Microbial stability of worm castings and sugarcane filter mud compost blended with biochar

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Abstract: Organic amendments such as worm castings and sugarcane filter mud compost can provide nutrient rich substrates for enhanced plant growth. Physico-chemical and microbial stability of these substrates might be enhanced with the addition of biochar. A series of experiments was carried out to determine the stability of microbe populations in both worm castings and sugarcane filter mud compost with the addition of biochar made from sugarcane bagasse. Storage studies up to 150 days were carried out with biochar/worm castings and biochar/sugarcane filter mud compost blends on a volume basis (100/0; 90/10; 75/25; 50/50; 25/75; 10/90; 0/100). Physico-chemical properties, such as micro and macro nutrient composition, pH, ash and carbon contents, amongst others were monitored throughout storage time as well as microbe counts. No major deleterious effects to the microbial population were found by adding biochar to either substrate, despite decreasing moisture levels for increased biochar additions. Biochar might be providing nutrients needed by microbes, as well as possibly bind bacterial waste products that would otherwise be toxic to the microbes. Larger scale studies are warranted as well as longer storage time to optimize shelf stability.

ABOUT THE AUTHORS

Some of the major research objectives of the authors of this paper and their team include developing commercially-viable technologies that: (i) reduce or eliminate undesirable effects of high viscosity, color and starch on sugar processing/refinery efficiency; (ii) increase stability and lengthen storage of sugar feedstocks for the manufacture of sugars, advanced biofuels, and bioproducts; and (iii) in collaboration with industrial partners, improve and enable commercial production of marketable products from residues and by-product streams associated with postharvest sugar crop processing. This paper relates to both production of value-added biochar from sugarcane processing residues and the beneficial use as blending mixture with organic amendments including sugarcane filter mud compost. Byproducts of post harvesting and processing utilization are needed for effectively handling waste streams but also desirable in terms of economic opportunity for the sugarcane processors and refiners and beneficial to organic amendment markets.

PUBLIC INTEREST STATEMENT

Organic amendments such as worm castings and sugarcane filter mud compost can provide nutrient rich substrates for enhanced plant growth. Adding biochar to these amendments can enhance their physico-chemical and microbial stability. A series of blends including different proportions of each amendment and biochar were stored up to 150 days. No major deleterious effects were observed by adding biochar to either substrate, despite decreasing moisture levels for increased biochar additions. Biochar might be providing nutrients needed by microbes, as well as possibly bind bacterial waste products that would otherwise be toxic to microbes. Larger scale studies and longer storage times are warranted to optimize shelf stability of organic amendments. Study results are encouraging for the viability of successful commercialization of biochar and organic amendment mixtures for their withstanding of storage conditions, as well as realizing a viable outlet for these by-products.
1. Introduction

Commercial potting mixtures for plant growth utilize many different substrates to beneficially improve soil properties while increasing plant growth, yield and overall health. Increased importance is being given to compost materials and organic amendments as components in commercial potting substrates because these materials usually are rich in required nutrients, increase cation exchange capacity, and improve water-holding capabilities (Tomati, Galli, Grappelli, & Di Lena, 1990). Worm castings are one such substrate that has gained significant interest in the last few decades. These materials, called vermicomposts, are being used as organic fertilizers, soil amendments, and potting substrate components (Bachman & Metzger, 2008). Hidalgo, Matta, and Harkess (2006) when using various substrates to grow marigold flowers (Tagetes erecta), found out that worm castings had a greater nutrient content than other substrates including peat moss and led to the highest plant dry weight. Through their canal forming and living underneath the soil surface, earthworms have long been thought of creators of soil porosity and mixing, and their activity in soil favors root growth, plant development and crop yield (Grappelli, Tomati, Galli & Vergari, 1985). It is also well known that they are able to change chemical, physical and microbiologic characteristics in the soil. When studying microbiological, physical and chemical changes in earthworm casts ageing in the field, Parle (1963) suggested that earthworms and their casts stimulated soil fertility by increasing soil aggregate stability via bacterial polysaccharides and by enhancing the rate of organic matter breakdown via micro-flora. Shan et al. (2011) determined that both worms and their casts decreased the availability in soil of nonylphenol, a type of endocrine-disrupting xenobiotic found in dispersants, detergents, and emulsifiers. According to Lee (1985), N-containing products of earthworm metabolism are returned to the soil in casts, urine, mucoproteins, and dead earthworm tissue. Soil microbial activity is stimulated during gut transit and the resulting increase in mineralization rate can result in much greater nutrient availability (particularly that of N, P and S) in casts than soil (Blair, Parmelee, & Lovelle, 1995). Preferential ingestion of fragments of decaying plant material with a high nutrient content also contributes to the greater nutrient availability in casts (Lee, 1985). In addition earthworm castings are dispersed within the soil. Earthworm castings are also important protective and dispersal vehicles for soil microbes and nutrients (Weyers & Spokas, 2011). Taken altogether, earthworms have been recognized as ecosystem engineers, or organisms that can have a profound influence on the structure and functioning of soils (Lavelle, Bignell, & Lepage, 1997).

