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Biogas digestate as a renewable fertilizer: effects of digestate application on crop growth and nutrient composition

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Abstract

Biogas digesters convert waste matter into a natural gas-like fuel and a nutrient-rich digestate by-product. This digestate has the potential to be used as a soil amendment to benefit crop production with or without biochar, a purported nutrient sponge. In a greenhouse study of several crop species, the effects of digestate fertilization on crop growth, photosynthetic efficiency, vegetable production and chemical nutrient levels were tested. Results indicate that increasing potency of the applied digestate fosters higher growth and fruit production rates of several studied plants but to a lesser degree than a conventional fertilizer. More potent digestate application increases antioxidant capacity, total phenolics content and ascorbic acid levels in kale compared to the control chemical fertilizer test groups but has confounding results on legume nutrient levels. Additionally, the combined application of biochar and biogas digestate added to compost and used as potting media positively impacts crop germination. This work has relevance to agrarian communities that could benefit from recycling livestock and food waste into fuel and a renewable fertilizer.

Introduction

Biogas is the common name used to describe the gas produced from anaerobic digestion (AD) of organic matter. Biogas is primarily composed of methane and carbon dioxide, and as a result can be burned to generate heat, electricity or motive power. AD initially received attention as a viable solution to generating energy and resources from organic waste in the mid-1980s, with most current innovations coming from China, India and Western Europe (Abbasi *et al.*, 2012; Ji *et al.*, 2017). Over the past few decades, biogas production has expanded in municipal areas as businesses have recognized the benefits of organic waste codigestion into fuel (Angelidaki and Ellegaard, 2003). AD is highly regarded as a mechanism to harness clean energy from agricultural waste, while cutting down potential environmental risks of untreated waste (Barrena Gómez *et al.*, 2006; Holm-Nielsen *et al.*, 2009; He *et al.*, 2018; Scarlat *et al.*, 2018). AD systems also preserve key NPK (nitrogen-phosphorus-potassium) nutrients from the feedstock, producing a nutrient-rich digestate in addition to methane (Bibby *et al.*, 2010; Bond and Templeton, 2011; Fermoso *et al.*, 2018; Mukhuba *et al.*, 2018). Biogas generation increases the productivity of arable land while achieving a positive energy balance, two qualities desirable in renewable agro-ecosystems (Michel *et al.*, 2010; Pugesgaard *et al.*, 2014; Råberg *et al.*, 2017; Scarlat *et al.*, 2018).

A by-product of biogas generation is the nutrient-rich digestate, also commonly referred to as effluent or slurry. A knowledge gap was identified in 2017 regarding the efficacy of using digestate as a resource, specifically mentioning the potential for AD digestate to function as a fertilizer (Ji *et al.*, 2017). Previous research has indicated that biogas digestate can quickly release bioavailable N in field settings (Möller *et al.*, 2008; Gunnarsson *et al.*, 2011). There is anecdotal evidence from farmers in central Pennsylvania who have observed increased biomass production after application of biogas digestate to forage crops (Kelsey, 2018). AD digestate application has resulted in similar biomass production, protein/lipid content and amino acid composition of the edible microalgae Spirulina when compared to a conventional growing medium, indicating its potential to act as a substitute fertilizer (Hultberg *et al.*, 2016). Fertilization with a modified biogas digestate has increased the yields of celery (Wang and Sun,2007), Chinese cabbage (Zhu, 1985), lettuce (Xu *et al.*, 2003), green peppers (Zhou *et al.*, 2007) and many other vegetables (Liu *et al.*, 2009). Co-application of conventional fertilizer and animal waste bio-slurry increases the yield of tomato (*Solanum lycopersicum* L.) plants (Ferdous *et al.*, 2018). While these investigations provide a solid foundation for reuse of digestate as a fertilizer, additional work must be conducted to assess digestate's wider viability as a fertilizer given differing treatments, study species and experimental conditions. Further, prior work is often limited in the crop types and affected properties, making it difficult to compare results among studies.

