Land Applied Residual Effects on Soil Biogeochemical Properties

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Abstract

The production of bioethanol using lignocellulosic feedstock can result in the co-production of high nutrient quality bioenergy residues that can be land applied as soil amendments. Residues can reduce the need for synthetic fertilizers, provide plant available nutrients, limit nutrient leaching, and improve carbon (C) sequestration potential in the soil. However, there is little to no literature exploring the effects of residue application on microbial activity and how the resulting microbial stimulation affects the biogeochemical transformation and nutrient release of C and nitrogen (N) in the soil. To determine how the land application of bioenergy residues affects C and N dynamics, the physico-chemical and nutrient composition of several bioenergy residues were characterized and then applied to grassland soil samples during a laboratory incubation study. Residue-amended soil samples from a field study were also collected. Residue-amended samples were used to measure aerobic microbial respiration, enzyme activity, microbial biomass carbon, as well as nutrient contents. Residue amendment stimulated microbial activity, organic N mineralization rates, and the release of plant available nutrients. Plant-based residues, especially, stimulated microbial activity, suggesting that the residues could be used instead of, or in conjunction with, conventional synthetic fertilizers to provide plant available nutrients. Land applying bioenergy residues as a soil amendment promotes sustainable land practices, while reducing waste residue disposal issues.

1. Introduction

Rising energy prices, along with concern about petroleum supplies and greater environmental awareness, have stirred increased demand for biofuels for energy consumption (Goldemberg et al., 2014; Dodder et al., 2015; Guo et al., 2015). Although first generation biofuels have been explored extensively, their sustainability has been questioned, and second-generation biofuels have been considered an improved energy alternative that can overcome the limitations of first generation biofuels (Antizar-Ladislao and Turrion- Gomez, 2008). Second-generation biofuels, particularly those derived from lignocellulosic material, consist of non-food biomass including agricultural residues, forest residues, municipal solids, and industrial wastes (Naik et al., 2010).

Like first generation biofuel production, the production of second generation biofuel involves hydrolyzing polysaccharides into sugars, followed by fermentation to create bioethanol (Coyle, 2007). In addition to the production of biofuel, the production process results in vast amount of bioenergy residue (here forward, known as residue or residues) that needs to be properly utilized or disposed. The amount of residue produced with biofuels is significant, with approximately 20 liters of stillage produced for each liter of ethanol (Wilkie et al., 2000). There are many ways in which biofuel residues can be reused, however, one of the most beneficial is for land application as a soil amendment to supply some of the plant nutrients.

If land applied in appropriate amounts, residues can be a source of nutrients and organic matter, increasing the biodiversity and activity of microorganisms in the soil. Residue application influences a range of biogeochemical processes in soils including organic matter (OM) and nutrients cycling (Bulluck et al., 2002; Rivero et al., 2004; Tilman et al., 2009; Brown et al., 2011; Ding et al., 2014). Residue application can enhance microbial activity, specifically the activity of extracellular enzymes that help decompose OM (Galvez et al., 2012).

Applying organic residues as soil amendments can influence and improve soil physical and chemical properties, therefore enhancing soil health. Residue application can also improve water-holding capacity (WHC), bulk density, and C storage of the soil (Johnson et al., 2007). In the past decade, there have been a number of investigations that have observed the environmental effects of land applying bioethanol residues onto soil (Shi; García-Gil et al., 2000; Johnson et al., 2007; Birikorang et al., 2013; Wang et al., 2014). Yet, there is a need for more research pertaining to the effects of residue application on the biogeochemical transformations of C and N, particularly how residue application pertains to microbial activity and nutrient release

for plant use. This research is particularly important now, as our society searches for alternative fuel options and means to sequester C in earth systems in order to mitigate climate change.

The overall goal of this investigation was to determine the effects of bioenergy residue application on select soil biogeochemical properties, and nutrient release. Objectives in this study were:

i. Characterize the physico-chemical properties of select second generation bioenergy residues.
ii. Determine the short-term effects of residue application on soil enzyme activities and microbial respiration.
iii. Determine the effects of land application of residues to a grassland ecosystem on soil microbial activities and nutrient release.

In this study, we hypothesized that physico-chemical characteristics of residues determine the rate of soil microbial activities and nutrient release. Specifically, we hypothesized that plant-based residues containing large amounts of organic matter and more labile C are used readily in the soil and stimulate microbial respiration and enzyme activities, as well as nutrient release.

2. Materials and Methods

2.1 Characterization of Bioenergy Residues

2.1.1 Sample Collection

The following amendments including bioenergy residues were used in this research: (1) wet cake residue, (2) raw sugarcane bagasse, (3) biosolids, (4) compost, (5) vermicompost, and (6) anaerobic digestate. These amendments were chosen because they are high nutrient content, lignocellulosic materials that have been recycled and been commonly used as organic soil amendments.

We obtained the wet cake residues from the Stan Mayfield Bio Refinery Pilot Plant in Perry, FL. The biofuel residues were produced from sugarcane bagasse feedstock and received as liquid stillage with solid material (known as wet cake). Raw sugarcane bagasse (primary feedstock) from the Bio Refinery was also used in the study to determine changes in soil biogeochemical

properties. In addition, other commonly applied soil amendments such as compost, biosolids, and synthetic fertilizer (ammonium nitrate) were also used in the study to compare the performance with the bioenergy residues.

The biosolids used in this study is a commercial, class AA, thermally dried product called GreenEdge, derived from conventional wastewater treatment processes (Green Technologies, Inc.) that has been treated with sulfate of potash (Miller and O'Connor, 2009). Class AA biosolids are the highest quality biosolids, and are considered pathogen-free by the US Environmental Protection Agency (USEPA) (McIntosh and Oleszkiewicz, 1997) and can therefore can be land applied as an organic amendment. The vermicompost and anaerobic digestate were collected from the University of Florida Bioenergy Park. The vermicompost used in this study was derived from dairy cow manure that was composted for a minimum of 15 days at 13°C or higher. The composted manure was then fed to earthworms, after which the castings were harvested and separated through a 0.125 in mesh size screen. The anaerobic digestate used in this study was derived from a mesophilic anaerobic digester fed with flushed dairy manure. After collection, the digestate was stored in a large container in a walk-in cooler (6°C) for a week before characterization. The ammonium nitrate is a commercial high-N fertilizer (34% N; NPK: 34-0-0) purchased locally. All amendments were independently collected as one bulk sample.

2.1.2 Analytical methods

Amendments were well homogenized by mixing bulk samples in their containers by hand. Amendment pH was measured using a Fisher AR50 pH meter (Fisher Scientific, Pittsburg, PA). Subsamples were oven dried at 70°C to constant weight. The amendment dry weights were used to calculate moisture content. All physico-chemical analyses were reported based on the oven dried weights. After drying, amendments were passed through a 0.42 mm sieve and finely ground into powders using a SPEX SamplePrep 8000M Mixer/Mill (Thomas Scientific, Swedesboro, NJ) and stored in scintillation vials. Oven dried samples were used to determine total nitrogen (TN), total carbon (TC), and total phosphorus (TP) content. Oven dried samples were also used for determining Mehlich-1, 3, and 1 M HCl extractable metals and P. Fresh, non-dried samples were used to test for water extractable P, ammonium (NH_4^+-N), and nitrate (NO_3^--N), as well as potassium chloride (KCl) extractable NH_4^+-N. All analyses were run in lab-generated triplicates. Organic matter (OM) content was determined using loss-on-ignition (LOI)

combustion method (Heiri et al., 2001) Briefly, oved dried samples were heated in a muffle furnace at 550°C for 4 hours, resulting in the combustion of all organic matter, leaving behind mineral material.

To determine total C and total N, oven-dried, ground samples were combusted at 900°C using a Thermo Flash EA 1112 elemental analyzer (CE Elantech Inc., USA) at the Isotope Mass Spectrometer Lab in the Soil and Water Sciences Department (University of Florida). Total phosphorus was determined by placing 0.5 g of oven-dried and ground sample in a muffle furnace at 550 °C for 4 h and then digesting samples with 20 mL 6 M HCl solution on a RC-2240 Hot Plate (Thermo Scientific, Waltham, MA) (Andersen, 1976; Wright et al., 2008). Sample digestates were filtered through 41Whatman filter paper and the TP concentration of digested solutions determined by the ascorbic acid-molybdenum blue method using a spectrophotometer (Shimadzu Spectrophotometer UV-1800, Norcross, GA) (Kuo 1996).

Water extractable nutrients (P, NH_4^+-N, and NO_3^--N) were determined by adding 25 ml of DDI water to a wet soil equivalent of 2.5 g of dry soil in a 50-mL centrifuge tube (1:10 sample to solution mass ratio). Tubes were shaken at 125 rpm for an hour, and then centrifuged at 6000 rpm for 10 min. The supernatant was filtered using 0.45 µm membranes into 20 mL scintillation vials. Water extractable P was analyzed using a spectrophotometer. Water extractable NH_4^+-N and NO_3^--N was analyzed using an AQ2 Discrete Analyzer (Seal Analytical, Mequon, WI) based on methods by Kuo (1996).

KCl extractable NH_4^+-N, representing the inorganic fraction of N, was determined with 2 M KCl solution (Bremner and Keeney, 1966) and analyzed colorimetrically using an AQ2 Discrete Analyzer (Method 350.1, USEPA 1983). Wet soil (2.5 g) was mixed with 25 mL of 2 M KCl solution followed by 1 h of shaking at 125 rpm and 10 min of centrifugation at 6000 rpm. The subsequently filtered extract was analyzed for NH_4-N using a Alpkem Flow Solution IV (O.I. Analytical, College Station TX). Similarly, inorganic P was determined using 25 mL of 1 M HCl solution (Reddy et al., 1998). Samples were shaken at 125 rpm for 3 h and then centrifuged at 6000 rpm for 10 min, filtered, and supernatant was analyzed spectrophotometrically. Additionally, P and metals were extracted (K^+, Mg^{2+}, Ca^{2+}, and Fe^{3+}) using Mehlich-1 and Mehlich-3. All P concentrations were measured spectrophotometrically at the WBL in Gainesville, FL using Method 350.1 (USEPA 1983). Metal concentrations were measured using

ICP-AES at the Institute of Food and Agricultural Science, Analytical Research Lab at the University of Florida, Gainesville, following USEPA Method 200.7 (USEPA, 2001).

In addition to extractable nutrient analyses, ^{13}C-NMR spectroscopy was used to determine the C functional groups present in each of the soil amendments (Pichler et al., 2000; Piterina et al., 2009). Air dried and ground amendment samples were placed in individual rotors and the resulting spectra were divided into 4 common chemical shift regions (alkyl C; 0-45 ppm, O-alkyl C; 45-110 ppm, aromatic C; 110-160 ppm, and carbonyl C; 160-220 ppm). The relative intensities for each region were then determined by integration using an Avance III spectrometer (Bruker Bio-Spin) (McKnight Brain Institute at the University of Florida, Gainesville**)**. Spectroscopy data were collected using TopSpin software (ver 3.2 pl5).

2.2 Laboratory Incubation

2.2.1 Sample Collection

For this study, fresh surface (0-10 cm) soil cores were collected from a grassland ecosystem at the UF Plant Science and Research & Education Unit (PSREU) in Citra, Florida. The soil is classified as Kanapaha fine sand, classified as a loamy, siliceous, semiactive, hyperthermic Grossarenic Paleuquults (Shi). Thirty soil cores were collected and composited in a large plastic bag, placed on ice, and transported to the Wetland Biogeochemistry Lab (University of Florida). Bulk soil was stored in a walk-in cooler (4°C) for 2 days before experimentation and analysis. Soil was passed through a 2-mm mesh sieve to remove rock and root fragments and then the bulk soil was stored in air-tight containers. Untreated Citra field soil amended with different residues were used for the laboratory incubation study.

2.2.2 Experimental Setup

Glass Mason jars (473 ml) and serum bottles (120 ml) were used to incubate residue-amended soils in the short-term laboratory incubation study. Each jar contained 20 g of untreated, field moist soil amended with either wet cake (5.3 mg g^{-1}), raw sugarcane bagasse (23 mg g^{-1}), compost (15 mg g^{-1}), biosolids (1.8 mg g^{-1}), vermicompost (5.1 mg g^{-1}), and anaerobic digestate (3.5 mg g^{-1}).

Table 1. Amendment weights corresponding to the recommended loading rate. Carbon, N, and P weights represent the amount of nutrients in the amendment added to the soil.

Amendment	Total amendment dry weight (g)	Carbon (mg C in 20 g^{-1} soil)	Nitrogen (mg N in 20 g^{-1} soil)	Phosphorus (mg P in 20 g^{-1} soil)
Wet Cake	0.113	57.8	2.38	0.23
Raw Bagasse	0.476	234.7	2.38	0.17
Biosolids	0.038	14.6	2.38	0.57
Anaerobic Digestate	0.082	36.8	2.38	0.57
Vermicompost	0.125	28.2	2.38	0.24
Compost	1.400	222.6	2.38	0.52
Synthetic Fertilizer	0.113	0	2.38	0

Assuming bulk density = 1.4 g cm^3; soil volume = $1.5x10^9$ cm^3; soil weight = $2.1x10^9$ g

The residues were added at a rate of 250 kg N ha^{-1} (total N). This rate was chosen based on past studies that used similar N loading rates for producing elephantgrass in the southeastern United States (Woodard and Sollenberger, 2008; Fedenko et al., 2013; Reyes-Cabrera et al., 2017) This rate was estimated specifically for each residue by determining the total N content of each of the residues and then calculating the amount of N required to satisfy the loading rate in 20 g of soil contained in each jar (Table 1). In addition to the amendment treatments, non-amended soil control and synthetic fertilizer (ammonium nitrate) were also prepared for a total of 8 treatments. After the amendments were added, soils from each of the treatments was then used to prepare sample sets for nutrient analyses and enzyme activity analyses. After mixing the amendments with the untreated soil, the 40 serum bottles and 36 glass jars were covered with moist paper towels to minimize moisture loss but allow aeration, encouraging that aerobic conditions prevailed for the entire length of incubation. The containers were kept in large plastic bins arranged in a randomized block design in a climate controlled (25°C ± 0.50) laboratory setting for the 28 days of incubation.

Jars were removed from the incubation bins at day 0 (start of incubation) and day 28 (end of incubation) to analyze concentrations of soil nutrients, including water extractable P, NH_4^+-N, NO_3^--N, KCl extractable NH_4^+-N, Mehlich-1, Mehlich-3, and HCl extractable P and metals (K^+, Ca^{2+}, Mg^{2+}, and Fe^{3+}). Additionally, TN, TC, TP, pH, and LOI analyses were determined. All analyses consisted of 3 laboratory replicates for each of the 8 treatments.

2.2.3 Enzyme Analysis

Enzyme analysis was conducted on four soil amendment treatments, including control soil, wet cake, bagasse, and anaerobic digestate. These four treatments were chosen because they had the highest OM content (LOI%) and greatest amount of total C content. The activities of three enzymes associated with C, N, and P cycling in decomposition and mineralization of organic nutrients in soils were assessed. Enzymes were ß-1,4-glucosidase (BG), involved in catalyzing cellulose degradation; leucine aminopeptidase (LAP), involved in degradation of proteins; and acid phosphatase (PHO), involved in hydrolyzing monoester bonds (Sinsabaugh et al., 2008; Nannipieri et al., 2011; Singhania et al., 2013). activity was determined using a fluorometric method, previously described by (Inglett et al., 2011; Liao et al., 2013). One gram of amended field moist soil was removed from each of the selected treatment enzyme jars and diluted with DDI water to create a soil slurry homogenate sample. Assays were set up in enzyme microplates consisting of 96 wells. Subsamples of soil homogenate was incubated with fluorometric substrate, and buffer solution. Substrates used were methylumbelliferone (MUF) or AMC. Samples were incubated up to 3 h and sample fluorescence was measured at 360 nm excitation and 460 nm emission using plate reader a Bio-tek® model Gen5 (Biotek Instruments, Inc. Winooski, VT) to determine a kinetic curve. Standard curves were determined using 4-methylumbelliferone (MUF) or AMC and quench curves were also determined to account for potential soil quenching. Enzyme activity was reported as nmol enzyme released g^{-1} dry weight soil (dws) h^{-1}. Enzyme activities were measured at three times (days 8, 17, and 29) during a 29-day incubation period.