The main by-products of sugarcane processing into refined sugar are bagasse, molasses, fly ash, and filter press mud. As a waste byproduct from sugar factories, filter press mud or cake is a soft, spongy, amorphous dark brown material. Filter press mud contains valuable micronutrients as well as sugar, fiber, coagulated colloids, including cane wax, albuminoids, inorganic salts and soil particles. A typical composition includes 15–30% fiber, 5–15% sugar, 5–15% crude protein, 5–14% wax and fats and 9–20% ash (Rein, 2007). When this material is digested under anaerobic condition, the lignin, cellulose and waxes are converted to release micronutrients from press mud that are freely available for plant growth (Rakkiyappan, Gopalasundaram, & Radhamani, 2005). Composting of filter press cake with and without other additives such as fly ash, has been utilized and practiced in various sugarcane growing countries (Hudson, 1994). Seed germination rates for various vegetables were significantly improved when using soil mixed with composted filter cake and fly ash (Fearon, 1997; Rengifo, Ramirez, & Bruzón, 1996; Vélez, Garzón, & Bruzón, 1996). Composting of filter cake reduces C/N ratio improving mineralization of N (Alexander, 1972) and leads to approximately 50% mass reduction due to loss of moisture and some organic carbon (Rein, 2007). Fly ash is made of partially burnt sugarcane bagasse, it is carried over in boiler gases and collected by boiler scrubbers. It is frequently handled wet. It is mainly composed of silica (60%) but also contains various important minerals such as calcium, magnesium, iron, phosphorous, potassium and manganese (Chen & Chou, 1993). When composting mixtures of filter cake, sugarcane straw and sugarcane fly ash,
Klibansky, León, Brizuela, Altuna, and González (1993) obtained suitable values for C/N ratio, humic acid/fulvic acid ratio, humification index, and cation exchange capacity, without the need to add external inoculum, due to the high microbial charge of the raw material used. Filter cake and fly ash represent approximately 3 and 0.3% of the initial input and besides being a good source of organic matter, they contain appreciable amounts of macro and micro nutrients, therefore offering a sustainable waste disposal method and chemical fertilizer savings (Jha, Sinha, Alam & Pandey, 2017). With mechanical harvesting in particular, field trash and dirt increase in the incoming cane, leading to increased amounts of filter cake, up to 27 to 64 kg Mg$^{-1}$ of cane, with moisture content of 65 to 80% (Chen & Chou, 1993). Poole (1989) successfully used potting mixtures containing filter cake, bagasse and boiler ash from cane sugar factories to grow flowers in a greenhouse study.

Biochar utilization as soil amendment has received a significant amount of attention recently. There are numerous studies published in the literature reporting on the effects of biochar as means to improve soil fertility as well as mitigate climate change. Potential positive impacts include increased soil organic matter (SOM), more permanent soil carbon (C) sequestration, increased herbicide persistence, and improved soil physical properties (Laird, 2008). Biochar may also help regulate soil pH. Because biochar is commonly produced from agricultural and forestry residues, as well as fermentation and refinery wastes, its use represents a beneficial solution to waste disposal. As costs of organic-waste disposal have escalated progressively and the environmental regulations on their disposal have become increasingly restrictive, there is much greater interest in exploiting organic wastes as fertilizers, soil conditioners, and amendments or as energy sources, such as for methane production (Edwards, 2011). In Louisiana, excess sugarcane bagasse is available as feedstock for biochar production. Given the nature of biochar, it is of interest to determine if blending it into organic soil amendments leads to further improvements of their physico-chemical properties. Furthermore, it is necessary to determine if biochar additions help maintain the organic amendments microbial population thriving throughout storage. If microbe populations can be maintained post addition of biochar, these amendments can be successfully commercialized. This study was carried out to determine the effects of biochar additions to two organic amendments (worm castings and sugarcane filter mud compost) on the physico-chemical properties and the microbial population of the resulting blends throughout storage.