Compost and biochar are two supplemental resources farms utilize in attempts to increase crop yields. The viability of these inputs can be further improved through combined application with AD digestate. Composting (managed aerobic decomposition) is an alternative method for degrading organic matter into a nutrient-rich substance with many agricultural applications (de Bertoldi *et al.*, 1983). Composting organic matter recycles key nutrients (NPK) to the soil and regulates soil structure and humus balance (de Bertoldi *et al.*, 1983; Chen *et al.*, 2008; López-Cano *et al.*, 2016). While successful composting operations benefit from strict environmental parameters relating to aeration, temperature, moisture, C:N (carbon:nitrogen) ratio of added organic matter and pH (MacGregor *et al.*, 1981), there is some flexibility for the variability that occurs in dynamic conditions on working farms. However, unfinished compost can impair seedling growth and fertilization due to high amounts of conductive ions, including NO_3^-, Ca^{2+} and Mg^{2+} (Inbar *et al.*, 1993), and NH_4^+. Digestate from biogas systems has the potential to be utilized as an organic fertilizer akin to finished compost or raw manure as these resources have similar feedstocks: the biggest difference being that AD digestate contains a higher NH_4-NH^+ content and allows a higher N uptake efficiency than compost (Möller and Müller, 2012). While biogas systems are typically more complex to operate and capital-intensive than composting operations of similar scale, the AD pathway has the benefit of generating energy-rich CH_4 in addition to CO_2; whereas, aerobic composting produces only CO_2 and heat, most of which is lost to the environment.

Biochar is another renewable soil amendment formed by pyrolysis of organic matter under hypoxic conditions, producing a substance that retains nutrients in soil ecosystems and stabilizes soil structure (Cai *et al.*, 2016; Wu *et al.*, 2017). Fields in India applied with 30 mg ha^{-1} biochar and 56.25 m^3 ha^{-1} biogas digestate yielded 1300% higher radish (*Raphanus sativus* L.) harvests than control plots, and this yield was higher than either digestate or biochar alone (Sekar *et al.*, 2014). While biochar is not a substitute for AD digestate or finished compost, co-application of biochar and digestate or compost can further benefit crop production due to the biochar's action as a dynamic nutrient sink in the soil.

Digestate contains a more balanced nutrient profile than pure livestock waste slurries, resulting in lower demand for nutrient fortification from conventional fertilizers when attending to crop nutrient requirements (Massé *et al.*, 2011). Studies investigating how fertilization of crops with AD digestate impacted crop nutrition have produced confounding results regarding vitamin C, acidity and amino acid contents (Liu *et al.*, 2009). There is a high variability in these qualities due to differences in the study species, the analyte being tested and experimental conditions. In addition to plant nutritive qualities, photosynthetic efficiency (PE) is another plant metric that changes with the type of fertilizer application. PE is particularly useful in quantifying plant response to external stresses, including soil nutrient levels (Singh *et al.*, 1939).

Anaerobic biogas and digestate generation, aerobic composting, and biochar production are all appealing practices in renewable agriculture. They convert 'waste' material into useful products for energy and soil amendment. As described above, renewable soil amendments—digestate, compost and biochar—have been studied individually and occasionally in combination for their effects on crop production, beginning to address the research gap postulated by Ji *et al.* (2017). There is, however, less known about potential synergistic effects of combining these renewable fertilizers. Here, we investigate the applications of biogas digestate to greenhouse-grown cucumbers, green leaf lettuce, curly kale, bush beans and cotton poplars. We present its impacts on growth, fruit production and crop nutrient content. The impact of addition of biochar and varied amounts of digestate and food waste to compost piles is also presented.

Materials and methods

Digestate production via anaerobic biogas digester

Two fixed-volume 3785-L plug flow anaerobic digesters operated by the Dickinson College Farm (College Farm, lat 40°08′N, long 77°08′W) in Boiling Springs, PA generated the digestate slurry utilized in this study. Digesters were fed pulped mixed food waste sourced from the Dickinson College dining hall. The food waste consisted of pre- and post-consumer compostable food materials, including fruit and vegetable peels, waste food left on cafeteria trays and unused catering food, as well as other compostable products, such as napkins and corn-based disposable utensils. The waste material was processed into a homogenized pulp using a HOBART food waste pulper and spun to reduce water content. Analysis of the food waste (FW) performed by DiStefano and Schust (unpublished, 2016) found 0.111 g total solids/g FW, 0.101 g volatile solids/g FW, 0.217 g COD/g FW and 886 mg total nitrogen/L FW. After seasonal startup with cattle manure, each digester was fed three times per week with 25 kg of pulped food waste diluted to 190 L with water to target 7% solids in the digester influent. Supernatant liquid was recirculated from the outlet of the digester to the inlet line during each feeding to assist with mixing and introducing a robust microbe community to the food waste/water matrix.