2.2.4 Soil Respiration

To determine the effect of amendments on soil respiration, 40 microcosms were set up for the 8 treatments. For each treatment 5 replicates were prepared by weighing out 5 g of amended soil samples in serum bottles (120 mL). Microbial respiration rates in soil with residues were determined over a period of 4 weeks. Rates of soil respiration (CO_2 production over time) were measured twice a week during the first two weeks of the incubation study, and once a week for the remaining 2 weeks, totaling in 6 respiration measurements. Respiration measurements were conducted periodically over an 80-min time period. Concentration of CO_2 in headspace was determined using a Shimadzu gas chromatograph equipped with a thermal conductivity detector

(120°C injection, 40°C detector) (Shimadzu Scientific Instruments, Columbia, MD). Carbon dioxide was determined by calculating the linear regression of the CO_2 peaks and using the ideal gas law. Calculated gas concentrations in the headspace were reported as rates of accumulated CO_2-C (µg CO_2-C g^{-1} dws h^{-1}). Calibration curves were determined using standard gas mixtures (Scotty Specialty Gases, Plumsteadville, PA) using a 1.8 m (80/100) Porapak N column (Supelco Inc., Bellefonte, PA).

2.3 Residue Application Field Study

In this study, we investigated microbial activity as an indicator for biogeochemical cycling after the land application of lignocellulosic residues. This study provides a snapshot perspective on residue land application in a long-term study, as opposed to the laboratory study, which was a short-term application experiment. In addition, this study is useful because it takes place in a grassland ecosystem, where biofuel feedstock crops can be grown and recycled for bioethanol and residue amendment, creating a potentially cyclical and sustainable agricultural practice of amendment use and reuse. Soil samples used for this study were collected from a field site in Citra, FL, where grassland field plots had been treated with different soil amendments for 3 years (2013-2015). The treatments were the following:

1. Elephantgrass (*Pennisetum purpureum*) fertilized with a low rate of N fertilizer (ammonium nitrate; NH_4NO_3; 34-0-0) at a rate of 50 kg N ha^{-1} yr^{-1}
2. Elephantgrass fertilized with a low rate of N fertilizer (ammonium nitrate; NH_4NO_3; 34-0-0) and wet cake residue (10 Mg ha^{-1} yr^{-1})
3. Elephantgrass fertilized with a low rate of N fertilizer (ammonium nitrate; NH_4NO_3; 34-0-0) and pyrolyzed biochar (5 Mg ha^{-1} yr^{-1})
4. Elephantgrass fertilized with a high rate of N fertilizer (ammonium nitrate; NH_4NO_3; 34-0-0) at a rate of 250 kg N ha^{-1} yr^{-1} (Reyes-Cabrera et al., 2017).

The field experiment was organized in a block design, made up of 4 rows in which each row contained replicate microplots of all 4 treatments (4 treatments x 5 replicates = 16 microplots) (see supplementary materials). Within each of the wet cake-treated plots, 5.5 kg of wet cake (estimated 220 g N) was applied as dried, evaporated material (Shi 2015). The wet cake material

used in the field study differed slightly in chemical composition compared to the wet cake that was collected and used in our laboratory study. These differences in chemical composition most likely occurred due changes and optimization of fermentation techniques during the bioethanol production process (Shi 2015). The wet cake material used in this study had a C concentration of 486 g kg^{-1} and 40 g kg^{-1} N. The biochar applied to the field was made up of reclaimed pine bark char that had been pyrolyzed for 30 min at 760°C (Reyes-Cabrera et al., 2017). The biochar had a C concentration of 625 g kg^{-1} and 5 g kg^{-1} N.

Four soil cores (0-15 cm) were collected from each of the plots stored in Ziploc bags placed on ice to be transported to the Wetland Biogeochemistry Laboratory in Gainesville, Florida, where they were stored in a walk-in cooler (6°C) for 24 h before the samples were prepared for soil incubation study. Soil samples were sieved through a 2-mm mesh sieve to remove rock and root fragments. Initial physico-chemical and biogeochemical analyses of soil samples included moisture content, LOI, pH, TN, TC, TP, MBC, KCl extractable NH_4^+-N, water extractable P, NH_4^+-N, and NO_3^--N. Phosphorus and metal (K^+, Ca^{2+}, Mg^{2+}, and Fe^{3+}) concentrations were measured using 1 M HCl acid, Mehlich-1, and Mehlich-3 solutions using methods USEPA 365.1 and 200.7, respectively (USEPA, 1983 and USEPA, 2001).

Microbial biomass carbon (MBC) was measured on the soil samples using the fumigation-extraction method (Vance et al., 1987). Microbial biomass carbon was analyzed using a 5050A TOC auto-analyzer (Shimadzu Corp., Columbia, MD; EPA method 415.1). A combined extraction efficiency factor of K_{EC} = 0.37 was applied (Sparling et al., 1990). Enzyme assays were also determined in this study. N-acetylglutamate (NAG) and α-1,4-glucosidase (AG) were also measured, in addition to PHOS, LAP, and BG. Enzyme activity was determined as described earlier. Enzyme activity was measured only once, two days after sample collection.

Aerobic respiration was measured using the same method described previously. Respiration was measured four days after field collection. Five grams of soil from each of the 16 plots were placed into serum bottles (4 reps from each treatment totaling in 16 respiration samples). Respiration was measured in a 96-min time period to determine the respiration rate of each treatment. In another short respiration experiment, 5 g of soil from each of the 16 plots were placed into clean serum bottles and incubated over a 7-day period. Respiration was measured from each sample at days 0, 4, and 7 using a 90-160 min respiration period.

2.4 Statistical Analysis

To ensure Quality Assurance/Quality Control (QA/QC), three replicates of each treatment were incubated. Additionally, method blanks and two samples random samples were selected for each analysis as analysis replicates. A standard soil (provided by the Soil Fertility Lab in Gainesville Florida) was also used to ensure the accuracy of the total P analysis. Two method blanks, 1 matrix spike, and 1 continuing calibration check standard (CCCS) were also included in analyses. For each analysis, a standard linear regression (R^2 values > 0.995) was determined.

All data were checked for normality and analyzed statistically ($\alpha = 0.05$) using JMP statistical software (version 12.2.0; SAS Institute Inc., Cary, NC). For the lab incubation study, regression analyses were used to determine if there was a linear relationship between each variable with total C content (Table 1). Canonical discriminant analysis was used to assess the differences between the characterized residues. One-way analysis of variance (ANOVA) was used to evaluate significant differences in treatment means, followed by Tukey's HSD at a significance (α) level of 0.05 to establish statistically significant differences between residue treatments. Regression analyses were used to identify the parameters that most impacted the rates respiration and enzyme activities. Data from the incubation and field study were also analyzed using multi-factor ANOVA to compare the means of the data. Simple correlations and regression analysis were used to evaluate any linear relationships between the characteristics of the amendments and rates of enzyme activities, rates of respiration, and MBC. Multivariate correlation was used to determine which variables affect microbial activity enzyme activity, respiration, and MBC, therefore affecting the turnover of nutrients in the incubated samples and in the field.

3. Results

3.1 Physico-chemical Characteristics of Residues

The residues studied in this investigation varied widely in chemical composition (Table 2). The amounts of total C, N, and P in each of the treatments were significantly different ($P<0.05$). Canonical discriminant analysis was used to compare the residues and determine their differences based on the parameters characterized (Figure 1). In this figure, one can easily see that the wet cake and biosolids, in particular, varied significantly in physico-chemical properties, compared to the other residues. The mean content for the listed variables increases from left to

right. Table 3 shows the phenotypic correlations between the original variables and the canonical variates. The variables listed at the top of the table are high correlations; they are the driving force for the discrimination between residues. The analysis reports that the driving forces in the first canonical variate are KCl extractable NH_4^+ ($R^2 = 0.95$), Water extractable NH_4^+ ($R^2 = 0.97$), and HCl extractable P ($R^2 = 0.98$).

Wet cake and bagasse had the greatest OM and TC contents. Wet cake had a TC value of 510 ± 7.1 g C kg^{-1} residue followed by bagasse with a TC value of 493 ± 16 g C kg^{-1} residue. Anaerobic digestate and biosolid had the next highest TC contents, followed by vermicompost, and compost (449 ± 1.5, 386 ± 7.5, 225 ± 23, and 159 ± 45 g C kg^{-1} soil, respectively). Wet cake had an OM content of 98 ± 0.36% and bagasse had an OM content of 97 ± 0.04%. Anaerobic digestate and biosolid also had high OM contents, followed by compost, and vermicompost with lower OM contents (87 ± 0.38, 75 ± 0.20, 40 ± 1.6, and 38 ± 0.10%, respectively). Though some of the residues showed similarities in C concentration, the ^{13}C-NMR data showed differences in biochemical makeup of C functional groups in the residues (Table 4) (Figure 2). Wet cake had the highest aryl-C concentration (29%), which is mostly made up of aromatic lignins, phenols, and aromatic ethers. Compost, vermicompost, and anaerobic digestate followed with an average aromatic C content of 22%. Biosolid and bagasse had the lowest aromatic C contents, averaging 10% aromatic C. Compared to the wet cake, bagasse had the second lowest concentration of aromatic C (11%), as well as the lowest concentration of alkyl-C (4.6%), and the highest O-alkyl concentration (82%). The other residues had much lower alkyl-C contents (average of 41% O-alkyl C).

Table 2. Physico-chemical characteristics of select bioenergy residues (mean ± standard error, n=3 source reps).

Parameter	Units	Treatment					
		Plant-derived Residue		Municipal Solid Waste Residue	Derived from Dairy Manure		
		Wet Cake	Raw Bagasse	Biosolid	Anaerobic Digestate	Vermicompost	Compost
pH		4.5 ± 0.12 ^{d}	6.9 ± 0.12 ^{b}	5.9 ± 0.09 ^{c}	8.1 ± 0.09 ^{a}	5.7 ± 0.06 ^{c}	8.0 ± 0.15 ^{a}
Moisture Content	%	70 ± 0.16 ^{b}	6.3 ± 0.04 ^{e}	8.0 ± 0.05 ^{e}	93 ± 0.22 ^{a}	51 ± 0.36 ^{c}	39 ± 1.0 ^{d}
LOI	%	98 ± 0.36 ^{a}	97 ± 0.04 ^{a}	75 ± 0.20 ^{c}	87 ± 0.38 ^{b}	38 ± 0.10 ^{d}	40 ± 1.6 ^{d}
Total C	g kg^{-1}	510 ± 7.1 ^{a}	493 ± 16 ^{ab}	386 ± 7.5 ^{c}	449 ± 1.5 ^{b}	225 ± 23 ^{d}	159 ± 45 ^{e}
Total N	g kg^{-1}	21 ± 0.80 ^{c}	5.0 ± 0.09 ^{d}	63 ± 1.1 ^{a}	29 ± 0.31 ^{b}	19 ± 0.76 ^{c}	1.7 ± 0.17 ^{e}
Total P	g kg^{-1}	2.0 ± 0.11 ^{c}	0.35 ± 0.01 ^{d}	15 ± 0.55 ^{a}	7.0 ± 0.15 ^{b}	1.9 ± 0.10 ^{c}	0.37 ± 0.01 ^{d}
C:N		24 ± 0.87 ^{bc}	99 ± 1.7 ^{a}	6.1 ± 0.11 ^{e}	15 ± 0.19 ^{cd}	12 ± 1.6 ^{de}	104 ± 5.0 ^{a}
C:P		256 ± 16 ^{c}	1412 ± 46 ^{a}	26 ± 0.49 ^{de}	64 ± 1.4 ^{de}	120 ± 17 ^{d}	436 ± 2.1 ^{b}
N:P		11 ± 0.49 ^{b}	14 ± 0.23 ^{a}	4.3 ± 0.12 ^{c}	4.1 ± 0.14 ^{c}	10 ± 0.61 ^{b}	4.6 ± 0.38 ^{c}
KCl Extractable NH_4^+	g kg^{-1}	8.4 ± 0.16 ^{a}	0.03 ^{d}	5.4 ± 0.09 ^{b}	0.5 ± 0.04 ^{c}	0.004 ^{d}	0.003 ^{d}
Water Extractable							
P		0.48 ± 0.01 ^{b}	0.05 ^{d}	0.65 ^{a}	0.31 ± 0.02 ^{c}	0.30 ± 0.02 ^{c}	0.05 ^{d}
NH_4^+	g kg^{-1}	8.5 ± 0.10 ^{a}	0.04 ± 0.01 ^{d}	4.5 ± 0.03 ^{b}	0.55 ± 0.05 ^{c}	0.01 ^{d}	0.01 ^{d}
NO_3^-		0.003 ^{d}	0.004 ^{d}	4.0 ± 0.07 ^{a}	0.44 ± 0.07 ^{c}	3.6 ± 0.02 ^{b}	0.004 ^{d}

Levels not connected by the same are significantly different (Tukey's test, α=0.05)

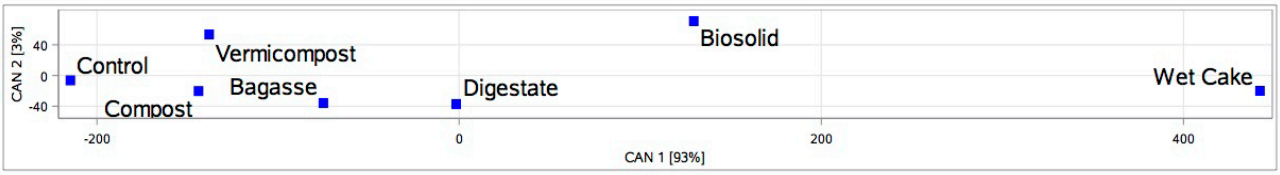

Figure 1. Canonical discriminant analysis shows the relationship between the residues.