2. Materials and methods

2.1. Sample collection and preparation

2.1.1. Biochar manufacture
Biochar, BC, produced from excess unutilized sugarcane bagasse was supplied by American Biocarbon, LLC in Cora Texas, White Castle, LA. American Biocarbon has a continuous 1 ton/hr pyrolysis/torrefier unit that processes excess sugarcane bagasse at an approximate pyrolysis temperature of 600°F. The residence time in the pyrolysis furnace is approximately 10 min.

2.1.2. Worm castings sample collection
Worm castings from African Nightcrawlers (Eudrilus eugeniae) were grown in a bedding of decomposing cow manure and hay. A mixture of worms and manure was placed into plastic containers in the shade at ambient temperature and in the dark. The duration of worm feeding was 14 days. The worm castings (WC) were then separated from the worms and the eggs by selective screening based on size (worms>>worm eggs>>worm castings). Two different collections of worm castings were obtained from Jack Investine at Investine Farms in Kentwood, LA for each of two storage studies. Worm castings were utilized within one week of collection. Worm castings physical and chemical properties are displayed in Table 1.
2.1.3. Sugarcane filter mud compost collection
Sugarcane raw juice goes through a clarification process to remove impurities, color and turbidity. Filter cake is the by-product of clarification. Factories typically add bagasse fly ash to the filter cake prior to disposal and refer to it as mud, having enhanced value as soil conditioner. When composted, the resulting product is referred to as mud compost (MC), produced for an improved commercial value. A pile composed of 50% sugarcane filter mud and 50% sugarcane bagasse was composted in 12 ft wide and 6–7 feet high piles turned every 3 days with temperature reaching 150 °C. Composting was completed within 21 to 30 days. Composted sugarcane filter mud was supplied by Les Ewing, White Castle, LA and was utilized within one week of collection (physical and chemical properties are displayed in Table 1).

2.1.4. Storage study of blends of worm castings and sugarcane filter mud compost with biochar
A series of storage experiments was carried out by blending biochar with either worm castings or sugarcane filter mud compost at different blending rates. Blends were prepared based on volume due to the fact that all three compounds displayed significantly different bulk densities (BC bulk density = 0.125; WC bulk density = 0.607; MC bulk density = 0.927). In the first study, the following blends of worm castings and biochar (100/0; 95/5; 90/10; 75/25 and 0/100 WC/BC) were made. Mixtures with a total volume of 30 mL (total weight ranging from 3.75 g to 18.21 depending on blend) were blended thoroughly prior to incubation at room temperature in the dark and placed in

Table 1. Various physical and chemical properties for worm castings, sugarcane filter mud compost and biochar samples

