cultivar for *Gemmatimonadaceae* and *Gemmatimonas* (Table 7) and for *Anaerolineaceae* and *Gemmatimonadaceae* sp. (Table 8). *Anaerolineaceae* population increased significantly in the rhizosphere of 'J6/66' when NPK was applied compared with the control and the other amendments. *Gemmatimonadaceae* populations increased significantly when PL and NPK were applied compared with control. *Gemmatimonadaceae* significantly increased in the rhizosphere of 'Whatley/Loretan', when NPK was applied compared with control and the other organic amendments. Megabloom significantly decreased *Gemmatimonadaceae* population compared with control, NPK and PL in the rhizosphere of 'Whatley/Loretan', but increased significantly in the rhizosphere of 'NCC-58' when NPK and organic amendments were applied compared with the control (Table 8).

'TU Purple' and 'Whatley/Loretan' significantly affected the phylum *Gemmatimonadetes* at every taxonomical level, suggesting that these cultivars produce exudates that may attract certain bacterial groups. Bacteria belonging to this phylum are frequently detected in a variety of environments and are noted as one of the nine most common from the soil in 16S rRNA gene libraries. Janssen (2006) and Khodadad et al. (2011) also found that these bacteria are abundant in carbon-rich soils, further suggesting that these bacteria may have influenced carbon content in the rhizosphere of these two cultivars. *Gemmatimonadetes* play a role in phosphorus cycling (Zhang et al., 2003), as they improve phosphorus removal in wastewater, and could play a similar role in the soil. The varying RA of *Rhodospirillaceae* sp. among the cultivars is worthy of note, as the capacity to fix molecular nitrogen is ubiquitous among members of *Rhodospirillaceae*, whereas the

Table 6. Effect of cultivars on bacterial relative abundance in sweetpotato rhizosphere at the genus and species taxonomic level.

	TU Purple	J6/66	NCC-58	Whatley/Loretan
Genus				
<i>Mycobacterium</i>	1.94 a ^z	1.60 ab	1.84 a	1.39 b
<i>Sinobacter</i>	0.91 b	1.03 b	0.95 b	1.72 a
Species				
<i>Bradyrhizobium elkanii</i>	0.79 b	0.94 b	0.79 b	1.38 a
<i>Rhodospirillaceae</i>	0.64 b	0.71 b	0.81 ab	1.09 a

^zMeans within columns within genus and species accompanied by the same letter are not significantly different according to Tukey-Kramer's test at 0.05 level of probability.

Table 7. Effect of fertilization and cultivar on bacterial composition in sweetpotato rhizosphere at the genus taxonomic level.

Genus	Treatment	Cultivar	Estimate
<i>Gemmatimonadaceae</i>	Poultry litter ^z	NCC-58	2.3646 a
	Control	NCC-58	0.6962 b
	NPK	NCC-58	2.2638 ab
	Megabloom	NCC-58	1.7106 ab
<i>Gemmatimonadaceae</i>	Poultry litter	Whatley/Loretan	2.7972 a
	Control	Whatley/Loretan	2.9307 a
	NPK	Whatley/Loretan	3.8371 a
	Megabloom	Whatley/Loretan	1.1969 b
<i>Gemmatimonas</i>	Poultry litter	Whatley/Loretan	1.072 b
	Control	Whatley/Loretan	1.3797 b
	NPK	Whatley/Loretan	1.3473 b
	Megabloom	Whatley/Loretan	2.8128 a

^zMeans within columns and within genus accompanied by the same letter are not significantly different according to Tukey-Kramer's test at 0.05 level of probability.

Table 8. Effect of fertilizer and organic amendments and cultivar on bacterial composition in sweetpotato rhizosphere at the species taxonomic level.

Species	Treatment	Cultivar	Estimate
<i>Anaerolineaceae</i>	Poultry litter ^z	J6/66	1.47 b
	Control	J6/66	0.84 b
	NPK	J6/66	2.21 a
	Megabloom	J6/66	1.37 b
<i>Gemmatimonadaceae</i>	Poultry litter	J6/66	2.61 a
	Control	J6/66	1.76 b
	NPK	J6/66	2.53 a
	Megabloom	J6/66	2.44 ab
<i>Gemmatimonadaceae</i>	Poultry litter	NCC-58	2.36 a
	Control	NCC-58	0.70 b
	NPK	NCC-58	2.26 a
	Megabloom	NCC-58	1.71 a
<i>Gemmatimonadaceae</i>	Poultry litter	Whatley/Loretan	2.80 b
	Control	Whatley/Loretan	2.93 b
	NPK	Whatley/Loretan	3.84 a
	Megabloom	Whatley/Loretan	1.20 c

^zMeans within columns within species accompanied by the same letter are not significantly different according to Tukey-Kramer's test at 0.05 level of probability.

efficacy of the process varies among species (Madigan et al., 1984). This group, along with *Bradyrhizobium elkanii* (another known N-fixer) was also highest in the 'Whatley/Loretan' rhizosphere (also lowest in SOC and highest in β -NAG activity, although not significant). The abundant concentration of the closely related *Azospirillum* sp. (known AOB) is interesting, as this species is well known as associative nitrogen fixers, which can partly explain the ability of sweetpotato to grow in N-deficient marginal soils (Mortley and Hill, 1990; Trotman et al., 1993).

The findings suggest that organic fertilizer and variety influenced selected microbial populations and enzymatic function within the soil rhizosphere of sweetpotato. The effects of organic amendments on bacterial composition varied as population differed significantly under the different organic amendments as bacteria involved in C and N cycling were dominant at every taxonomic level. Organic amendments had no significant impact on SOC but variety did, with soil from 'Whatley/Loretan' rhizosphere having lower SOC than other cultivars. Although variety did not affect enzyme activity, all amendments

significantly increase ACP, ALKP, β -NAG, and β -Glu enzyme activity compared with the control, but there was no significant difference among treatment. The phyla *Actinobacteria* and *Gemmatimonadetes* were also found in the samples, which could explain the increased activity seen in β -GLU and β -glucosaminidase enzymes. Based on these findings, we gather that bacteria associated with C and N cycling under aerobic conditions can dominate in the rhizosphere of sweetpotato. Most importantly, these findings gave a better understanding of microbiome associated with sweetpotato that may not have been previously connected with this storage root crop. Not only were we able to elucidate the storage root microbiome of these particular cultivars, but also demonstrate differential responses of organic and inorganic fertilizers possibly allowing for more studies investigating alternative fertilization technology as well as nutrient uptake and the soil, plant, microbe interface.

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