Table 3. Phenotypic correlations between the original variables and the canonical variates in a canonical discriminant analysis

Observations	Variable	Can1	Can2
1	LOI	0.694688	-0.249995
2	TN	0.474337	0.639965
3	KCl-NH_4^+	0.954363	0.201760
4	Water ext. NH_4^+	0.966261	0.135628
5	Water ext. NO_3^-	0.009557	0.953797
6	CN ratio	-0.314734	-0.546083
7	HCl ext. P	0.979459	0.080255
8	Mehlich-1 P	0.641596	0.544280
9	Mehlich-3 P	0.385683	0.232335

Table 4. ^{13}C-NMR characteristics of select bioenergy residues

Functional groups	Units	Control	Treatment					
		Citra Soil	Wet Cake	Raw Bagasse	Biosolid	Compost	Anaerobic Digestate	Vermicompost
Alkyl C	%	-	11 [e]	4.6 [f]	42 [a]	16 [d]	23 [c]	27 [b]
O-Alkyl C	%	-	56	82	36	59	51	45
Aromatic C	%	-	29	11	9.4	23	20	22
Carboxyl C	%	-	3.7 [d]	2.5 [e]	13 [a]	1.7 [f]	5.8 [c]	6.7 [b]
Ratio of select functional groups								
Alkyl C : O-Alkyl C			0.20	0.06	1.2	0.27	0.45	0.60
O-Alkyl C : Aromatic C			1.9 [e]	7.5 [a]	3.8 [b]	2.6 [c]	2.6 [c]	2.0 [d]

Levels not connected by the same letter are significantly different (Tukey's test, α=0.05)

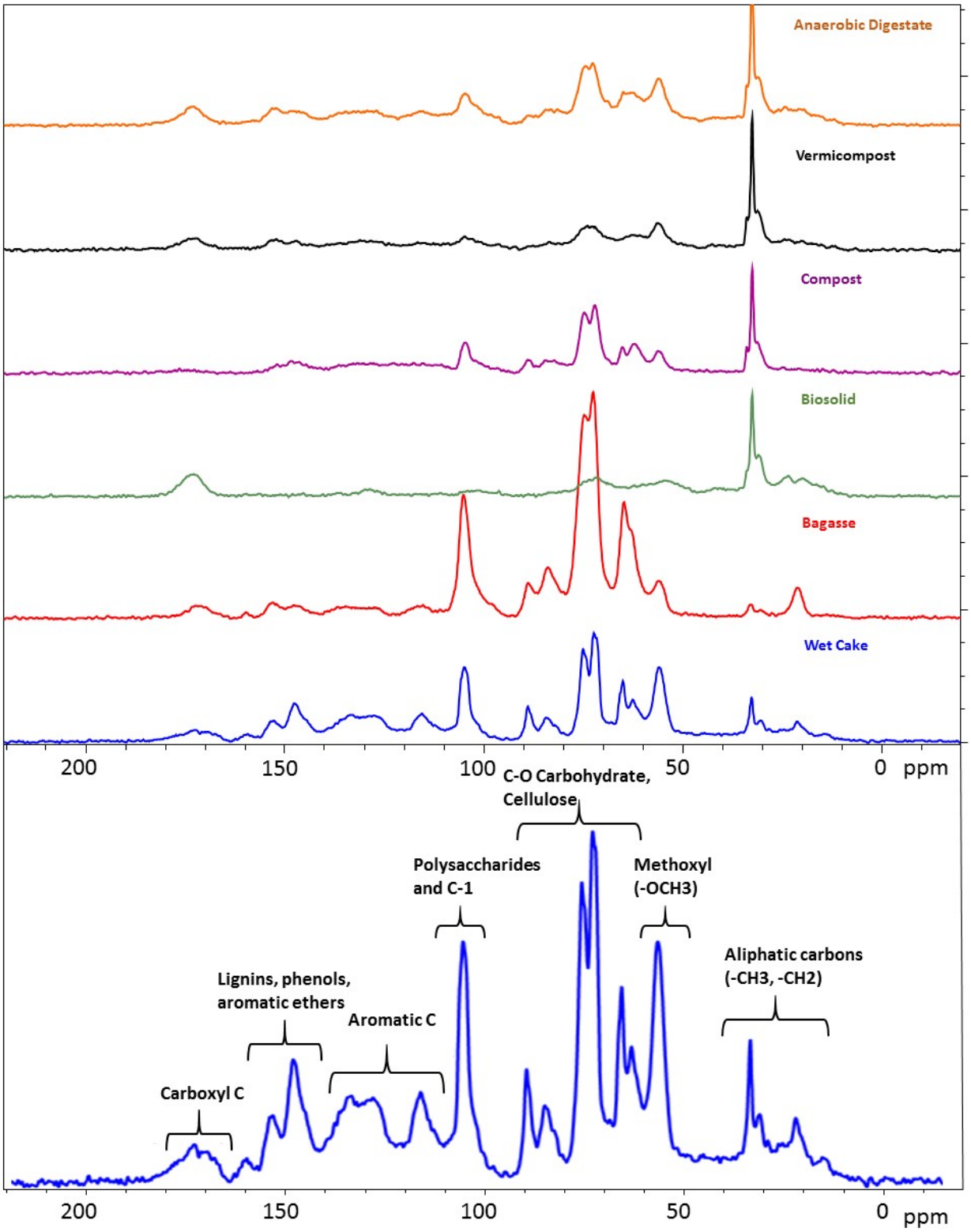

Figure 2. ^{13}C NMR spectra for 6 bioenergy residue amendments. With labeled C functional groups on wet cake residue.

^{13}C-NMR data showed differences in biochemical makeup of C functional groups in the residues (Table 2) (Figure 2). Wet cake had the highest aryl-C concentration (29%), which is mostly made up of aromatic lignins, phenols, and aromatic ethers. Compost, vermicompost, and anaerobic digestate followed with an average aromatic C content of 22%. Biosolid and bagasse had the lowest aromatic C contents, averaging 10% aromatic C. Compared to the wet cake, bagasse had the second lowest concentration of aromatic C (11%), as well as the lowest concentration of alkyl-C (4.6%), and the highest O-alkyl concentration (82%). The other residues had much lower alkyl-C contents (average of 41% O-alkyl C).

The biosolids had the greatest TN content of all the residues (63 ± 1.1 g N kg^{-1} residue), followed by anaerobic digestate, wet cake, and vermicompost (29 ± 0.31, 21 ± 0.8, and 19 ± 0.76 g N kg^{-1} residue, respectively). Bagasse and compost had the lowest TN contents (5.0 ± 0.09 and 1.7 ± 0.17 g N kg^{-1} residue, respectively). KCl extractable NH_4^+-N was highest in wet cake and biosolid (8.4 ± 0.16 and 5.4 ± 0.09 g NH_4^+-N kg^{-1} residue, respectively) accounting for approximately 40 and 8.6% of the total N in wet cake and biosolids, respectively (Table 2). KCl extractable NH_4^+-N was very low in anaerobic digestate, bagasse, vermicompost and compost (average of 0.14 g NH_4^+-N kg^{-1} residue). The amount of KCl extractable NH_4^+-N found in anaerobic digestate, bagasse, vermicompost and compost was approximately 1.7, 0.6, 0.02, and 0.18% of their respective total N contents. Wet cake and biosolid residues also had the greatest water extractable NH_4^+-N (8.5 ± 0.10 and 4.5 ± 0.03 g NH_4^+-N kg^{-1} residue, respectively), accounting for approximately 40 and 7.1% of the total N in wet cake and biosolids, respectively. Water extractable NH_4^+-N in the other residues was also very low (average of 0.15 g NH_4^+-N kg^{-1}). Biosolid and vermicompost had the greatest concentration of water extractable NO_3^--N (4.0 ± 0.07 and 3.6 ± 0.02 g NO_3^--N kg^{-1} residue, respectively) accounting for 6.4 and 19% of the total N in biosolids and vermicompost, respectively. The remaining residues also contained a very small amount of NO_3^--N (average of 0.11 g NO_3^--N kg^{-1} residue). Values for NO_3^--N in wet cake, bagasse, anaerobic digestate, and compost made up 0.01, 0.08, 1.7, and 0.24% of the total N of the residues in that order.

Biosolid and anaerobic digestate residues had the greatest amount of TP (15 ± 0.55 and 7.0 ± 0.15 g P kg^{-1} residue, respectively). Wet cake and vermicompost had similar TP concentrations (2.0 ± 0.11 and 1.9 ± 0.10 g P kg^{-1} residue, respectively), followed by compost and raw bagasse

that very low TP concentrations (0.37 ± 0.01 and 0.35 ± 0.01 g P kg^{-1} residue, respectively). Wet cake and biosolid had the greatest concentration of HCl extractable P (2.1 ± 0.01 and 1.2 ± 0.02 g P kg^{-1} residue, respectively) (see supplementary material), making up approximately 100 and 13% of total P for wet cake and biosolid respectively. The remaining residues made up an average of 0.24 g P kg^{-1}). Bagasse, anaerobic digestate, vermicompost and compost had concentrations of HCl P that accounted for 4.4, 12, 17, and 2.0% of the total P of those residues in that order. Results from water extractable P as well as Mehlich-1 and 3 extractions showed that biosolid had the greatest P concentrations (the three extractions for biosolid yielded an average of 0.67 g P kg^{-1} residue), accounting for 4.3, 20, and 24% of total P for biosolid. Phosphorus extractions for wet cake and anaerobic digestate P resulted in an average of 0.37 and 0.44 g P kg^{-1} residue, respectively. The data showed that the other residues had very little extractable P concentrations.

3.2 Laboratory Incubation Study

Nutrient content changes between day 1 and day 28 of the incubation study showed that the residue nutrients were being consumed, transformed, and cycled by the microbial community. Comparing the physico-chemical characteristics that were measured at the start of incubation versus the end of incubation yielded several results. During the incubation, LOI decreased, as was expected as the microbes used the available substrate for metabolic purposes (see supplementary materials). The pH of residue-amended soils did not show a significant change over the course of the incubation study. The pH of the untreated soil was measured at 8.1 ± 0.07 and is likely high due to past treatments of lime amendment on the lab before the study site was established (Shi 2015).

Expected amounts of total C, N, and P were calculated based on the amount of C, N, or P in the soil plus the C, N, or P added from the amendment. These expected values were compared to the observed total nutrient values measured on day 1 and 28 (see supplementary material).

Over the course of the incubation study, TC decreased in the untreated Citra soil, biosolid, vermicompost, and compost treatments over time. All treatments decreased in TN content while TP increased in some treatments and decreased in others. Total P increased in wet cake, bagasse, and vermicompost. Total P decreased in the untreated Citra soil, biosolid, and compost. Total P content in anaerobic digestate remained the same.

Inorganic NH_4^+-N (2 M KCl extraction) decreased in all treatments, with the exception of the untreated Citra soil, which had a slight increase in available NH_4^+-N. Water extractable P increased slightly in the untreated Citra soil and compost treatments, and decreased slightly in the rest of the treatments. Water extractable NH_4^+-N increased slightly in bagasse, compost, and vermicompost treatments, and decreased in the rest of the treatments. Water extractable NO_3^--N increased in the untreated Citra soil, wet cake, biosolid, compost, and anaerobic digestate. Water extractable NO_3^--N decreased slightly in the bagasse and vermicompost treatments. Metal analyses indicated that metal concentrations remained relatively the same throughout the incubation (see supplementary material).

Carbon dioxide production during respiration by soil microbes was greatly impacted by the different organic residues that were applied to the soil samples. The respiration data show that the more organic-rich (high organic matter) residues had the highest respiration rates at the start of the incubation study (Figure 3). Wet cake and bagasse treatments (highest TC and LOI contents) and were found to have the highest respiration rates to start, followed by a rapid decline in respiration rate over a short period of time. Though measurements were taken up to 28 days, data after 10 days did not show any changes in respiration rates. In addition, the residues with high organic matter had the greatest amount of accumulated CO_2-C by the end of the study (Figure 4). The amount of CO_2-C accumulation is indicative of the amount of CO_2-C respired. In our study, the wet cake treatment had the greatest amount of accumulated CO_2 after each measurement, suggesting that this residue had a greater effect in stimulating the soil microbial community compared to the other residue treatments, resulting in greater CO_2 production. Following wet cake, biosolid and anaerobic digestate had the next highest amounts of CO_2 accumulated. There was a positive correlation between TC of the amendments and the respiration rate of the incubated samples ($R^2 = 0.59$, $P < 0.05$); the greater the total C concentration the greater the respiration rate. Unsurprisingly, there was also a positive correlation between LOI and respiration rate ($R^2 = 0.60$, $P < 0.05$).

Additionally, we reported the total amount of C determined from the soil plus amendment, as well the percent of C lost via respiration calculated from the total C (Table 5). The percentage of total C lost in the residues studied was less than or equal to 2%, except for the control and ammonium nitrate residues, which lost 24 ± 1.9 and 55 ± 2.8% total C respectively. The percentage of total C lost in the residues studied here are minimal compared to the amount of C

lost in a similar study by Cayuela et al., (2010). In their study, Cayuela et al., found that the second generation residues investigated had released approximately 60% of the C applied to the soil over the course of 60 days. It is possible that residues studied in our investigation would have released more C than 2%, but the amount of CO_2-C released decreased dramatically after 10 days of application in our incubation study. This information is particularly important in understanding how the application of residues can potentially impact or mitigate climate change through the emissions of greenhouse gases.

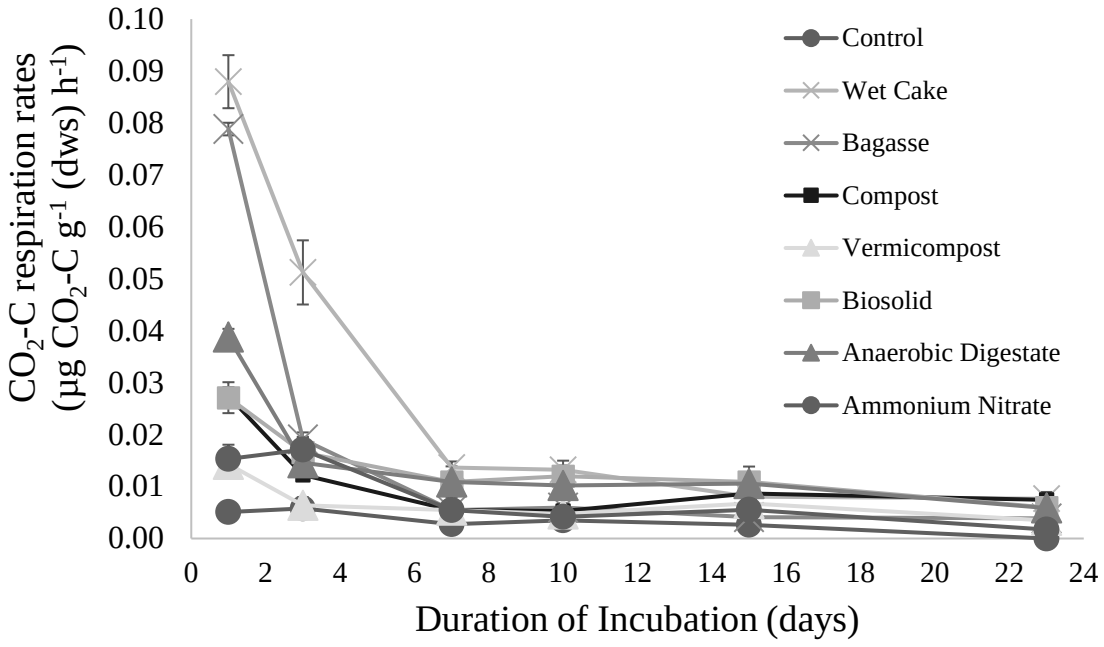

Figure 3. Carbon dioxide microbial respiration rates in a soil-amended samples incubated for a 28-day period (no change occurred after 15 days). Respiration measurements were taken up until day 23. Data represent the mean values with standard errors.