| Sample | Worm castings | Mud compost | Biochar |
|--------|---------------|-------------|---------|
| Nitrogen |               |             |         |
| Total N (%) | 1.83 | 0.15 | 0.71 |
| Organic N (%) | 1.66 | 0.15 | 0.70 |
| Ammonium N (%) | – | <0.001 | 0.003 |
| Nitrate N (%) | 0.17 | – | – |
| Macroelements |               |             |         |
| P (%) | 0.67 | 0.30 | 0.07 |
| P as P2O5 (%) | 1.54 | 0.70 | 0.17 |
| K (%) | 0.67 | 0.59 | 0.39 |
| K as K2O (%) | 0.81 | 0.72 | 0.46 |
| S (%) | 0.61 | – | 0.19 |
| Ca (%) | 3.17 | 0.74 | 0.77 |
| Mg (%) | 0.81 | 0.29 | 0.150 |
| Na (%) | 0.145 | 0.048 | 0.043 |
| Microelements |               |             |         |
| Fe (ppm) | 9,863 | 10,166 | 4,443 |
| Mn (ppm) | 1,024 | 301 | 99 |
| Other properties |               |             |         |
| Moisture (%) | 65.63 | 37.44 | 6.60 |
| Total solids (%) | 34.37 | 62.56 | 93.4 |
| Organic matter (%) | 39.57 | 7.26 | 89.19 |
| Ash (%) | 60.52 | 92.71 | 10.81 |
| Total Carbon (%) | 20.95 | 5.34 | 45.09 |
| H:C molar ratio | 1.1 | | |
| Chloride (%) | 0.12 | <0.01 | – |
| pH | 5.9 | 6.0 | 5.3 |
| Conductivity (mS/cm)* | – | 0.22 | |
| Physical properties |               |             |         |
| Bulk density (lbs/cu yd) | 0.500 | 0.740 | 0.150 |
| Surface area, m²/g | Negl. | Negl. | 1.52 ± 0.02 |

*1:5 soluble salts.
sealed 125 mL Nalgene bottles in order to provide enough headspace. Blends were incubated at 22°C and samples were taken on 0, 42, and 83 days of storage for physico-chemical and microbial analysis. Based on the results from the first study, a second study ensued with the following blends of worm castings and biochar (100/0; 90/10; 75/25; 50/50; 25/75; 10/90; 0/100 WC/BC) for a period of 150 days. Samples were pulled on day 0, 30, 60, 90, and 150 day of storage for physico-chemical and microbial analysis. A third study included sugarcane filter mud compost and biochar blends but did not contain worm castings (100/0; 90/10; 75/25; 50/50; 25/75; 10/90; 0/100 MC/BC). Samples were taken on days 0, 30, 80, and 140 of storage for physico-chemical and microbial analysis.

2.2. Physico-chemical Analysis of worm castings, sugarcane filter mud compost and biochar

Proximate analysis (ASTM method D5142-09) was performed on a Thermo-gravimetric Analyzer (TGA701) to determine moisture, ash, volatile matter (VM), and fixed carbon and data reported as average of triplicate samples on a dry basis (except for moisture), for worm castings, filter mud compost and biochar samples. Moisture was determined as the weight loss after heating sample under N₂ atmosphere in open crucible to 107°C until stable sample weight. Volatile matter was determined as weight loss after heating sample under N₂ atmosphere in covered crucible to 950°C for 7 min. Ash was calculated from remaining mass after heating sample under O₂ atmosphere in open crucible to 750°C and holding until stable weight. Fixed carbon was calculated by difference. Samples of biochar, worm castings, and sugarcane filter mud compost were sent out to Midwest Laboratories, Inc. (Omaha, Nebraska) and Soil Control Lab (Watsonville, California) for various physical and chemical analyses, including major, secondary and minor nutrients, total organic matter, total carbon, pH, conductivity, bulk density and, heavy metal analysis. Data is reported in Tables 1 and 2. Ultimate analysis of biochar (CHNSO) was determined by dry combustion/TCD (CHN); percent sulfur content was determined by O₂ flask combustion/titration and percent oxygen content was determined by pyrolysis/gravimetric determination (Micro-Analysis, Inc., Wilmington, DE) (Table 3).

Surface area measurements were obtained from nitrogen adsorption isotherms at 77 °K using a Nova 2200 Surface Area Analyzer (Quantachrome Corp., Boynton Beach, FL, USA). Data reported is average of duplicate samples. Specific surface areas (BET, Brunner-Emmett-Teller) were taken from adsorption isotherms using the BET equation (Table 1).