When digester gas production or pH decreased over a 2-day span, fresh microbes were introduced through addition of cow manure to a normal feeding cycle. Once an anaerobic digester was noted to be producing ample methane-rich gas within the proper VA/Alk range, 38 L of digestate was removed during recirculation for nutrient component analysis and use in the subsequent crop experiments. Additional information regarding digester construction, maintenance and gas quality can be found in the supplemental document.

Compost bioassay

During early June 2018 on the Dickinson College Farm, ten compost piles were constructed from mixtures of dry deciduous leaves, food waste, water, digestate and biochar. They were then divided into five test groups with two replicates of each composition (Table 1). The ratio of food waste to dry leaves in control treatments was 60 L FW: 180 L leaves. In experimental piles, as the quantity of added food waste was decreased, digestate was added in amounts representing the volume produced by the AD system when fed with the missing volume of food waste. This was to have the same total nutrient additions to each pile—the difference being whether or not the feedstock passed through the anaerobic digester. Piles were maintained for the duration of the summer with regular watering and turning. Each pile was constructed by layering leaf matter, digestate and food waste and biochar into piles, fluffed with a digging fork to ~1 m^3 to maximize the rate of decomposition via aeration.

All food waste and biochar were added to respective piles on the initial day of construction. Biochar was produced on site by slowly burning kiln-dried, untreated oak wood scraps from a local flooring

Table 1. Compost assay pile composition

Pile label	Pile composition
4FW	60 L food waste
	180 L leaf matter
4F	60 L food waste equiv. of digestate
	180 L leaf matter
2FW2F	30 L food waste
	30 L food waste equiv. of digestate
	180 L leaf matter
4FB	60 L food waste equiv. of digestate
	180 L leaf matter
	19 L biochar
2FW2FB	30 L food waste
	30 L food waste equiv. of digestate
	180 L leaf matter
	19 L biochar

Labels correspond to compost feedstock. FW, food waste; F, digestate; B, biochar.

Table 2. Chemical analysis of digestate utilized as feedstock in the compost and greenhouse bioassays

Test	As received analysis	Test	As received analysis
Nitrogen, N %	0.107	Iron, Fe ppm	15.5
Ammoniacal-N %	0.041	Aluminum, Al ppm	11.1
Phosphorus, P %	0.006	Manganese, Mn ppm	0.743
Potassium, K %	0.015	Copper, Cu ppm	0.114
Sulfur, S %	0.002	Zinc, Zn ppm	0.902
Magnesium, Mg %	0.004	Boron, B ppm	<0.100
Calcium, Ca %	0.019	Moisture %	99.5
Sodium, NA ppm	99.9	Solid %	0.5

Wet analysis of the slurry was performed by Waypoint Analytics in Leola, PA. Results were reported on a w/w% basis unless stated otherwise. This analysis indicated that the digestate was phosphorus poor but contained dilute but useful amounts of potassium, nitrogen and select micronutrients.

company in an oxygen-limited steel vessel. Charcoal was reduced to a coarse powder by packing the charred wood into burlap sacks and driving over the sacks repeatedly with a tractor. Digestate was gradually added to piles in the appropriate treatment groups by carefully pouring 19-L increments of freshly produced digestate on top of existing piles three times per week—adding all of the digestate equivalent to replaced food waste on the day of first construction would have resulted in liquid saturation and runoff. Nineteen liters of water was added to piles that did not require digestate addition during these feedings. Once sufficient digestate was added to piles requiring digestate addition, they were moistened with 19 L of water three times per week. Pile core temperatures were recorded daily before 9:00 am.