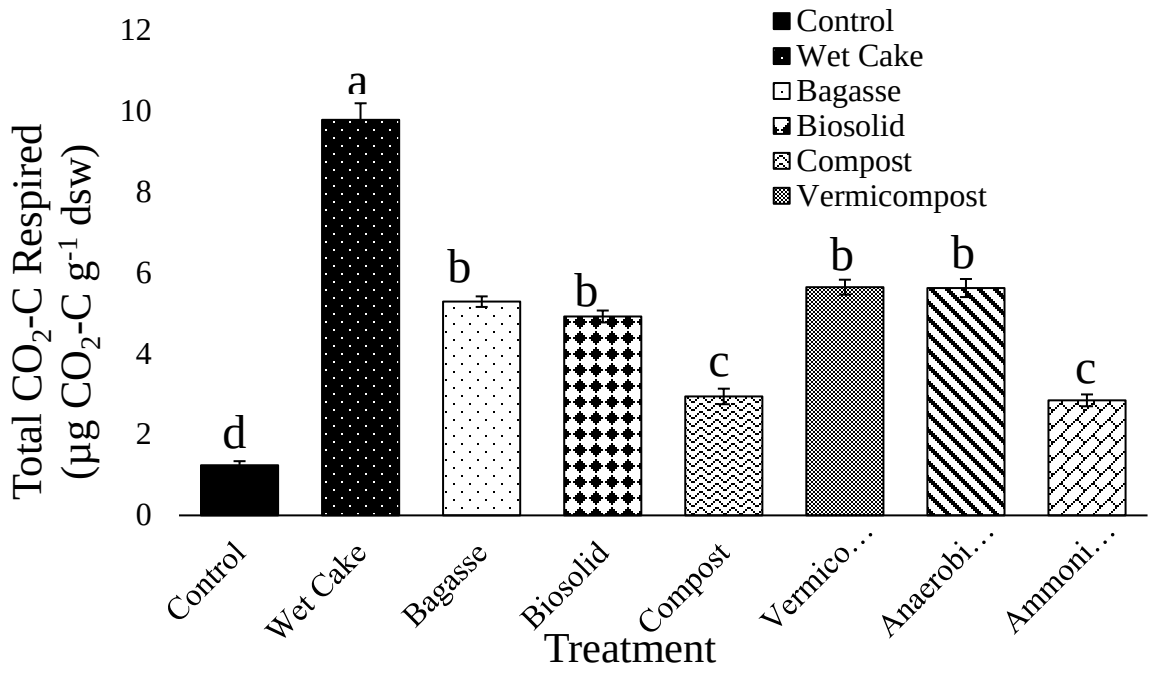

Figure 4. Total amount of CO_2-C respired after 28 days of incubation in the laboratory incubation study. Data represent the mean values with standard errors. Levels not connected by the same letter are significantly different (Tukey's test, α=0.05).

Table 5. Percent of total C lost as CO_2-C after 28 days of incubation, calculated from 5 g soil in aerobic glass serum bottles (mean ± standard error, n=3 source reps).

Treatment	Total C (amendment + soil) mg	Percent of Total C Lost %
Control	26	0.005
Wet Cake	53 ± 0. 22	0.019
Raw Bagasse	139 ± 0.61	0.004
Biosolid	33 ± 0.15	0.017
Compost	49 ± 0.11	0.010
Vermicompost	37 ± 0.11	0.008
Anaerobic Digestate	43 ± 0.28	0.013
Ammonium Nitrate	26	0.011

Results of the enzyme assays showed that all three amendment applications (wet cake, bagasse, and anaerobic digestate) resulted in a rapid increase in enzyme activity, followed by an eventual decrease in activity with time (Figure 5). All amendments maintained higher enzyme

activity than the control throughout the incubation. Enzyme activity seemed to peak 17 days after residue application in both BG and PHO-measured samples, whereas LAP activity continued to increase in the LAP-measured samples (though the rate decreased) throughout the 27 days of incubation.

The bagasse followed by wet cake had the highest PHO activity during the incubation study. Anaerobic digestate had a much lower range of activity (37 to 127 nmol AMC g^{-1} dsw h^{-1}). The untreated Citra soil showed very little PHO activity (max of 67 nmol AMC g^{-1} dsw h^{-1}). PHO was found to have a positive correlation with the N:P ratio of the amendments ($R^2 = 0.70$, $P < 0.05$). Amendments with higher N:P ratios were found to have higher PHO activity.

All treatments showed an increase in LAP activity, with the bagasse residue showing the most significant increase (bagasse ranging from 209 to 2051 nmol AMC g^{-1} dsw h^{-1}). Wet cake and anaerobic digestate followed with approximately half as much LAP activity as seen in the bagasse treatment. The activity of BG followed a similar trend to the PHO activity. Both the wet cake and bagasse treatment resulted in the highest BG activities throughout the incubation study. This data complements the laboratory respiration data, showing that wet cake and bagasse had the greatest respiration activity (Figures 5 and 3, respectively). The activity of BG in both anaerobic digestate and control treatments were low (both treatments below 43 nmol AMC g^{-1} dsw h^{-1}).

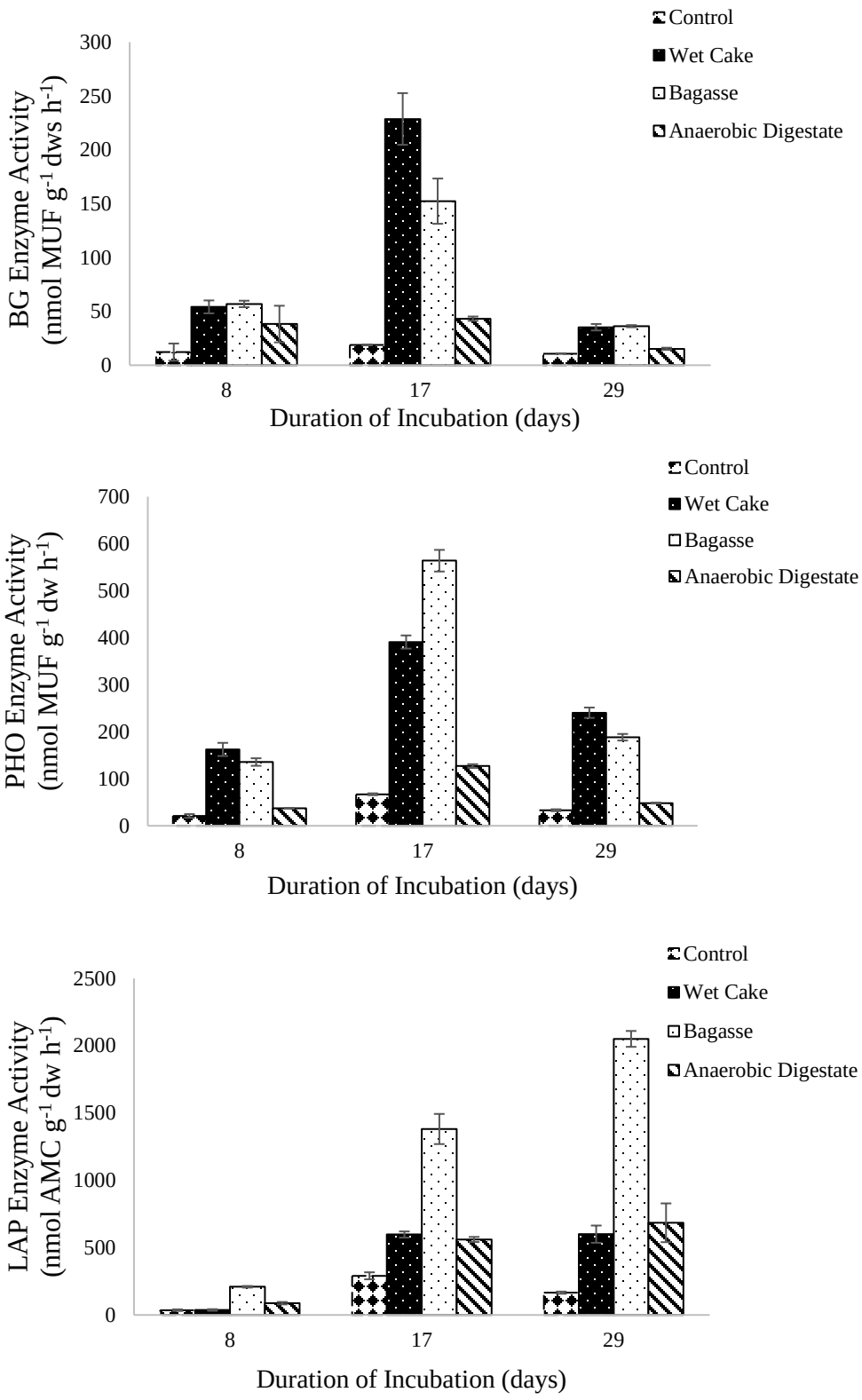

Figure 5. Rates of enzyme activity of β-glucosidase (Di Toppi and Gabbrielli 1999), phosphatase (middle), and leucine aminopeptidase (bottom) in select soil-amended samples during laboratory incubation study over a period of 29 days. Data represent the mean values with standard errors.

3.3 Residue Application Field Study

The nutrient data in this study showed that the amendment of wet cake residue resulted in higher nutrient contents compared to the synthetic N and biochar treatments. The nutrient data showed that the wet cake treatment applied to the grassland soil provided a higher amount of organic matter than the other treatments, as indicated by the LOI and TC values (2.7 ± 0.02 % and 7.5 ± 0.83 g C kg^{-1} soil, respectively) (Table 6). The biochar treatment resulted a similar amount of LOI and TC (6.9 ± 0.82% and 2.5 ± 0.25 g C kg^{-1} soil, respectively). Total C for the low and high N treatments were similar (averaging 5.5 g C kg^{-1} soil).

The wet cake treatment also resulted in the highest TN of all the field treatments (0.58 ± 0.07 and 0.45g N kg^{-1} soil). The low N treatment, biochar, and high N treatments showed less TN in the soil samples (average of 0.35 g N kg^{-1} soil). The wet cake and low N treatments both had the lowest C:N ratios (13), followed by the biochar and high N treatments (19 and 20, respectfully). Wet cake had the greatest concentration of inorganic NH_4^+-N, as suggested by the KCl extractable analysis as well as the greatest concentrations of water extractable NH_4^+-N (3.6 ± 0.46 and 3.2 ± 0.13 mg kg^{-1}, respectively). Biochar had lower KCl and water extractable NH_4^+-N (2.4 ± 1.0 and 2.6 ± 0.64 mg kg^{-1}, respectfully). Both the wet cake and biochar treatments provided the greatest concentration of water extractable NO_3^-- N (3.7 ± 1.1 and 3.7 ± 1.8 mg kg^{-1}, respectively).

All treatments were low in TP (averaging 47 mg P kg^{-1} soil), with the high N treatment providing the least amount of TP. Despite similarities in TP concentration, the water extractable data showed that wet cake had the highest concentration of water extractable P (11 ± 1.6 mg P kg^{-1} soil), followed by the biochar, high and low N treatments (averaging 11 mg P kg^{-1} soil). The wet cake treatment also had the highest C:P and N:P values, suggesting a greater amount of P in relation to C and N nutrients in the residue-amended soil.

Table 6. Physico-chemical characteristics of residue-amended soils (mean ± standard error, n=3 source reps).

Parameter	Units	Treatment			
		Low N	Low N + Wet Cake	Low N + Biochar	High N
pH		7.5 ± 0.08 [a]	6.7 ± 0.27 [b]	7.3 ± 0.13 [ab]	7.3 ± 0.11 [ab]
Moisture Content	%	6.1 ± 0.35 [ab]	5.4 ± 0.58 [ab]	7.7 ± 1.1 [a]	5.9 ± 0.37 [ab]
LOI	%	1.8 ± 0.23 [a]	2.7 ± 0.02 [a]	2.5 ± 0.25 [a]	1.5 ± 0.32 [a]
Total C	g kg^{-1}	5.9 ± 0.41 [a]	7.5 ± 0.83 [a]	6.9 ± 0.82 [a]	5.1 ± 0.21 [a]
Total N	g kg^{-1}	0.45 ± 0.06 [ab]	0.58 ± 0.07 [a]	0.36 ± 0.06 [bc]	0.25 ± 0.01 [c]
Total P	mg kg^{-1}	53 ± 4.7 [b]	50 ± 4.1 [b]	50 ± 4.8 [b]	35 ± 6.1 [b]
C:N		13 [b]	13 [b]	19 [b]	20 [b]
C:P		111 [a]	150 [a]	138 [a]	146 [a]
N:P		8.5 [ab]	12 [a]	7.2 [ab]	7.1 [b]
KCl Extractable NH_4^+	mg kg^{-1}	1.2 ± 0.33 [a]	3.6 ± 0.46 [a]	2.4 ± 1.0 [a]	1.7 ± 0.51 [a]
Water Extractable					
P		2.7 ± 0.23 [ab]	11 ± 1.6 [a]	5.4 ± 0.55 [ab]	3.3 ± 0.70 [b]
NH_4^+	mg kg^{-1}	1.6 ± 0.13 [a]	3.2 ± 0.40 [a]	2.6 ± 0.64 [a]	2.6 ± 0.23 [a]
NO_3^-		2.2 ± 0.25 [a]	3.7 ± 1.1 [a]	3.7 ± 1.8 [a]	2.7 ± 0.15 [a]

Levels not connected by the same letter are significantly different (Tukey's test, α=0.05)

The respiration data show that the wet cake had the highest initial respiration rate at the start of the short incubation (Figure 6). The biochar and N treatments had similar initial respiration rates (averaging 0.01 µg CO_2-C g^{-1} dws h^{-1}). All treatments showed a decline in respiration after 4 days of incubation. Figure 6 shows the respiration rates measured on the first day of the incubation study. From this figure, it is clear that wet cake treatment resulted in the greatest amount of CO_2-C respired. Figure 7 shows the rates of respiration from the three treatments on day 1. The rate of respiration for wet cake was twice as high as the rates of the other treatments.

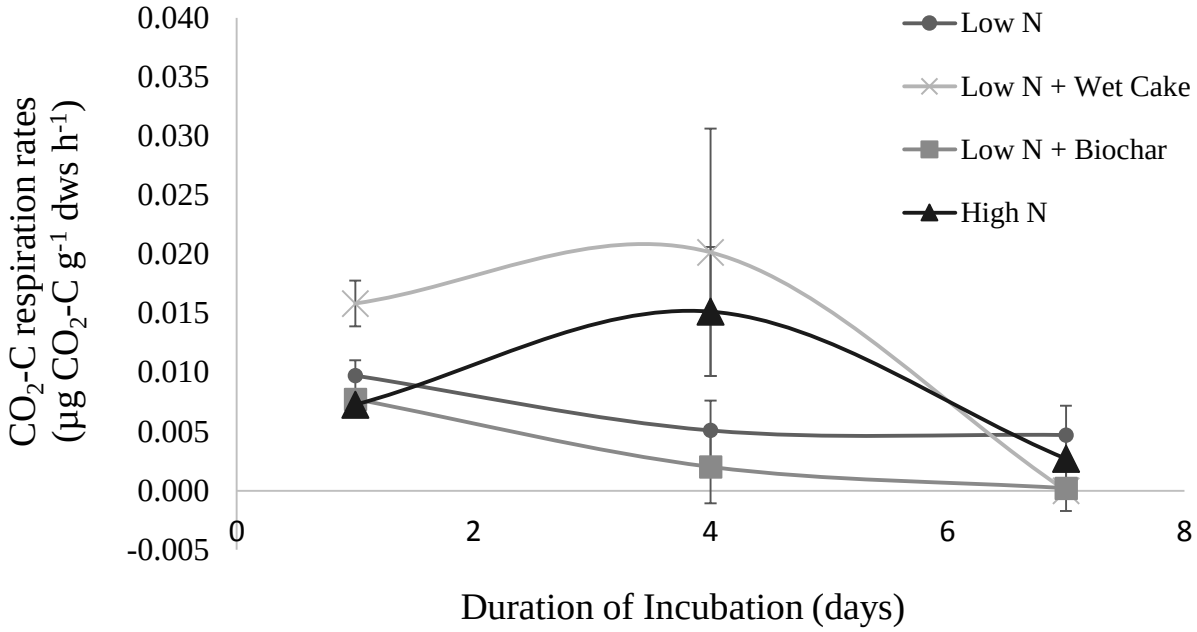

Figure 6. Respiration rates of low N, wet cake, biochar, and high N amendments during a 7-day incubation period. Data represent the mean values with standard errors. Levels not connected by the same letter are significantly different (Tukey's test, α=0.05).