Particle size analysis (PSA) was conducted on a Partica Laser Scattering Particle Size Distributor Analyzer LA-950V2 by HoribaTM (Kyoto, Japan). Approximately 5 g of sample were dispersed into 25 mL of deionized water and vortexed for 1 min for complete dispersion and removal of clumps. Each sample was then added to the liquid feeder of the instrument using a dropper, until the % transmittance value was in the valid range, using Horiba LA-950 software (Ver. 5.). PSA results are reported in Table 4.

| Table 2. Heavy metal analysis (dry weight basis) |
|-----------------------------------------------|
| Heavy metal (ppm) | Worm castings | Mud compost | Biochar |
| Cd | <0.50 | n.d. | <0.05 |
| Cr | 25.1 | 34.0 | – |
| Hg | <0.05 | n.d. | <0.05 |
| Pb | <5.0 | 8.2 | <5.0 |
| Mo | n.d. | 1.1 | |
| Ni | 11.3 | 11.7 | 3.2 |
| Se | <10.0 | n.d. | <5.0 |
| Zn | 437.6 | 93.4 | 32.1 |
| Cu | 75.1 | 22.7 | – |
| As | 2.62 | 3.44 | <5.0 |

Note: n.d. = below detection limit.
2.3. Microbial count
For microbial analysis, 0.5 g subsamples of worm castings, biochar, and worm castings/biochar combinations were weighed into sterile 50 mL conical centrifuge tubes on the specified day post-incubation. Sterile 0.85% NaCl was added at a concentration of approximately 0.1 g/mL, and the tubes were vortexed for 30 s. An aliquot was then serially diluted in sterile 0.85% NaCl. The serial dilutions were plated on nutrient agar (NA, Difco, Sparks, MD, USA) to enumerate a broad range of non-fastidious microorganisms, using the spread plate method. Inoculated NA plates were incubated at 30°C. Colonies were enumerated after incubation for a minimum of 24 h.

3. Results and discussion
3.1. Biochar addition to worm castings
In the first 42 days of storage, the microbial count of the 100% worm castings dropped from 3.53x10^6 to 5.2x10^5 colony forming units (cfu) per mL (Figure 1). An additional drop in microbial count to 1.2x10^5 cfu/mL was observed on day 83. On day 42 there were microbes observed in the 100% biochar sample but only on one of the replicated plates, and no microbes were observed from biochar samples on day 83. Additionally, the reduction in microbial counts for the blends that include biochar was less than that seen for the 100% worm casting sample. Particularly for the first 42 days of storage, adding biochar at any of the blends appeared to stabilize the microbial population. It is possible that biochar modifies the community of soil microorganisms as well as their activity by providing a suitable habitat for them (Pietikäinen & Fritze, 2000). Particle size distribution for the biochar was narrower and had a lower mean size than that for the worm castings (Table 4). It is possible that biochar modifies the community of soil microorganisms as well as their activity by providing a suitable habitat for them (Pietikäinen & Fritze, 2000). Particle size distribution for the biochar was narrower and had a lower mean size than that for the worm castings (Table 4).

### Table 3. Ultimate analysis of biochar on % dry basis and H:C and O:C molar ratios

|   | C   | H   | N   | O   | S   | H:C  | O:C  |
|---|-----|-----|-----|-----|-----|------|------|
|   | 45.1| 2.48| 0.41| 15.0| 0.47| 0.686| 0.261|

### Table 4. Laser scattering particle size distribution analysis of the two substrates (worm castings and sugarcane filter mud compost) and biochar

| Biochar | Worm castings | Mud compost | Biochar |
|---|---------------|-------------|---------|
| Median, µm | 51.3 | 25.8 | 174 |
| Mean size, µm | 338 | 47.1 | 203 |
| Standard deviation, µm | 504 | 53.2 | 164 |
| Diameter on cumulative, µm | | | | |
| 5% | 6.88 | 7.06 | 13.7 |
| 10% | 8.43 | 8.70 | 21.7 |
| 20% | 11.2 | 11.4 | 51.8 |
| 30% | 14.8 | 14.4 | 90.1 |
| 40% | 22.9 | 18.6 | 134 |
| 60 % | 93.4 | 37.3 | 214 |
| 70 % | 192 | 52.1 | 259 |
| 80 % | 899 | 72.4 | 322 |
| 90 % | 1119 | 111 | 428 |

2.3. Microbial count
For microbial analysis, 0.5 g subsamples of worm castings, biochar, and worm castings/biochar combinations were weighed into sterile 50 mL conical centrifuge tubes on the specified day post-incubation. Sterile 0.85% NaCl was added at a concentration of approximately 0.1 g/mL, and the tubes were vortexed for 30 s. An aliquot was then serially diluted in sterile 0.85% NaCl. The serial dilutions were plated on nutrient agar (NA, Difco, Sparks, MD, USA) to enumerate a broad range of non-fastidious microorganisms, using the spread plate method. Inoculated NA plates were incubated at 30°C. Colonies were enumerated after incubation for a minimum of 24 h.