Once pile temperatures had stabilized (<2°C change between consecutive measurements), piles were turned and remade into a cubic shape, allowing composting microbes to digest new substrate. This continued until piles no longer showed increasing temperatures after turning. Pile temperature stabilization after turning indicated that the composting process had approached completion. Compost was sifted through a 1-cm^2 mesh screen to remove larger elements remaining in the soil. To gauge compost health, germination percentage of cucumbers (*Cucumis sativus*, var. Dasher II) grown in each treatment group's compost was tested. Cucumber was chosen as the study specimen due to high sensitivity to soil environments, accurately reflecting the ability of the potting media and nutrient inputs to nurture seedling growth. Ten seeding trays were prepared: each tray contained 40 cucumber seeds in sifted compost from one of the test piles. Trays were misted with water daily for a week. After 1 week, germination percentage was calculated by dividing the number of plants that had sprouted by the total number of seeds planted.

Greenhouse bioassay

Seedlings of the following plants were planted in flats on site in early June 2018: cucumbers (*C. sativus*, var. Dasher II), lettuce (*Lactuca sativa*, green leaf type), kale (*Brassica oleracea*, curly type) and green beans (*Phaseolus vulgaris*, bush type). Cotton poplar trees (*Populus* sp.) grown in 4 L pots from rooted cuttings were also used in the bioassay. The plants chosen represent many different crops and other vegetation types grown on a typical produce farm, including cucurbits, leafy greens, brassicas, legumes and trees.

Thirty-eight liters of digestate was collected from one digester in mid-June after AD operations were observed to be functioning healthily. A representative subsample of digestate was sent to Waypoint Analytical labs in Leola PA for chemical analysis of digestate components (Table 2). Digestate dilutions for feeding test groups were calculated by converting the digestate NPK ratio as reported by Waypoint Analytical to a 1:15 dilution of the NPK ratio 2.9: 3.5:0.3% w/w, matching the NPK ratio of an organic fish hydrolysate fertilizer (Organic Gem brand) commonly used by farms. A 1:1 ratio of digestate:water would have given the same nutrient content per volume added as the applied fish emulsion fertilizer. Previous experiments have indicated that digestate:water ratios greater than 1:1 inhibit root elongation and seed germination in cabbage and ryegrass (Kaparaju *et al.*, 2012). More dilute fertilizer ratios were chosen for test groups to observe how low-dose fertilizer application impacts growth and to avoid overfertilizing and 'burning' plants. Test groups that required digestate were fed the corresponding dilution once per week until saturation. All plants were watered daily.

Once crops had germinated, flats were transported to a thermostatically controlled greenhouse where seedlings were transplanted into larger containers filled with soilless media. The five crops were separated into six test groups, indicating the applied feeding regime. The test groups were 1:5, 1:5P, 1:20, 1:40, 1:40P and OS+ (Osmocote plus). Cotton poplar test groups were limited to 1:5, 1:20, 1:40 and OS+. Test group ratios indicate parts digestate:parts water applied during each feeding. Groups followed by a 'P' were fortified with 240 mL organic phosphorus fertilizer (bone meal) per 19 L soilless media during transplant. OS+ is a slow release conventional fertilizer, used as a control feeding regimen. Fifteen milliliters of OS+ pellets were added to the potting media surface for respective test groups during transplant. Bush beans grown on plants were harvested, massed and frozen for chemical analysis at three points during the growing period. Kale plant height was recorded at 4 weeks of growth, and multiple leaves from each plant were harvested and frozen for chemical analysis. Bean mass and kale height were chosen as measured indicators of crop production as these metrics represent the amount of edible vegetables produced by plants.

Photosynthetic efficiency

PE was measured for ten plants in each test group after a month of growth, ensuring plant leaves were large enough to cover the testing aperture. PE was measured using a 3-cm^2 reaction chamber head on a LiCOR-6800 testing system, and values were calculated by dividing the change in photosynthetic rate (FV) by the initial photosynthetic rate (FM). In determining FV/FM, the LiCOR system measured initial photosynthetic rate before pulsing a bright light in the chamber head and measuring the final photosynthetic rate.