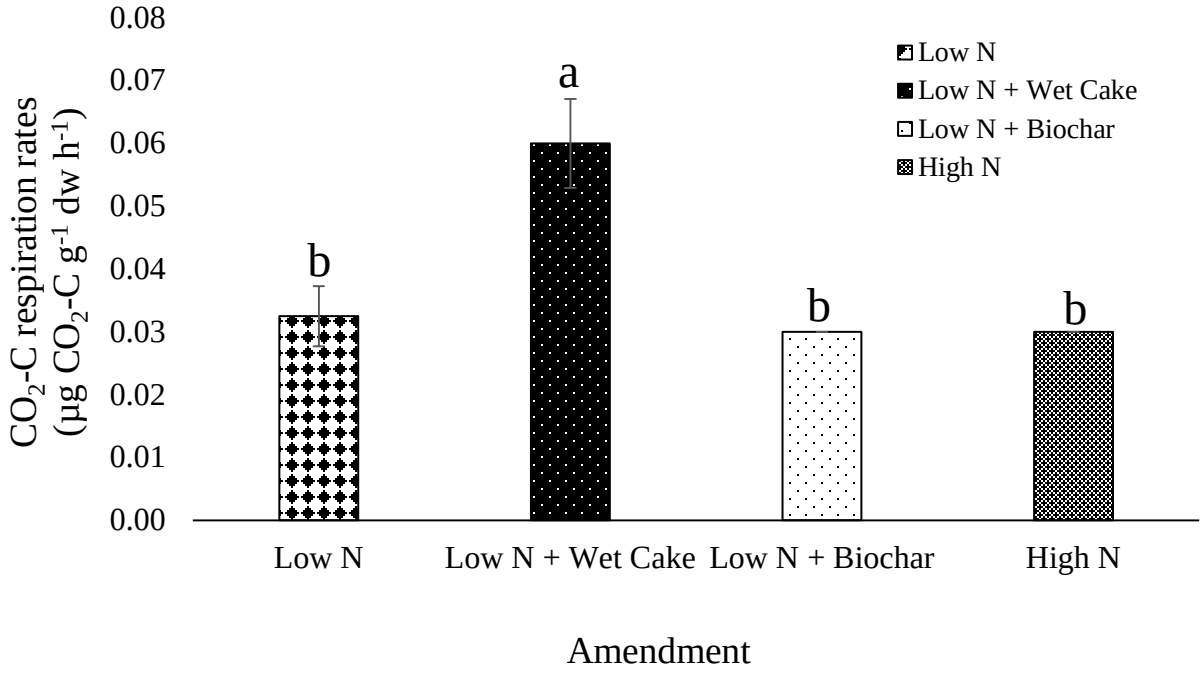

Figure 7. Rate of carbon dioxide respiration in soils amended with low N, wet cake, biochar, and high N. Data represents the mean values with standard errors. Data represent the mean values with standard errors. Levels not connected by the same letter are significantly different (Tukey's test, α=0.05).

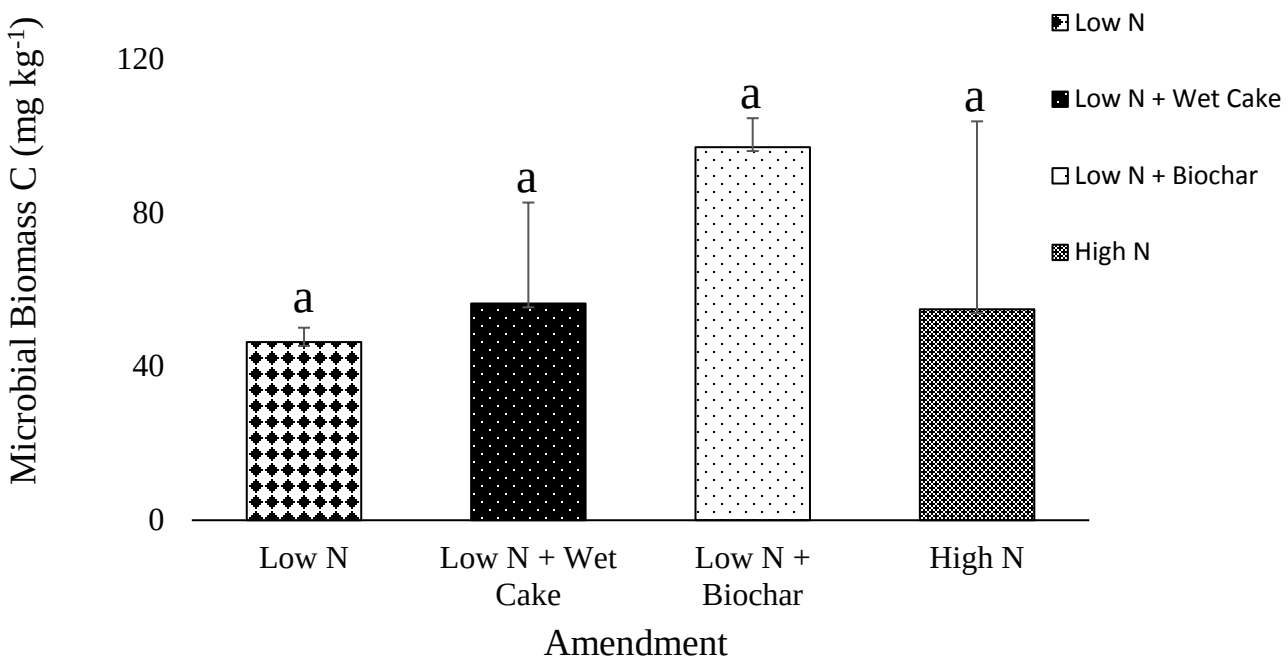

Figure 8. Microbial biomass carbon measurements in soils with low N, wet cake, biochar, and high N amendments. Data represent the mean values with standard errors. Levels not connected by the same letter are significantly different (Tukey's test, α=0.05).

The biochar treatment accumulated the most MBC (97 ± 8.0 mg kg^{-1}) (Figure 8). The wet cake, high N, and low N treatments were found to have approximately half as much MBC as the biochar treatment (56 ± 4, 55 ± 49, and 46 ± 4, mg kg^{-1}, respectively). More C in the wet cake-amended soil seemed to be respired as CO_2, as opposed to the biochar C that was being held in the microbial biomass. In this study, the ratio of C respired as CO_2 over the amount of C held in the microbial biomass was calculated for each treatment (see supplementary materials). The wet cake treatment had the highest ratio of CO_2 respired to C held as MBC, suggesting that more C was being respired than stored in the microbial biomass compared to the biochar-treated soil. On the other hand, the biochar treated plot had the lowest ratio of CO_2 respired to C held as MBC, most likely due to the recalcitrance of the C in the biochar. The C in the biochar was not easily usable by the soil microbes and therefore less C was respired, but instead maintained in the biomass of the microbial community. The data however showed that the amount of MBC measured in each of the field N treatments were not significantly different.

MBC showed a positive relationship with NAG (R^2=0.39, P>0.05) and BG (R^2=0.48, P>0.05) enzymes. Field soils with higher total C and LOI were found to have greater MBC values, suggesting that amendments with high organic matter and available C from the total C could be valuable for providing C to soil microbial communities in the form of biomass.

The results from the enzyme assays show that the wet cake treatment had the greatest concentration of enzyme activity for all the enzymes tested (Figure 9). The wet cake showed the greatest PHO enzyme activity, followed by the high N treatment (345 ± 112 and 298 ± 153 nmol g^{-1} h^{-1}, respectively). The low N and biochar treatments had the lowest PHO activity. The biochar treatment had the same amount of TP as the wet cake treatment, yet, the biochar treatment showed less PHO activity possibly due to the small amount of TP in the high N treatments.

Wet cake and high N treatment also had the highest rates of LAP activity (122 ± 17 and 93 ± 28 nmol g^{-1} h^{-1}, respectively), followed closely behind by the biochar and low N treatments. NAG activity followed the same trend as the LAP data, though the NAG activity in wet cake was significantly greater than the other residues (55 ± 13 nmol g^{-1} h^{-1}), while the other residues averaged 23 nmol g^{-1} h^{-1}. Wet cake had a much higher rate of BG activity than the other residues (89 ± 30 nmol g^{-1} h^{-1}), while the other residues averaged 17 nmol g^{-1} h^{-1}. The activity of AG showed the same trend as BG; wet cake had a higher rate of AG (7.0 ± 2.2 nmol g^{-1} h^{-1}), followed by the much lower activity of the other residues, averaging 3.7 nmol g^{-1} h^{-1}. However, the data show the average rate of LAP, BG, AG, and PHO in the field treatments did not differ significantly.

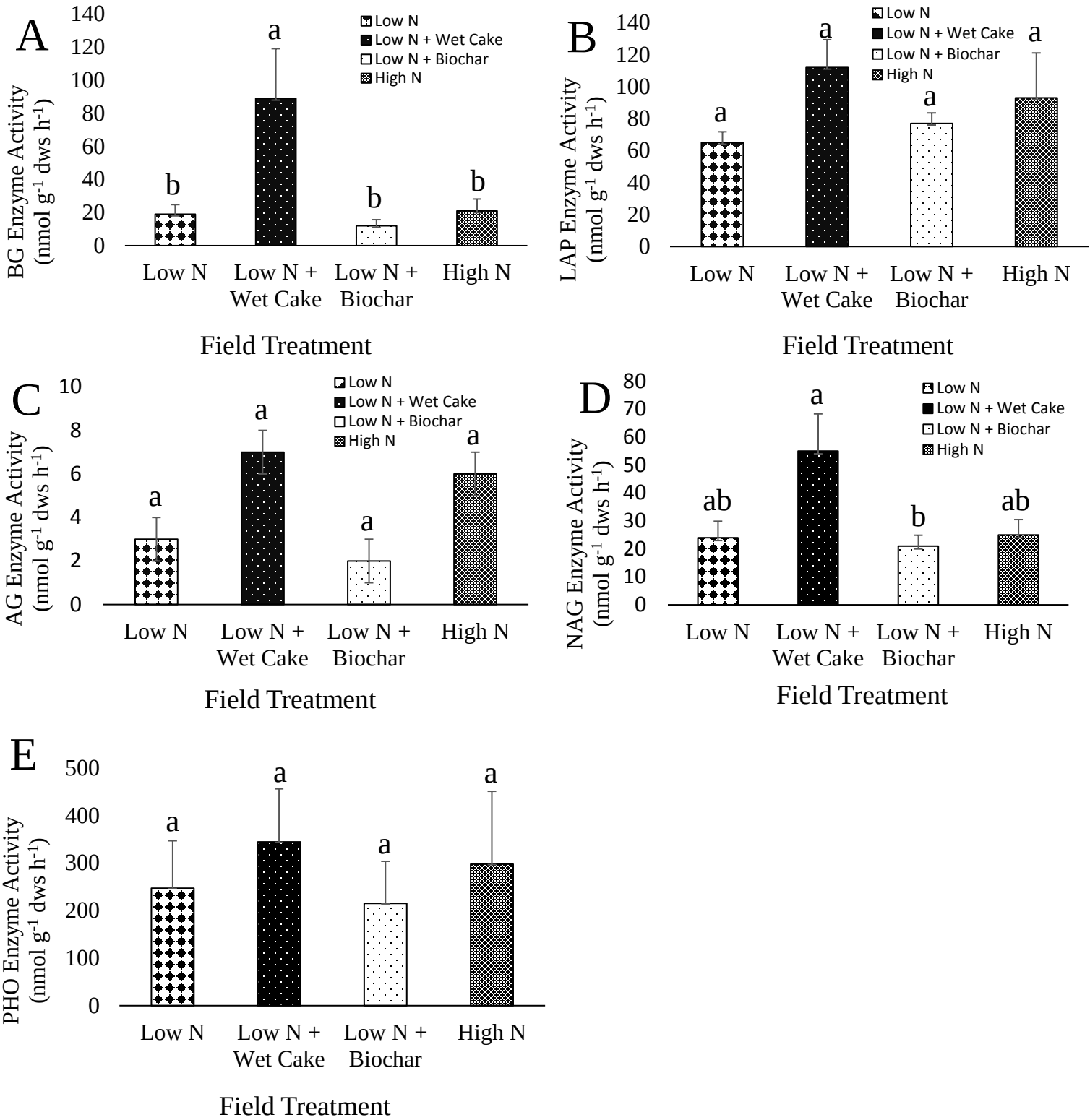

Figure 9. Enzyme activities of 5 extracellular enzymes associated with nutrient cycling. A) β-glucosidase; B) leucine aminopeptidase; C); α-glucosidase D) N-acetylglutamate; E) PHOS: phosphatase.

Discussion

Population increases and diminishing fossil fuel reserves have caused an increase in demand of alternative energy resources to replace conventional fuels. The increased interest in biofuels have led to a surge in research relating to biofuels, bioenergy technology, and the reuse of co-produced bioenergy residues as a soil amendment. However, a gap in the research exists pertaining to the impact of second generation bioenergy residues after land application on C and N cycling in the soil. This research is particularly important as sustainable practices and soil health become increasingly prioritized. Therefore, this study aimed to characterize select second generation bioenergy residues as potential soil amendments and to determine their behavior in the soil as they influence nutrient cycling in the soil. Of all the amendments investigated in this study, the wet cake material was of particular importance due to its potential recalcitrant nature and its ability to behave as a slow-release organic amendment providing plant-available nutrients. The characterization study shows that the wet cake residue is indeed unique because of the high amounts of bioavailable N and P.

The data in the first study shows what we expected; the residues have great variability in their chemical properties. Data collected in the characterization study showed that the plant-derived residues, wet cake and bagasse, had the greatest amounts of total C and OM contents, suggesting that these residues could have a greater potential to provide labile substrate for soil microbial use compared to the other residues. Our ^{13}C-NMR data further verified our findings that the residues varied significantly in the makeup of C functional groups. The spectra showed that wet cake had the greatest concentration of aryl-C groups. This suggests that wet cake could be more recalcitrant than the other residues and that the quality of C in wet cake may not be as high as the C quality of some of the more labile residues. Bagasse, on the other hand, had the lowest concentration of recalcitrant C (alkyl-C), along with the greatest concentration of labile C (O-alkyl C), suggesting that the bagasse residue may be the most labile residue in our study because it is made up of the highest quality of C. Alkyl C functional groups are made up of recalcitrant or persistent metabolic aliphatic species that are derived from waxy, lipids, and the decomposition of carbohydrates. O-alkyl C functional are made up of plant-derived polysaccharide compounds, methoxyl or C-O groups of carbohydrate and cellulosic material that can be broken down easily.

The ratio of functional groups could also be indicative of the potential lability of the organic substrates. The ratio of O-alkyl C to aromatic C, for example is a commonly used ratio that can describe the lability of organic matter. The O-alkyl C : aromatic ratio (O:A) for wet cake was 1.9, the lowest of all O:A ratios for the characterized residues, suggesting that there was more aromatic C groups in the wet cake than O-alkyl C groups (Table 4). Bagasse, however, has the highest O:A ratio, suggesting that the bagasse is made up of much more O-alkyl C groups than aromatic C, which could cause faster decomposition compared to the other residues.