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increased, almost doubled, from 3.72E06 to 6.99E06 cfu/mL on day 42, but this was followed by a decrease to 8.0E04 cfu/mL on day 83. Blending worm castings with biochar did not appear to cause a substantial reduction in moisture content (Figure 2(A)), and this likely contributed to the stabilization of the microbial populations over time. In a recent review of biochar effects on soil biota, Lehmann et al. (2011) found that in most studies involving biochar addition to soils, microbial biomass has been found to increase as a result of biochar additions, with significant changes in microbial community composition and enzyme activities that may explain biogeochemical effects of biochar on element cycles, plant pathogens, and crop growth. Yet, very little is known about the mechanisms through which biochar affects microbial abundance and community composition. Moisture content of the 100% biochar sample was markedly lower than that of the blends supporting the microbial data of very to minor cell count of day zero and day 83. The volatile matter of biochar was 66% greater than that of worm castings on day zero (Figure 2(B)) as well as biochar’s
contribution of organic matter (Table 1). It is possible that the volatile organic compounds are readily accessible as a nutrient source for the microbes. A small but noticeable decrease in the percent of volatiles during storage was also observed. This is potentially due to consumption of these low volatility carbon compounds by the microbes. Numerous studies have shown that biochar application onto soil significantly increases the supply of nutrients, the organic matter content and it improves soil structure and buffer abilities (Głąb, Palmowska, Zaleski, & Gondek, 2016). Additionally, Sun, Lan, Xu, Meng, and Chen (2016) found that the presence of inherent volatile organic compounds in the biochar and respective adsorbed soluble organic matter changed the structure and metabolic capacities of composting microbial communities protected in biochar pores, compared to those of the adjacent composting material. The activity of the microorganisms colonizing the biochar particles was also supported by the stabilization effect of biochar (Sanchez-Monedero et al., 2018). Worm castings contained approximately five times the amount of ash content than the biochar at the start of the experiment and this ratio remained approximately the same throughout storage (Figure 2(C)). Relative contributions of several nutrients, e.g. nitrogen, potassium, sulfur, calcium, iron and sodium were proportionally higher than what would be expected from total mineral content alone (Table 1). From Table 2 it can be seen that biochar does not contribute heavy metals when blended with worm castings.