Ascorbic acid and thiamine analysis

Ascorbic acid and thiamine content were determined for frozen leaf or fruit samples isolated from greenhouse grown curly kale and bush bean plants using a method adapted from Sami *et al.* (2014). In brief, ~10 g of each test group plant tissue was blended in a 1:1 ratio with 10 mL deionized water. Mixtures were stirred at reflux for 30 min and filtered to remove solids. The solutions were re-filtered with a 0.2-µm syringe filter and transferred to HPLC vials. Standard solutions containing both ascorbic acid and thiamine were made to calibrate response over a 10–250 µM range of each analyte. Chromatograms for ascorbic acid and thiamine were recorded on an 1100 Series Agilent 91312A HPLC using a 4.6 × 150 mm Agilent eclipse DB-C18 column with a 5-µm inner diameter. The mobile phase used was a 2.5 pH phosphate buffer, flowing at 0.8 mL min^{-1}. Ten microliters of each solution was injected onto the column and monitored at 267 nm. Sample runtime was 10 min. Peaks for ascorbic acid and thiamine were identified concurrently, with ascorbic acid and thiamine eluting at 3.2 and 2.4 min, respectively.

Phenolics and antioxidant analysis

Total phenolics and antioxidants were quantified for frozen leaf samples isolated from curly kale grown in the greenhouse bioassay. Total phenolic content was analyzed through an adapted Folin–Ciocalteau's phenolics method (Ainsworth and Gillespie, 2007). Ten grams of leaf tissue was blended in 100 mL deionized water for each test group. In total, 0.150 mL of plant sample, deionized water or epicatechin standard was added to a test tube containing 1.0 mL of 1:10 diluted Folin–Ciocalteau reagent. Epicatechin standards were made to calibrate response over a range of 25–200 µM. After reacting for 7 min, 1.0 mL of 7% w/v sodium carbonate was added. Samples were incubated in a 40°C water bath for 30 min. After 30 min, the absorbance of each solution was recorded with a UV-VIS spectrometer at 765 nm.

A modified TEAC (Trolox equivalence antioxidant capacity) assay was used to quantify total antioxidant content (Re *et al.*, 1999). Five grams of ground curly kale leaf tissue was suspended in 10 mL deionized water, filtered and stored at 4°C in the dark. The absorbance of 2.9 mL of 7 mM ABTS$^+$ at 734 nm was taken. In total, 33, 66 and 99 µL of each test solution was added to ABTS$^+$, then diluted to 3 mL total volume with deionized water. The absorbance values of each sample were taken again after 7 min. The change in absorbance for each sample was plotted against the concentration of analyte added to calculate %-inhibition for each test group. Values were compared against Trolox standard made to calibrate response over a 50–200 µM range.

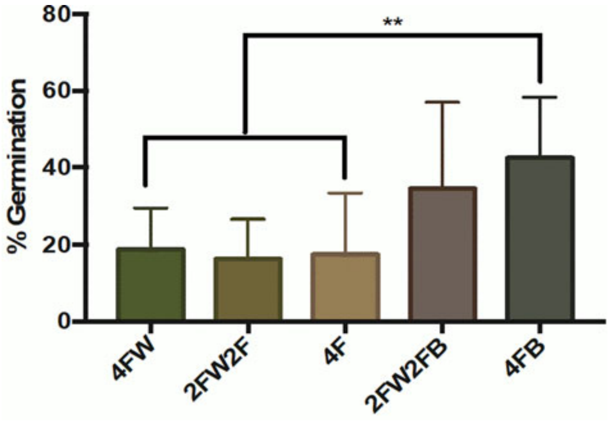

Fig. 1. Percent germination of cucumbers grown in different compost treatments. Biochar significantly increased crop germination. There were no significant differences in germination rates between the piles that contained biochar, or between the piles that lacked it.

Statistical analysis

Statistical analysis of data was performed using a two-tailed *t*-test to determine the reliability of the hypothesis. P values ≤0.05 were considered to be significant.