The differences in C content and quality of the residues can explain the observed differences in microbial response after amendment application. Respiration data in the laboratory incubation study showed that the residues with high OM content (wet cake and bagasse) resulted in a greater stimulation in microbial response to amendment addition either through the decomposition of the organic matter in the residues or microbial stimulation from a priming effect. The field study showed the same results; amendment of wet cake residue led to the greatest microbial stimulation. By the end of the laboratory incubation study, the data showed that the soils that accumulated the largest amounts of CO_2-C were those that were amended with low C quality residues, containing the greatest amount of stable functional groups (wet cake, 29% aromatic C and anaerobic digestate, 20% aromatic C). In general, lower quality C should result in less microbial stimulation. Given the low C quality of the wet cake residue, it was expected that the recalcitrant nature of the C in the wet cake would result in less microbial activity. However, in both the laboratory and field incubations, wet cake amendment resulted in the most microbial stimulation. It is possible that the wet cake, while made up of more stable C functional groups than the characterized residues, contained enough labile substrate to stimulate microbial activity. These results may also be in accordance with the results of Tejada et al. (2009), who observed that soils amended with residues with higher concentrations of fulvic acids resulted in higher cumulative CO_2 respired. Though we did not measure fulvic acid concentrations fulvic acids are associated with stable, more recalcitrant organic matter.

In the field study, however, the biochar material, which generally contain stable aromatic C groups, had the lowest respiration rates, likely because the biochar did not provide enough labile material for the microbes to stimulate microbial activity in the field soils (Nanda et al., 2016). The respiration results suggest that the microbes were easily able to use the available organic substrate in the wet cake and high N treatments, allowing for the transformation of organic C to

inorganic CO_2-C. These results coincide with the results of Monaco et al. (2008), Cayuela et al. (2010) and Odlare et al. (2008), where amendments supplying additional inputs of C resulted in higher respiration values. Also, the quality of C in the amendment influenced respiration; higher quality C resulted in higher respiration rates, as indicated by the wet cake respiration rate (Figure 3).

In addition to stimulation of respiration, the organic matter in the residues can contain compounds that can act as substrates for the enzymatic activity (Bastida et al., 2008), promoting the release of extracellular enzymes to breakdown and acquire the nutrients in the residue. The enzyme activity of our residues is significantly smaller than the activity measured in other similar studies (Albiach et al., 2000; García-Gil et al., 2000; Odlare et al., 2008; Tejada et al., 2009). which is likely due to differences in residue composition, loading rates, and soil nutrient contents. Despite smaller measurements in enzyme activity compared to other enzyme studies, our results showed the same trends; all the residues (wet cake, bagasse, and anaerobic digestate) led to an increase in enzyme activity compared to the control soil, with wet cake showing the most stimulation in enzyme activity. Wet cake had the highest rates of PHO, BG, AG, and NAG activity, and second highest rates of LAP activity. These enzymes are associated with the decomposition of substrates and acquisition and mineralization of C, N, and P.

The enzyme data could be indicative that the wet cake residue has the most available C and N contents, therefore stimulating a greater amount of extracellular enzyme production than the other residues. The short burst of enzymatic activity likely results from the decomposition of the organic matter in the applied residues. Our enzyme data is in accordance with Tejada et al. (Tejada et al.), where researchers found an increase in nutrient-cycling enzymes after land applying a diverse variety of organic residues. our data suggest reflects the idea that microbial stimulation and activity depends on the source of C and N added to the soil (Šnajdr et al., 2008). Microbes generally regulate the production and release of extracellular enzymes to obtain available or limiting nutrients. Therefore, fluctuations in enzyme activities can reflect the patterns of microbial nutrient limitations or abundance (Bowles et al., 2014).

The results of MBC could also be attributed to C content quality. In the field study, treatments with high LOI and total C had the greatest amounts of MBC and enzyme activity involved in nutrient transformation and release. Linear regression showed that MBC was positively correlated with TC (R^2=0.25, P>0.05), as seen in other microbial studies using organic

amendments (Bowles et al., 2014). The data show that both organic amendments (wet cake and biochar) had greater MBC values than the synthetic fertilizer amendments (Figure 8), suggesting that some organic amendments may allow microbes to use available nutrients for biomass acquisition. This result is consistent with Bowles et al. (2014), who also observed that organic amendments with high nutrient content (C and N) promoted high soil C, MBC, and increased enzyme activities. This information suggests that high organic contents and available C can promote the biogeochemical cycling of nutrients through microbial activity.

Conclusions

The variability measured between the chemical properties of the residues gave us reason to believe that the differences in residue properties could affect the behavior of the residues when land applied, affecting microbial activity, nutrient mineralization, resulting in changes in the movement and transformation of nutrients in the soil. In terms of the plant-based residues, our results show that the wet cake residue as well as the raw bagasse could indeed be valuable soil amendments, due to the high amount of OM and plant available nutrients. Our results are in accordance with several scientific articles that argue the effectiveness of wet cake residue as a soil fertilizer. From our research, we can conclude that the lignocellulosic bioenergy residues (particularly the wet cake material) tested are indeed nutrient-rich and have the potential to provide plant essential nutrients to the soil. As a result, these residues may stimulate microbial activity, influencing the nutrient cycling of important elements in the soil.

The data from the lab and field study confirm our hypotheses that residue-amended soils would show an increase in microbial activity, particularly in aerobic respiration, MBC, and enzyme activity. In addition, all of our studies confirmed our third hypothesis that the physico-chemical characteristics could determine the use of residues by microbes. Particularly that the plant-based residues with more OM and labile C could be used more readily in the soil, resulting in stimulation of microbial activity. Our results show that the amendment of soils with bioenergy residues results in the stimulation of soil microbial activity (via aerobic respiration, enzyme release, and amount of C held in microbial biomass) and changes soil in nutrient content. The organic-rich residues (particularly the wet cake and bagasse) showed the greatest stimulation in microbial activity, evidenced by the high rates of respiration throughout the incubation study as

well as the total amount of CO_2-C respired. These residues also showed the greatest rate of enzyme activity.

Enzyme data from 5 enzymes associated with nutrient cycling (PHO, LAP, NAG, BG, and AG) indicate that organic substrates stimulate microbial activity, resulting in the release of enzymes to break down complex organic matter and the incorporation and synthesis of nutrients into the microbial biomass. The microbial activity measured in this study (MBC, respiration, and enzyme activity) can help to better understand how soil amendments can affect microbial communities and the transformation and movement of nutrient cycles through the soil ecosystem. By comparing the effects of organic amendments and traditional synthetic fertilizers, we can determine which amendments can have the greatest effects on nutrient cycling and soil quality. When compared to another popular organic amendment (biochar), the wet cake-amended soils still showed greater increases in microbial activity, even several months after land application.

By determining MBC of the wet cake and biochar treatments, we found that biochar held more C in microbial biomass than the wet cake. Yet, the wet cake released the most CO_2-C of all the treatments. We can conclude that though biochar is a high C organic amendment, it may not be the best amendment for stimulating microbial activity and C transformation in a sandy, low OM soil. The data from the lab and field study show that higher OM bioenergy residues with relatively labile C can stimulate microbial activity, which can affect the nutrient cycling of both C and N in the soil. Though the complex C in biochar may not be bioavailable for microbial use, the biochar may contribute to greater C sequestration in the soil compared to the more labile, high OM residues, resulting in improved soil health.

In terms of N mineralization, the application of the residues to the control soil resulted in an increase in total N in all residue-amended soils. We did not see an increase of NH_4^+-N and NO_3^--N in all residue-amended soils. Bagasse, vermicompost, and compost treatments led to an increase in water extractable NH_4^+-N. Biosolid, compost, and anaerobic digestate treatments resulted in an increase in NO_3^--N. These increases in plant available N may be indicative of N mineralization in the residue-amended soils through the transformation of N from organic to inorganic, plant-available forms. Though this was only a short-term study, we would expect to see an increase in plant available forms of N in most of the residue-amended soils that also showed a stimulation of microbial activity.

Our results emphasize the importance of reusing second generation bioenergy byproducts as potential soil amendments. Especially at this point in time, where the exploration of alternative biofuels could allow for the sustainable reuse of bioenergy byproducts that can be used to provide plant available nutrients in the soil. These bioenergy residues vary significantly in physico-chemical characteristics and can therefore result in different C and N transformations in the soil. Plant-derived, or lignocellulosic, residues in particular may play an important role in stimulating microbial activity, resulting in the mineralization of plant available nutrients. These residues could replace or be used in conjunction with synthetic fertilizers to reduce synthetic fertilizer production and use. More independence from synthetic fertilizer could result in a reduction of GHGs released during the production of fertilizers, while also reducing the amount of nutrient leaching lost in the soil. The results of this study are important for farmers and land-owners that are investing in the use of organic amendments for land application.

Further research should be directed in the areas of nutrient leaching (nitrate, ammonium, and phosphate) and nitrous oxide emissions to help researchers better understand the environmental implications and possible adverse effects of land applying bioenergy residues. In addition, long-term studies of second generation bioenergy residue land application can determine the overall effect of residues on crop production and soil health. C-NMR and microbial function assays could be used to assess residue-amended soils immediately after application and throughout a long period of residue land application. This could give researchers an idea about the long-term changes in the biogeochemistry of the soil, determining whether or not recalcitrant material and stable SOM is potentially accumulating in the soil. Other parameters such as ligno-cellulose index, water-holding capacity, and bulk density can be used in longer-term studies to assess any improvements in the soil structure. These longer-term effects are important for understanding how second-generation residues can affect or improve the quality of the soil long-term.

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Supplementary Materials

Table 7. Metal content of select bioenergy residues extracted using HCl, Mehlich 1, and Mehlich 3 solutions.

Parameter	Units	Treatment					
		Plant-derived Residue		Municipal Solid Waste Residue	Derived from Dairy Manure		
		Wet Cake	Raw Bagasse	Biosolid	Anaerobic Digestate	Vermicompost	Compost
HCl Extractable***							
P	g kg^{-1}	2.3 ± 0.02 ^{c}	1.3 ^{e}	15 ± 0.04 ^{a}	6.4 ± 0.05 ^{b}	1.1 ± 0.01 ^{d}	0.20 ± 0.01 ^{e}
K^+	g kg^{-1}	0.43 ± 0.01 ^{e}	1.4 ± 0.03 ^{d}	21 ± 0.11 ^{a}	11 ± 0.04 ^{b}	2.0 ± 0.03 ^{c}	0.34 ± 0.03 ^{e}
Ca^{2+}	g kg^{-1}	2.7 ± 0.03 ^{c}	3.5 ± 0.14 ^{c}	24 ± 0.10 ^{b}	22 ± 0.18 ^{b}	9.6 ± 0.12 ^{bc}	62 ± 9.8 ^{a}
Mg^{2+}	g kg^{-1}	0.33 ± 0.01 ^{e}	0.58 ± 0.01 ^{e}	4.3 ± 0.02 ^{b}	6.0 ± 0.05 ^{a}	1.3 ± 0.02 ^{c}	1.0 ± 0.13 ^{d}
Fe	g kg^{-1}	0.42 ^{d}	0.23 ^{e}	8.9 ± 0.04 ^{a}	2.1 ± 0.02 ^{b}	0.59 ± 0.01 ^{c}	0.57 ± 0.06 ^{c}
Mehlich-1 Extractable***							
P	g kg^{-1}	2.56 ± 0.05 ^{b}	0.07 ^{d}	6.27 ± 0.06 ^{a}	0.70 ± 0.06 ^{c}	0.83 ± 0.05 ^{c}	0.04 ^{d}
K^+	g kg^{-1}	0.55 ± 0.01 ^{c}	0.35 ± 0.03 ^{b}	23 ± 0.37 ^{a}	1.8 ± 0.16 ^{b}	1.8 ± 0.1 ^{b}	0.34 ^{c}
Ca^{2+}	g kg^{-1}	2.8 ± 0.05 ^{d}	3.0 ± 0.09 ^{d}	16 ± 0.11 ^{b}	7.9 ± 0.72 ^{d}	3.3 ± 0.17 ^{c}	20 ± 0.08 ^{a}
Mg^{2+}	g kg^{-1}	0.39 ± 0.01 ^{c}	0.53 ± 0.01 ^{c}	3.6 ± 0.05 ^{a}	1.1 ± 0.11 ^{b}	0.97 ± 0.04 ^{b}	0.54 ^{c}
Fe	g kg^{-1}	0.017 ^{a}	0.09 ^{b}	0.16 ± 0.01 ^{a}	0.02 ^{c}	0.06 ^{d}	NDL
Mehlich-3 Extractable***							
P	g kg^{-1}	1.68 ± 0.03 ^{b}	0.08 ± 0.01 ^{d}	4.8 ± 0.15 ^{a}	0.64 ± 0.11 ^{c}	0.72 ± 0.05 ^{c}	0.03 ^{d}
K^+	g kg^{-1}	0.52 ±0.01 ^{b}	1.4 ± 0.35 ^{b}	21 ± 1.5 ^{a}	1.7 ± 0.32 ^{b}	1.9 ± 0.14 ^{b}	0.41 ^{b}
Ca^{2+}	g kg^{-1}	0.97 ± 0.02 ^{c}	1.3 ± 0.14 ^{c}	9.1 ± 0.49 ^{b}	4.5 ± 0.91 ^{c}	2.2 ± 0.11 ^{c}	30 ± 2.0 ^{a}
Mg^{2+}	g kg^{-1}	0.28 ± 0.01 ^{cd}	0.48 ± 0.11 ^{bcd}	2.9 ± 0.22 ^{a}	0.95 ± 0.17 ^{b}	0.86 ± 0.06 ^{b}	0.63 ± 0.01 ^{bc}
Fe	g kg^{-1}	0.17 ^{cd}	0.14 ± 0.04 ^{bc}	0.5 ± 0.0 ^{a}	0.18 ± 0.02 ^{bcd}	0.13 ± 0.01 ^{b}	0.08 ± 0.01 ^{cd}

Levels not connected by the same letter are significantly different (Tukey's test, α=0.05).

Table 8. Phosphorus content of select bioenergy residues using HCl, Mehlich 1, and Mehlich 3 solutions.

Parameter	Units	Treatment					
		Plant-derived Residue		Municipal Solid Waste Residue	Derived from Dairy Manure		
		Wet Cake	Raw Bagasse	Biosolids	Anaerobic Digestate	Vermicompost	Compost
HCl Extractable P	g kg^{-1}	2.1 ± 0.01 ^{c}	0.08 ^{b}	1.2 ± 0.02 ^{a}	0.57 ± 0.02 ^{d}	0.11 ± 0.01 ^{d}	0.19 ^{d}
Mehlich-1 Extractable P	g kg^{-1}	0.39 ^{b}	0.04 ^{d}	0.75 ± 0.02 ^{a}	0.34 ^{c}	0.16 ^{c}	0.04 ^{d}
Mehlich-3 Extractable P	g kg^{-1}	0.25 ^{b}	0.03 ^{d}	0.62 ^{a}	0.68 ± 0.01 ^{b}	0.12 ^{c}	0.05 ^{d}

Levels not connected by the same letter are significantly different (Tukey's test, α=0.05).