Once it was determined that biochar at the highest concentration did not have an inhibitory effect on microbes, a decision was made to conduct another study with blends including biochar as high as 90% on a volume basis. This second experiment was also conducted for a longer period of time, 150 days. Microbial counts decreased as percent of biochar added to the blend increased because the source of the microbes is the worm castings (Figure 3). For all blends of worm castings to biochar, the initial microbial count was not greatly reduced, up to 150 days of storage. Moisture content of the worm castings was 57.1% and biochar moisture content was 9.1% leading to a decrease in moisture content as the percentage of the drier biochar in the blend increased (Figure 4(A)). Nonetheless, microbes were still viable at the moisture level with the highest percentage of biochar (90%) which had a moisture content of 27.5%. Over storage time, moisture of each blend remained stable and this is likely due to biochar’s high water holding capacity of 3.7 g water per gram of dry sample. Volatile matter (Figure 4(B)) increased as the amount of biochar in the blend increased from 37.9% at the 90% worm castings and 10% biochar to 58.3% for the 10% worm castings and 90% biochar, due to the fact that it was present in much higher amounts in the biochar. Volatile matter remained relatively stable during storage for all blends. The same can be said for stability of ash content as it appeared to remain stable during storage for each blend (Figure 4(C)). It is possible that the release
of a variety of organic molecules from fresh biochar may in some cases be responsible for increases or decreases in abundance and activity of soil biota (Lehmann et al., 2011). Looking at the pH for all blends, the biochar stabilized a drop in pH during the 150 day storage period (Figure 5). A decrease in pH for worm castings alone was observed (Figure 5). Microbes produce waste products that accumulate and normally reduce pH. The biochar appeared to neutralize this effect and positively stabilize the microbial population. This was consistent with the pH levels observed for the first experiment (data not shown). Due to the porous nature of biochar and therefore a close interaction with microorganisms, pH can have an important influence on their activity. Sanchez-Monedero et al. (2018) reported on the capacity of biochars to adsorb toxic compounds that can inhibit microbial growth and therefore stabilize microbial populations. Observations on microbial dynamics lead to the conclusion of a possible improved resource use due to co-location of various resources in and around biochars with sorption and thereby inactivation of growth-inhibiting substances likely playing a role for increased abundance of soil biota (Lehmann et al., 2011). Biochar addition may affect the soil biological community composition and has been shown to increase soil microbial biomass (Liang et al., 2010; O’Neill et al., 2009). Biochar surface area, despite being low (1.52 m²/g) will likely be a positive contribution to microbes by providing structure as well as nutrient retention over time. Biochar influences nutrient cycling and retention of mineral nutrients which is likely to increase nutrient availability in the long term (Lehmann, Gaunt, & Rondon, 2006; Lehmann & Rondon, 2006).
addition to sustaining microbe population, the blending of worm castings with biochar increased the amounts of fixed carbon from an average of 3.1% for the worm castings alone to 18.19% for the 90% biochar blend (Figure 4(D)). A higher carbon content can be beneficial for organically poor soils. Additionally, biochar helps to lower bulk density (Table 1) which helps promote aeration in soils and therefore facilitate root growth and dispersion. Biochar did not add substantial amounts of mineral nitrogen to the worm castings (Table 1). Although the influence of biochar on nitrogen transformation processes in soil is complicated (Singh, Hatton, Singh, Cowie, & Kathuria, 2010), it has been reported that biochar can increase mineral nitrogen concentration and availability (Noguera et al., 2010) possibly by increasing cation exchange capacity and therefore retention of cations such as ammonium (Lehmann et al., 2003). Worm castings are rich in various major and minor nutrients (Table 1) and although not substantial, blending them with biochar contributed additional nutrients. More importantly biochar provides a synergistic interaction with worm castings by increasing the availability and retention of nutrients so that worm castings and biochar blends can lead to the building up of a much larger nutrient stock in the long term (Barot, Ugolini, & Brikci, 2007; Boudsocq, Lata, Mathieu, Abbadie, & Barot, 2009).

Knowing that worm castings are a beneficial soil amendment and finding that blending biochar into worm castings helped to stabilize their beneficial properties, it was of interest to investigate whether this was also true for the beneficial properties of another possible soil amendment, filter mud compost as a by-product from sugarcane processing.