Results and discussion

Compost bioassay

Compost treatments applied to each test group are given in Table 1. Cucumber germination was significantly higher for the test group fed a mixture of digestate and biochar than all groups that lacked biochar ($P < 0.01$, $N = 50$, Fig. 1). Both groups that contained biochar resulted in roughly double the germination rates of test groups that did not contain biochar (Fig. 1). There were no statistical differences between groups not containing biochar, or between the two groups that contained biochar. Low average germination rates (<60%) are attributed to seed age. Composting processes were facilitated by the presence of food waste, as the piles constructed with food waste (4FW, 2FW2F, 2FW2FB) reached higher temperatures than piles containing solely digestate, indicative of higher microbial activity. Proper aeration due to frequent pile turning likely increased pile temperature (Fernandes *et al.*, 1994). Higher temperatures in the food waste-containing piles can potentially be explained by a larger mass of organic matter being present in the piles at any given time, providing ample substrate for microbial respiration. However, this trend did not translate to producing compost more fit for seedling germination at the time of the bioassay.

Fertilization with compost from food waste-containing piles resulted in lower cucumber germination than fertilization with compost from digestate and biochar piles. This was likely due

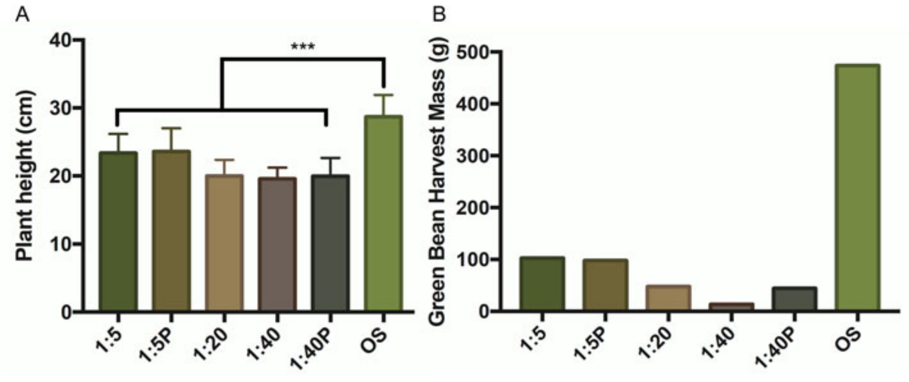

Fig. 2. Average curly kale height (A) and cumulative bush bean harvest mass (B) from different fertilizer treatments. Potent digestate solutions benefited crop height and fruit production compared to dilute treatments, but less than the chemical control fertilizer. Test group labels correspond with parts digestate: H_2O in feeding regimen, with P indicating phosphorus fortification during transplanting.

Table 3. Comparing micronutrient concentrations in Osmocote+ to digestate produced at the College Farm

Micronutrient	Osmocote+ concentration (ppm)	DCF digestate concentration (ppm)
Magnesium	13,000	4000
Sulfur	60,000	2000
Boron	200	Not detected
Copper	500	0.113
Iron	4600	15.5
Manganese	600	0.743
Molybdenum	200	Not tested
Zinc	500	0.902

Digestate nutrient composition was determined by subsample analysis at Waypoint Analytic (Leola, PA, USA). Information for nutrient composition of Osmacote+ retrieved from: https://icl-sf.com/uploads/USA/Product%20Sheets/OH/OH1005%20A903226%20Osmocote%20Plus%20%2815-9-12%29%285-6 M%29%28Std%29%20Product%20Info%20Sheet.pdf and converted into ppm.

to compost immaturity; immature compost addition changes the aerobic conditions of the soil ecosystem, resulting in a low oxygen environment that is detrimental to root growth and subsequently crop germination (Harada and Inoko, 1980; Mathur *et al.*, 1993). It is likely that although pile temperatures stabilized, the organic matter was not fully decomposed. Piles constructed from food waste contained all necessary organic matter from the day of initial pile construction, in contrast to the digestate piles which experienced gradual addition of digestate over the course of the compost formation period. Food material in the digestate was already mostly broken down by microbes in the anaerobic digester, meaning less digestion needed to occur in compost piles to produce finished compost.