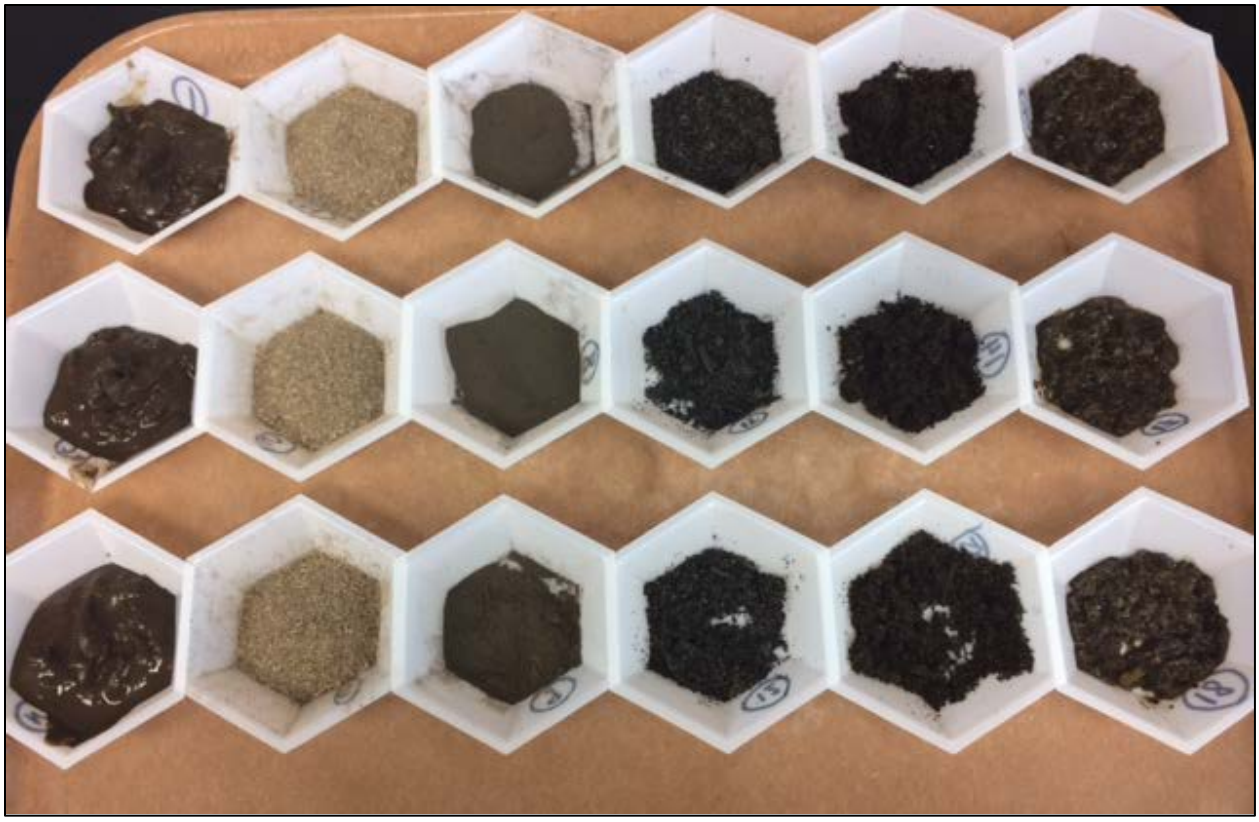

Figure 10. Residues prior to oven-drying. From left to right: wet cake, bagasse, biosolid, compost, vermicompost, anaerobic digestate

Table 9. Metal content of residue-amended soils before incubation using HCl, Mehlich 1, and Mehlich 3 solutions.

Parameter	Units	Control	Treatment					
		Citra Soil	Wet Cake	Raw Bagasse	Biosolid	Compost	Anaerobic Digestate	Vermicompost
HCl Extractable								
P		97 ± 6	107 ± 1	108 ± 7	99 ± 2	144 ± 13	104 ± 6	123 ± 1
K^+		8 ± 2	9 ± 1	39 ± 1	15 ± 2	43 ± 4	14 ± 1	45 ± 2
Ca^{2+}	mg kg^{-1}	700 ± 20	762 ± 11	850 ± 51	721 ± 15	897 ± 109	1530 ± 53	809 ± 3
Mg^{2+}		NDL	NDL	3 ± 2	NDL	NDL	5 ± 1	11 ± 1
Fe		46 ± 3	49 ± 2	58 ± 5	48 ± 22	80 ± 8	59 ± 2	56 ± 2
Mehlich-1 Extractable								
P		100 ± 5	103	93 ± 4	105 ± 1	91 ± 3	120 ± 3	94 ± 1
K^+		4	8 ± 1	45 ± 2	48 ± 2	10	55 ± 1	16 ± 1
Ca^{2+}	mg kg^{-1}	865 ± 22	928 ± 97	908 ± 25	851 ± 14	1950 ± 15	978 ± 58	920 ± 23
Mg^{2+}		11	14 ± 2	27 ± 1	18 ± 1	29 ± 1	40 ± 1	19 ± 1
Fe		10	13	14 ± 2	12	10	14 ± 1	10
Mehlich-3 Extractable								
P		124 ± 10	140 ± 2	135 ± 8	187 ± 3	146 ± 9	194 ± 4	169 ± 3
K^+		6	9	43 ± 1	55 ± 2	14 ± 1	58 ± 1	20 ± 1
Ca^{2+}	mg kg^{-1}	579 ± 12	610 ± 26	619 ± 25	732 ± 15	1043 ± 14	766 ± 18	758 ± 30
Mg^{2+}		12 ± 2	16 ± 1	28 ± 1	30 ± 1	32 ± 2	48 ± 1	28
Fe		49 ± 4	49 ± 1	57 ± 7	83 ± 3	69 ± 8	84 ± 3	76 ± 1

Table 10. Metal content of residue-amended soils after incubation using HCl, Mehlich 1, and Mehlich 3 solutions.

Parameter	Units	Control	Treatment					
		Citra Soil	Wet Cake	Raw Bagasse	Biosolid	Compost	Anaerobic Digestate	Vermicompost
HCl Extractable								
P		94 ± 3	110 ± 2	100 ± 3	124 ± 6	107 ± 3	128 ± 4	105
K^+		5 ± 1	10 ± 2	35 ± 2	39 ± 4	16 ± 4	46 ± 2	25 ± 5
Ca^{2+}	mg kg^{-1}	697 ± 12	731 ± 14	764 ± 5	756 ±27	1649 ± 50	801 ± 17	771 ± 10
Mg^{2+}		NDL	NDL	NDL	NDL	4 ± 1	12 ± 1	NDL
Fe		44 ± 2	49 ± 2	48 ± 1	65 ± 5	57 ± 2	57 ± 2	50
Mehlich-1 Extractable								
P		86 ± 3	95 ± 1	85	87 ± 1	105 ± 1	89 ± 2	108 ± 1
K^+		5	7	42 ± 1	16 ± 1	47 ± 2	10	48 ± 1
Ca^{2+}	mg kg^{-1}	816 ± 22	841 ± 20	897 ± 16	866 ± 28	907 ± 14	1927 ± 72	907 ± 14
Mg^{2+}		11 ± 1	12	26 ± 1	18 ± 1	36 ± 1	28 ± 1	36 ± 1
Fe		10 ± 2	10	10	8	11	12	11
Mehlich-3 Extractable								
P		156 ± 1	168 ± 8	134 ± 10	138 ± 1	167 ± 3	139 ± 2	174 ± 3
K^+		8	12 ± 1	46 ± 1	26 ± 9	54 ± 4	12	54 ± 1
Ca^{2+}	mg kg^{-1}	724 ± 18	742 ± 32	606 ± 87	547 ± 18	629 ± 49	947 ± 56	629 ± 49
Mg^{2+}		22 ± 2	22 ± 1	31 ± 2	22	44 ± 2	30 ± 1	44 ± 2
Fe		68 ± 2	74 ± 8	53 ± 10	50 ± 1	67 ± 3	61 ± 2	67 ± 3

Table 11. Physico-chemical characteristics of untreated Citra soil (control). Numbers represent mean ± standard error, n=3 source reps).

		Treatment
Parameter	Units	Untreated Soil
pH		8.1 ± 0.07
Moisture Content	%	3.3 ± 0.26
Total C	$g\ kg^{-1}$	5.2 ± 1.2
Total N	$g\ kg^{-1}$	0.20 ± 0.02
Total P	$g\ kg^{-1}$	0.42 ± 0.01
C:N		26 ± 3.0
C:P		13 ± 3.1
N:P		0.48 ± 0.06
KCl Extractable NH_4^+	$mg\ kg^{-1}$	1.4 ± 0.09
Water Extractable		
P	$mg\ kg^{-1}$	8.0 ± 0.58
NH_4^+		2.1 ± 0.20
NO_3^-		7.1

Table 12. Changes in pH values, moisture content, and loss on ignition (LOI) on day 1 and day 28 during the laboratory incubation study. The values represent the mean ± standard error (n=3).

Treatment	pH		LOI (%)	
	Incubation Time			
	Initial	Final	Initial	Final
Control Soil	8.1 ± 0.09 ^{bc}	8.5 ± 0.09 ^{a}	1.2 ± 0.32 ^{a}	1.6 ± 0.32 ^{a}
Wet Cake + Soil	7.6 ± 0.09 ^{d}	8.0 ± 0.15 ^{ab}	3.5 ± 1.8 ^{a}	2.0 ± 0.57 ^{a}
Raw Bagasse + Soil	7.8 ± 0.07 ^{cd}	7.9 ± 0.09 ^{ab}	4.0 ± 0.37 ^{a}	2.9 ± 0.56 ^{a}
Biosolid + Soil	8.2 ± 0.12 ^{abc}	7.6 ± 0.03 ^{ab}	1.6 ± 0.31 ^{a}	1.3 ± 0.33 ^{a}
Compost + Soil	8.5 ± 0.09 ^{a}	7.4 ± 0.07 ^{b}	1.9 ± 0.03 ^{a}	1.9 ± 0.54 ^{a}
Anaerobic Digestate + Soil	8.2 ± 0.07 ^{ab}	8.1 ± 0.09 ^{ab}	1.5 ± 0.27 ^{a}	1.2 ± 0.61 ^{a}
Vermicompost + Soil	7.9 ± 0.09 ^{cd}	7.6 ± 0.09 ^{ab}	1.8 ± 0.55 ^{a}	1.9 ± 0.56 ^{a}
Ammonium Nitrate + Soil	7.6 ± 0.12 ^{cd}	6.3 ± 0.12 ^{ab}	0.93 ± 0.02 ^{a}	1.6 ± 0.31 ^{a}

Levels not connected by the same letter are significantly different (Tukey's test, α=0.05).

Table 13. Total nutrient concentrations expected after amending soil samples with 250 kg N ha^{-1} and the actual observed concentrations on day 1 of the laboratory incubation study. The values represent the mean ± standard error (n=3).

Treatment	Total C (g kg^{-1})		Total N (g kg^{-1})		Total P (g kg^{-1})	
	Expected	Observed	Expected	Observed	Expected	Observed
Control Soil	5.2	5.2 ± 1.2 ^{b}	0.21	0.20 ± 0.02 ^{a}	0.42	0.42 ± 0.01 ^{a}
Wet Cake + Soil	7.9	7.0 ± 0.76 ^{b}	0.31	0.55 ± 0.03 ^{a}	0.43	0.41 ± 0.01 ^{a}
Raw Bagasse + Soil	16.5	21 ± 3.5 ^{a}	0.31	0.26 ± 0.08 ^{a}	0.43	0.48 ± 0.02 ^{a}
Biosolid + Soil	5.8	11 ± 0.69 ^{b}	0.31	0.42 ± 0.03 ^{a}	0.45	0.48 ± 0.02 ^{a}
Compost + Soil	7.5	8.0 ± 1.3 ^{b}	0.22	0.42 ± 0.25 ^{a}	0.43	0.43 ± 0.02 ^{a}
Anaerobic Digestate + Soil	6.9	3.8 ± 0.71 ^{b}	0.30	0.33 ± 0.06 ^{a}	0.44	0.41 ± 0.02 ^{a}
Vermicompost + Soil	6.3	7.4 ± 0.93 ^{b}	0.30	0.52 ± 0.11 ^{a}	0.43	0.46 ± 0.01 ^{a}
Ammonium Nitrate + Soil	5.2	4.3 ± 0.56 ^{b}	1.4	1.2 ± 0.28 ^{b}	0.42	0.42 ± 0.01 ^{a}

Levels not connected by the same letter are significantly different (Tukey's test, α=0.05).

Table 14. Changes to total C:N, C:P, and N:P ratios on day 1 and day 28 during the laboratory incubation study. The values represent the mean ± standard error (n=3).

Treatment	C:N		C:P		N:P	
	Incubation Time					
	Initial	Final	Initial	Final	Initial	Final
Control Soil	26 ^{b}	30 ^{c}	12 ^{ab}	9 ^{b}	0.48 ^{a}	0.31 ^{ab}
Wet Cake + Soil	13 ^{b}	63 ^{bc}	17 ^{ab}	22 ^{b}	1.3 $_{a}$	0.35 ^{ab}
Raw Bagasse + Soil	79 ^{a}	324 ^{a}	36 ^{a}	83 ^{a}	0.46 ^{a}	0.26 ^{b}
Biosolid + Soil	27 ^{b}	35 ^{c}	24 ^{ab}	20 ^{b}	0.87 ^{a}	0.58 ^{a}
Compost + Soil	19 ^{b}	111 ^{b}	19 ^{ab}	35 ^{b}	0.97 ^{a}	0.32 ^{ab}
Anaerobic Digestate + Soil	12 ^{b}	63 ^{bc}	9.0 ^{b}	14 ^{b}	0.79 ^{a}	0.23 ^{b}
Vermicompost + Soil	14 ^{b}	53 ^{c}	16 ^{ab}	12 ^{b}	1.1 ^{a}	0.23 ^{b}
Ammonium Nitrate + Soil	3.6 ^{c}	4.7 ^{d}	10 ^{b}	11 ^{b}	2.9 ^{b}	2.4 ^{c}

Levels not connected by the same letter are significantly different (Tukey's test, α=0.05).

Table 15. Total nutrient concentrations expected after amending soil samples with 250 kg N ha^{-1} and the actual observed concentrations on day 1 of the laboratory incubation study. The values represent the mean ± standard error (n=3).

Treatment	Total C (g kg^{-1})		Total N (g kg^{-1})		Total P (g kg^{-1})	
	Expected	Observed	Expected	Observed	Expected	Observed
Control Soil	5.2	5.2 ± 1.2 ^{b}	0.21	0.20 ± 0.02 ^{a}	0.42	0.42 ± 0.01 ^{a}
Wet Cake + Soil	7.9	7.0 ± 0.76 ^{b}	0.31	0.55 ± 0.03 ^{a}	0.43	0.41 ± 0.01 ^{a}
Raw Bagasse + Soil	16.5	21 ± 3.5 ^{a}	0.31	0.26 ± 0.08 ^{a}	0.43	0.48 ± 0.02 ^{a}
Biosolid + Soil	5.8	11 ± 0.69 ^{b}	0.31	0.42 ± 0.03 ^{a}	0.45	0.48 ± 0.02 ^{a}
Compost + Soil	7.5	8.0 ± 1.3 ^{b}	0.22	0.42 ± 0.25 ^{a}	0.43	0.43 ± 0.02 ^{a}
Anaerobic Digestate + Soil	6.9	3.8 ± 0.71 ^{b}	0.30	0.33 ± 0.06 ^{a}	0.44	0.41 ± 0.02 ^{a}
Vermicompost + Soil	6.3	7.4 ± 0.93 ^{b}	0.30	0.52 ± 0.11 ^{a}	0.43	0.46 ± 0.01 ^{a}
Ammonium Nitrate + Soil	5.2	4.3 ± 0.56 ^{b}	1.4	1.2 ± 0.28 ^{b}	0.42	0.42 ± 0.01 ^{a}

Levels not connected by the same letter are significantly different (Tukey's test, α=0.05).