3.2. Biochar addition to filter mud compost
Based on the results of the second worm castings experiment, the blend ratios of filter mud compost to biochar were maintained and the total storage time was 140 days. Filter mud compost was found to not be as rich a source of microbes as worm castings, with two orders of magnitude fewer cfu g⁻¹. During composting, readily degradable organic matter is used by microorganisms as C and N source, with compost consisting of transformed, slowly degradable compounds, intermediate breakdown products and the cell walls of dead microorganisms, which are classified together as humic substances (Saranraj & Stella, 2014). Over a 140 day storage experiment, the initial counts of microbes in sugarcane filter mud compost, did not decrease upon blending for each blend up to 90% biochar (Figure 6). For sugarcane filter mud compost, microorganisms such as bacteria thrive due to the available sugars, followed by fungi for the fibrous portion and further decomposition due to cacilli and actinomycetes (Chen & Chou, 1993). Initial moisture contents in the sugarcane filter mud compost...
compost biochar blends were much lower than those found for the worm castings/biochar blends. On the other hand, over time the initial moisture levels did not decrease (Figure 7(A)), consistent with what was also observed with worm castings. It appears that maintaining the initial moisture level is key for microbes to survive over the storage period. Research has demonstrated that aerobic microbial processes occur optimally at 60% water filled pore spaces; however, the soil water activity remains at 99% even as soils dry past the permanent wilting point (~1.5 MPa) (Harris, 1981). Given the range at which soil microorganisms thrive, subsets (e.g. heterotrophic, nitrifiers, bacteria, fungi) likely make use of available substrates in all but the most extreme soil conditions (White & Webber, 2017; unpublished). Additionally, biochar, due to its lower bulk density, is able to increase aeration, which might contribute to greater microbial activity (Haynes et al., 2003). Kopeć, Baran, and Mierzwa-Hersztek (2017) observed increased total numbers of bacteria and fungi with additions of biochar to sewage sludge composts. Sugarcane filter mud compost had much lower amounts of volatiles and much higher amounts of ash content initially (Figure 7(B) and (C)). This was expected since microbial activity of both mesophilic and thermophilic microbes during the different stages of composting leads to the consumption of organic carbon. Additionally, sugarcane filter mud compost contains approximately 20% fly ash contributing to its much lower particle size and narrow particle size distribution when compared to worm castings (Table 4). Blending biochar with sugarcane filter mud compost largely increased the organic content (Figure 7(B)). Noguera et al. (2010) determined that biochar is known to influence SOM dynamics as well as the release of mineral nutrients and their retention. Because C/N ratio was highest for biochar (11.5, 35.6 and 63.5 respectively for worm castings, sugarcane filter mud compost and biochar), blending it into either soil amendment led to increased ratios of C/N. It is known that C/N ratio affects microbial activity as microbes utilize carbon as energy source and nitrogen for building cell structure (Saranraj & Stella, 2014), however Golueke (1972) reported that C:N ratio of 50:1 to be well within optimum range. Over time, volatile and ash contents did not change (Figure 7(B) and (C)). For the case of sugarcane filter mud compost, blending with biochar led to increased amounts of nitrogen since it is present in higher amounts than those found in sugarcane filter mud compost. It also enhanced sugarcane filter mud compost by increasing its total carbon as well as organic matter content (Table 1, Figure 7(D)).

Biochar H/C and O/C molar ratios reflect respectively on its aromacity and polarity (Table 3). The latter of which, O/C at 0.26 usually correlates with the presence of oxygen containing surface functional groups (i.e. carboxyl groups) that can aid in cation exchange, CEC abilities in soils. Improved CEC possibly leads to enhanced adsorption of organic matter as well as moisture content onto the biochar surface. When blended with organic soil amendments the porous structure of the biochar
combined with its ability to adsorb soluble organic matter as well as inorganic nutrients can create a suitable habitat for microbes to thrive, grow and reproduce.

4. Summary
A series of experiments was carried out to determine the stability of microbe populations in both worm castings and sugarcane filter mud compost with the addition of biochar. No major deleterious effects were found by adding biochar to either substrate. It is possible that the biochar is stabilizing moisture levels and pH in the blends. Biochar contains various organic molecules that can lead to shifts in abundance and activity of microbes present in soil amendments. Additionally, although not verified in this study it is possible that the biochar may bind bacterial waste products that would otherwise be toxic to the microbe population, or biochar due to its porous nature, is benefiting storage of worm castings and sugarcane filter mud compost over time. Biochar can be viewed as a disordered mixture of C clusters and mineral inclusions, containing porous particles comparable to a soil aggregate that may provide similar functions such as protection of organic matter, habitat for soil biota, or retention of soil moisture and nutrients as described for aggregates made from minerals and organic matter (Lehmann et al., 2011). Based on the initial results on storage stability of blends of worm castings or sugarcane filter mud compost with biochar, it is warranted to expand this study to a larger scale and longer storage times in order to optimize shelf stability during storage.

Acknowledgments
The authors thank Jack Inestine, Kentwood, LA for providing samples of worm castings, Les Ewing for proving samples of sugarcane filter mud compost and Rick Buhr from American Biocarbon, LLC for providing the sugarcane bagasse biochar. The authors thank Renee Bigner for sample analysis. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture. USDA is an equal opportunity provider and employer.

Funding
This study was funded by the United States Department of Agriculture, Agricultural Research Service. No grants or other forms of outside funding were used.

Competing interests
The authors declare no competing interest.

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Citation information
Cite this article as: Microbial stability of worm castings and sugarcane filter mud compost blended with biochar, Isabel M. Lima & Maureen Wright, Cogent Food & Agriculture (2018), 4: 1423719.

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