Greenhouse bioassay

Crop height and fruit production increased with increasing potency of digestate fertilizer application (Fig. 2A and B). The control fertilizer (OS+) resulted in higher growth rates for curly kale plants than all other test groups ($P < 0.001$, $N = 12$, Fig. 2A). OS+ also increased vegetable harvest mass, as bush bean plants in the control group grew five times the mass of beans in the next largest harvest group (Fig. 2B). This discrepancy between crop production in control *vs* test groups can potentially be explained by differences in micronutrient presence between OS+ and the digestate. OS+ is a slow release chemical fertilizer, rich in NPK and micronutrients such as B, S, Cu, Fe, Mg and Zn that are all necessary to facilitate the fruiting process of these vegetables (Fageria *et al.*, 2002). Digestate samples used in this experiment contained micronutrients, but both the concentrations and ratios of various micronutrients were not sufficient to benefit crop growth (Table 3). The slow-release aspect of OS+ may have been optimized to deliver adequate amounts of nutrients over a sustained growth period; whereas, nutrients in digestate were immediately released into the soil upon addition. Biochar addition to soil provides potential to lengthen the nutrient release of N-rich fertilizers, including digestate, into soil environment, perhaps presenting a mechanism to increase the effectiveness of digestate as fertilizer (Ding *et al.*, 2016). Fortifying test groups with phosphorus had negligible effects on crop growth and vegetable production.

Photosynthetic efficiency

PE is a measure of plant stress levels. By comparing PE levels in ambient *vs* saturating light conditions, we quantified the change in photosynthesis as normalized to the initial photosynthetic rate. This ratio can also be expressed as FV/FM, where values near 1 indicate high efficiency. Bush beans fertilized by a high potency digestate solution photosynthesized at a significantly higher efficiency (0.88 for the 1:5 test group) than bush beans fertilized with a lower potency digestate solution (0.70 for the 1:40 test group), indicating that application of potent digestate solutions significantly increases photosynthetic efficiencies for this legume ($P = 0.01$, $N = 13$, Fig. 3A). Fortification of potting media with phosphorus for the 1:40 digestate dilution resulted in FV/FM values that were similar to more potent digestate test groups. No other significant differences in PE between test groups for any of the other plants were observed (Fig. 3B–D). PE was closest to 1 for curly kale (average values ranging between. 0.87 and 0.93) and cotton poplars (average values ranging between. 0.87 and 0.90), indicating healthy plants (Fig. 3B and C). Lower PE for bush bean (average values ranging between 0.70 and 0.88) and cucumber (average values ranging between 0.82 and 0.88) indicate more stressed plants, potentially due to higher nutrient requirements than the other study organisms (Fig. 3A and D).

Ascorbic acid and thiamine analysis

Ascorbic acid and thiamine were of interest because they contribute to anti-cancer activity, regulation of metabolic processes and