Table 16. HCl extractable metal concentrations on day 1 and day 28 during the laboratory incubation study. The values represent the mean ± standard error (n=3).

	P (mg kg^{-1})		K^{+} (mg kg^{-1})		Ca^{2+} (mg kg^{-1})		Mg^{2+} (mg kg^{-1})		Fe (mg kg^{-1})	
	Incubation Time									
Treatment + Soil	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
C	97 ± 5.7 ^{b}	94 ± 3.4	8.4 ± 2.2 ^{b}	4.6 ± 1.2	700 ± 20 ^{b}	697 ± 12	NDL	NDL	46 ± 3.1 ^{b}	44 ± 1.9
WC	107 ± 1.4 ^{b}	110 ± 1.7	9.3 ± 0.90 ^{b}	9.9 ± 2.1	762 ± 11 ^{b}	731 ± 14	NDL	NDL	49 ± 1.7 ^{b}	49 ± 1.8
RB	108 ± 6.7 ^{b}	100 ± 2.6	39 ± 0.80 ^{a}	35 ± 1.8	850 ± 51 ^{b}	764 ± 5	3.4 ± 0.60 ^{b}	NDL	58 ± 4.7 ^{b}	48 ± 1.5
Bs	99 ± 2.4 ^{b}	124 ± 6.0	15 ± 1.5 ^{b}	39 ± 4.2	721 ± 15 ^{b}	756 ±27	NDL	NDL	48 ± 1.6 ^{b}	65 ± 5.0
Co	144 ± 13 ^{b}	107 ± 3.0	43 ± 3.5 ^{a}	16 ± 3.7	897 ± 109 ^{b}	1649 ± 50	NDL	4.2 ± 1.2	80 ± 7.5 ^{a}	57 ± 1.6
AD	104 ± 5.6 ^{a}	128 ± 3.8	14 ± 1.2 ^{b}	46 ± 2.5	1530 ± 53 ^{a}	801 ± 17	4.8 ± 1.4 ^{ab}	12 ± 1.2	59 ± 2.2 ^{b}	57 ± 2.4
Vc	123 ± 1.4 ^{ab}	105 ± 0.50	45 ± 2.1 ^{a}	25 ± 4.8	809 ± 2.5 ^{b}	771 ± 10	11 ± 0.9 ^{a}	NDL	56 ± 1.5 ^{b}	50 ± 0.03
AN	96 ± 1.6 ^{b}	103 ± 2.03	6.6 ± 0.80 ^{b}	19 ± 11	730 ± 23 ^{b}	709 ± 16	NDL	NDL	46 ± 2.5 ^{b}	49 ± 0.79

C = control; WC = wet cake; RB = raw bagasse; Bs = biosolid; Co = compost; AD = anaerobic digestate; Vc = vermicompost; AN = anaerobic digestate. Levels not connected by the same letter are significantly different (Tukey's test, α=0.05).

Table 17. Mehlich-1 extractable metal concentrations on day 1 and day 28 during the laboratory incubation study. The values represent the mean ± standard error (n=3).

	P (mg kg^{-1})		K^{+} (mg kg^{-1})		Ca^{2+} (mg kg^{-1})		Mg^{2+} (mg kg^{-1})		Fe (mg kg^{-1})	
	Incubation Time									
Treatment + Soil	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
C	100 ± 4.8 ^{bc}	86 ± 2.6	4.5 ± 0.07 ^{e}	4.7 ± 0.42	865 ± 22 ^{b}	816 ± 22	11 ± 0.44 ^{d}	11 ± 0.55	10 ± 0.38 ^{ab}	10 ± 2.2
WC	103 ± 0.42 ^{bc}	95 ± 0.99	7.7 ± 0.52 ^{de}	7.1 ± 0.11	928 ± 97 ^{b}	841 ± 20	14 ± 1.9 ^{cd}	12 ± 0.40	13 ± 0.30 ^{ab}	9.7 ± 0.16
RB	93 ± 4.3 ^{bc}	85 ± 0.05	45 ± 1.6 ^{b}	42 ± 0.91	908 ± 25 ^{b}	897 ± 16	27 ± 1.5 ^{b}	26 ± 0.66	14 ± 1.7 ^{a}	9.9 ± 0.41
Bs	105 ± 1.4 ^{ab}	87 ± 1.2	48 ± 1.8 ^{b}	16 ± 0.55	851 ± 14 ^{b}	866 ± 28	18 ± 0.78 ^{c}	18 ± 0.77	12 ± 0.27 ^{ab}	8.5 ± 0.16
Co	91 ± 3.2 ^{bc}	105 ± 0.96	10 ± 0.39 ^{cd}	47 ± 1.6	1950 ± 15 ^{a}	839 ± 11	29 ± 0.97 ^{b}	18 ± 0.89	9.8 ± 0.30 ^{b}	12 ± 0.08
AD	120 ± 3.5 ^{a}	89 ± 2.5	55 ± 1.5 ^{a}	9.7 ± 0.21	978 ± 58 ^{b}	1927 ± 72	40 ± 1.5 ^{a}	28 ± 0.77	14 ± 1.1 ^{a}	10 ± 0.70
Vc	94 ± 0.87 ^{bc}	108 ± 0.74	16 ± 0.55 ^{c}	48 ± 0.59	920 ± 23 ^{b}	907 ± 14	19 ± 0.65 ^{c}	36 ± 0.55	9.9 ± 0.35 ^{b}	11 ± 0.30
AN	90 ± 2.8 ^{c}	80 ± 1.3	8.0 ± 0.68 ^{de}	5.3 ± 0.40	886 ± 47 ^{b}	837 ± 21	26 ± 1.8 ^{b}	25 ± 1.4	12 ± 0.77 ^{ab}	9.3 ± 0.16

C = control; WC = wet cake; RB = raw bagasse; Bs = biosolid; Co = compost; AD = anaerobic digestate; Vc = vermicompost; AN = anaerobic digestate. Levels not connected by the same letter are significantly different (Tukey's test, α=0.05).

Table 18. Mehlich-3 extractable metal concentrations on day 1 and day 28 during the laboratory incubation study. The values represent the mean ± standard error (n=3).

	P (mg kg^{-1})		K^+ (mg kg^{-1})		Ca^{2+} (mg kg^{-1})		Mg^{2+} (mg kg^{-1})		Fe (mg kg^{-1})	
	Incubation Time									
Treatment + Soil	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
C	124 ± 10 ^{c}	156 ± 1.2	5.8 ± 0.30 ^{e}	8.4 ± 0.15	579 ± 12 ^{d}	724 ± 18	12 ± 1.6 ^{c}	22 ± 1.5	49 ± 4.1 ^{c}	68 ± 2.4
WC	140 ± 2.1 ^{bc}	168 ± 8.3	9.0 ±0.39 ^{de}	12 ± 1.1	610 ± 26 ^{cd}	742 ± 32	16 ± 0.68 ^{c}	22 ± 1.3	49 ± 0.98 ^{c}	74 ± 7.9
RB	135 ± 8.1 ^{c}	134 ± 10	43 ± 0.74 ^{b}	46 ± 1.3	619 ± 25 ^{cd}	606 ± 87	28 ± 1.1 ^{b}	31 ± 2.1	57 ± 7.0 ^{bc}	53 ± 9.8
Bs	187 ± 3.9 ^{a}	138 ± 1.4	55 ± 2.4 ^{a}	26 ± 8.9	732 ± 15 ^{b}	547 ± 18	30 ± 0.93 ^{b}	22 ± 0.98	83 ± 3.1 ^{a}	50 ± 1.5
Co	146 ± 9.2 ^{bc}	167 ± 2.7	14 ± 1.1 ^{d}	54 ± 4.1	1043 ± 14 ^{a}	629 ± 49	32 ± 1.9 ^{b}	26 ± 0.38	69 ± 8.3 ^{abc}	64 ± 3.5
AD	194 ± 3.9 ^{a}	139 ± 1.9	58 ± 0.97 ^{a}	12 ± 0.24	766 ± 18 ^{b}	947 ± 56	48 ± 0.79 ^{a}	30 ± 0.98	84 ± 2.5 ^{a}	61 ± 2.4
Vc	169 ± 2.7 ^{ab}	174 ± 3.2	20 ± 0.69 ^{c}	54 ± 1.2	758 ± 30 ^{b}	629 ± 49	28 ± 1.1 ^{b}	44 ± 1.7	76 ± 1.3 ^{ab}	67 ± 2.8
AN	151 ± 3.7 ^{bc}	145 ± 1.0	11 ± 0.86 ^{de}	7.8 ± 0.62	715 ± 26 ^{bc}	651 ± 20	29 ± 1.1 ^{b}	32 ± 1.6	63 ± 3.0 ^{abc}	59 ± 0.59

C = control; WC = wet cake; RB = raw bagasse; Bs = biosolid; Co = compost; AD = anaerobic digestate; Vc = vermicompost; AN = anaerobic digestate. Levels not connected by the same letter are significantly different (Tukey's test, α=0.05).

Figure 11. Elephantgrass plots at Citra, amended with 4 treatments: Low N, Low N + wet cake, Low N + biochar, and High N.

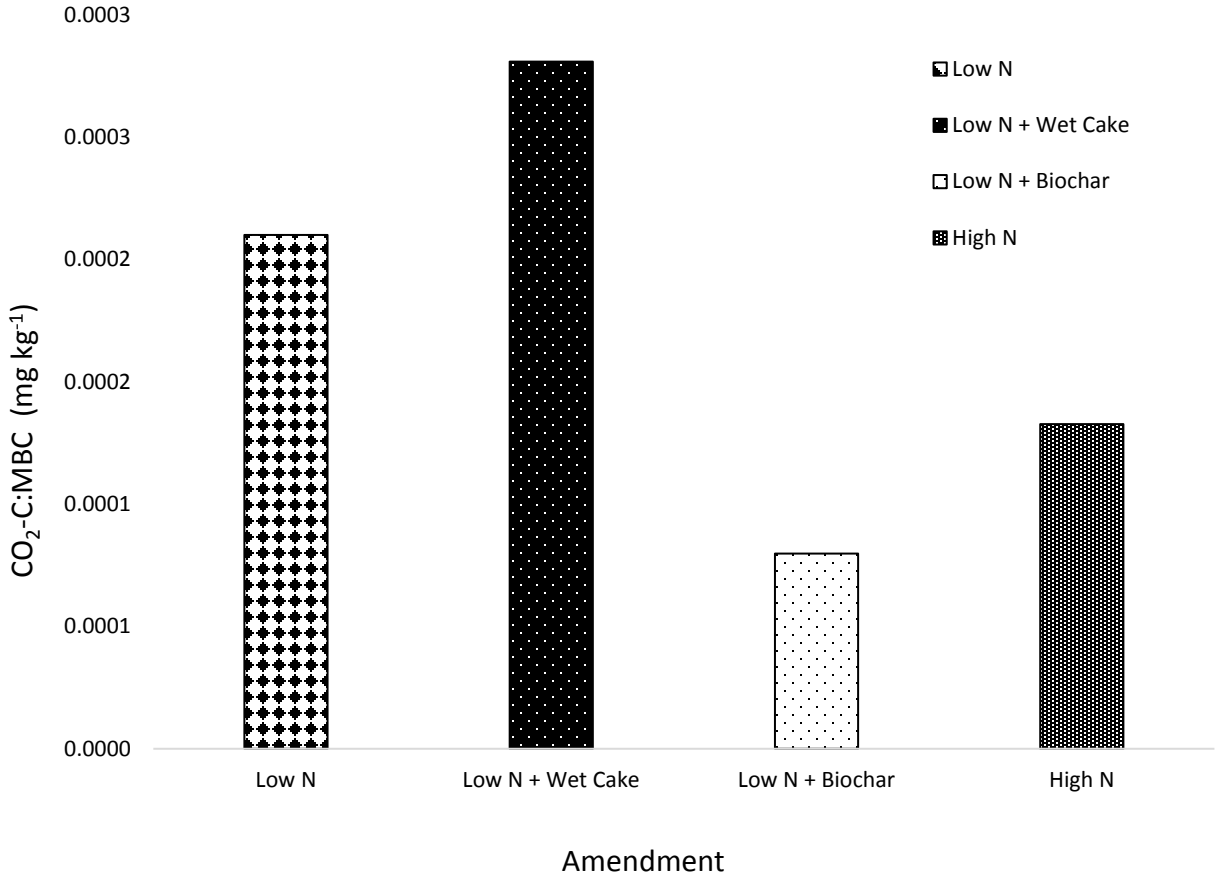

Figure 12. Amount of CO_2-C respired:MBC of low N, wet cake, biochar, and high N-amended soils.

Table 19. Metal content of field-amended bioenergy residues using HCl, Mehlich 1, and Mehlich 3 solutions.

Parameter	Units	Control	Treatment			
		Citra Soil	Low N	Low N + Wet Cake	Low N + Biochar	High N
HCl Extractable						
P		96 ± 2.4 [a]	90 ± 12 [a]	114 ± 13 [a]	100 ± 9.7 [a]	68 ± 11 [a]
K^+		14 ± 16	NDL	245 ± 86 [a]	110 ± 33	NDL
Ca^{2+}	mg kg^{-1}	665 ± 17 [a]	692 ± 49 [a]	571 ± 43 [a]	625 ± 40 [a]	482 ± 32 [a]
Mg^{2+}		NDL	NDL	NDL	NDL	NDL
Fe		43 ± 4.5 [a]	52 ± 11 [a]	59 ± 6.5 [a]	54 ± 8.8 [a]	45 ± 7.8 [a]
Mehlich-1 Extractable						
P *		69 ± 3.5 [ab]	46 ± 6.4 [ab]	80 ± 3.0 [a]	73 ± 7.0 [ab]	41 ± 9.4 [b]
K^{+} **		NDL	1126 ± 209 [ab]	873 ± 95 [abc]	1531 ± 345 [a]	503 ± 222 [bc]
Ca^{2+}	mg kg^{-1}	684 ± 21 [a]	615 ± 50 [a]	567 ± 57 [a]	658 ± 43 [a]	493 ± 15 [a]
Mg^{2+}		5.6 ± 0.3	NDL	NDL	18 ± 5.0	NDL
Fe		5.6 ± 0.2 [a]	3.6 ± 0.7 [a]	6.9 ± 3.2 [a]	6.6 ± 1.9 [a]	4.6 ± 1.9 [a]
Mehlich-3 Extractable						
P *		0.12 ± 0.01 [a]	80 ± 8.1 [b]	117 ± 4.6 [ab]	107 ± 6.5 [ab]	91 ± 6.6 [ab]
K^{+} **		0.006 [c]	2.1 ± 0.8 [abc]	19 ± 2.1 [a]	15 ± 3.3 [ab]	7.7 ± 0.9 [bc]
Ca^{2+} ***	mg kg^{-1}	0.58 ± 0.01 [a]	282 ± 9.9 [b]	244 ± 19 [b]	263 ± 14 [b]	291 ± 29 [b]
Mg^{2+} ***		0.01 [b]	11 ± 0.6 [b]	11 ± 1.7 [b]	39 ± 2.6 [a]	9.1 ± 1.6 [b]
Fe		0.05 [a]	49 ± 4.4 [a]	48 ± 5.7 [a]	46 ± 5.0 [a]	43 ± 5.7 [a]

Levels not connected by the same letter are significantly different (Tukey's test, α=0.05).