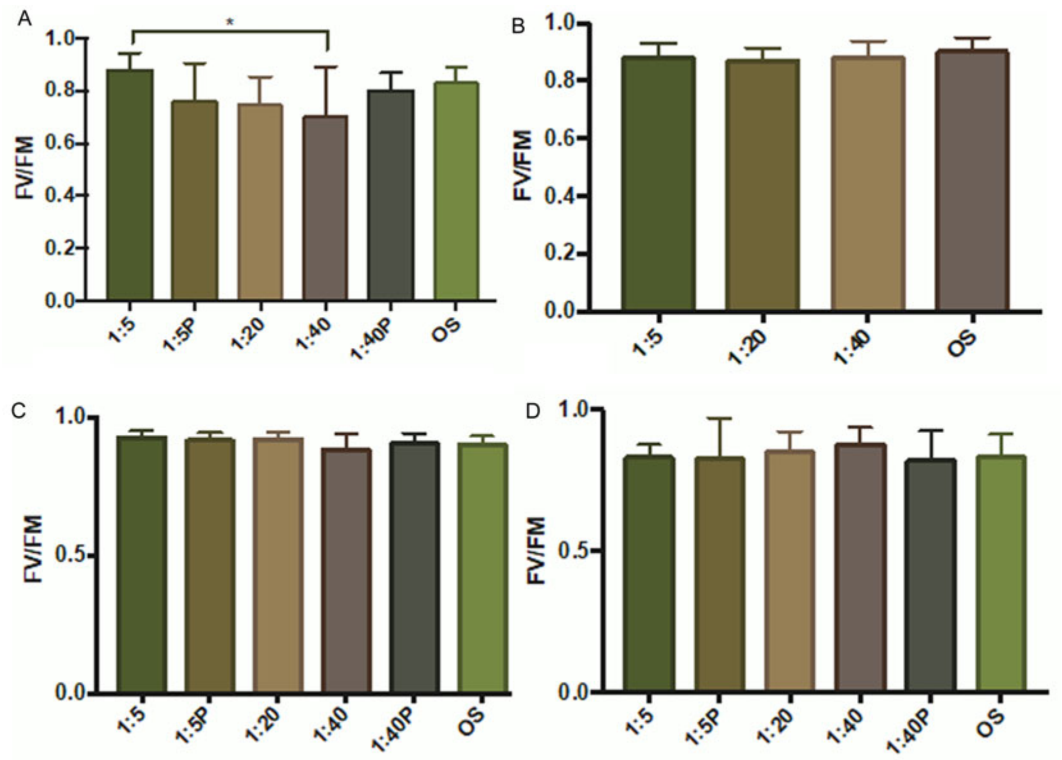

Fig. 3. Photosynthetic efficiency (PE) of bush bean (A), cotton poplar (B), curly kale (C) and cucumber (D) plants grown in different fertilizer treatments. Test group ratios indicate parts digestate:H_2O in fertilizer with OS+ as a control chemical fertilizer. PE increased with increasing digestate potency in bush bean test groups.

proper neurologic functioning (Cameron and Pauling, 1973; Sriram *et al*., 2012). In both bush bean and curly kale analysis, ascorbic acid content/g plant tissue for all test groups fertilized with digestate were higher than levels in plants grown with OS+ (Fig. 4A and B). Curly kale plants in more potent digestate treatments (1:5, 1:5P and 1:20) resulted in higher ascorbic acid content (>4.0 mmol ascorbic acid/g plant tissue) than more dilute fertilizer treatments (<3.2 mmol ascorbic acid/g plant tissue), indicating an impact of digestate concentration on ascorbic acid content (Fig. 4A). Although differences between test groups were not found to be significant, further analysis of digestate application on ascorbic acid in plant tissue has the potential to tease out significant trends for these plants. Ascorbic acid content in bush bean test groups peaked at 0.0016 mmol ascorbic acid/g plant tissue with a dilute 1:40P treatment, confounding impacts of digestate concentration on resulting ascorbic acid content (Fig. 4B). Thiamine content in bush beans followed the same trend as ascorbic acid, with the highest thiamine content (3.1 mmol thiamine/g plant tissue) observed for the 1:40P group (Fig. 4C). However, the OS+ trial group contained more relative thiamine than the 1:5P group (Fig. 4Cs). Both ascorbic acid and thiamine content in the 1:40 group for bush bean was omitted from comparison to other groups as insufficient mass was grown for sample preparation. Ascorbic acid content in curly kale increased with increasing digestate potency, with the 1:5P group resulting in the highest ascorbic acid concentration/g plant tissue. While phosphorus addition benefited ascorbic acid levels in potent digestate solutions, it did not have a similar result on more dilute digestate solutions. Thiamine content in bush beans was highest in the 1:40P test group, but there were no significant differences between any test groups.

Phenolics and antioxidant analysis

Phenolics and antioxidants are additional chemical compounds that comprise part of a healthy diet. Antioxidants have received attention due to claimed anti-aging properties and other health benefits. While the robustness of these claims is still being established, antioxidants reduce free radicals in the body that would otherwise serve to impair metabolic functioning (Finley *et al*., 2011). Phenolics contribute to antioxidant activity, implying their importance to human health (Liyana-Pathirana and Shahidi, 2006).

Both total phenolics and antioxidant capacity for curly kale were higher in test groups fed potent digestate solutions (1:5, 1:5P) than groups fed dilute digestate solutions, indicating an impact of increasing digestate potency on increasing antioxidant capacity and phenolic content in curly kale leaves (Fig. 5A and B). Antioxidant capacity in curly kale leaves in the 1:5P digestate treatment was significantly higher than antioxidant capacity in dilute digestate treatdments, 1:20 1:40, 1:40P ($P < 0.01$, $N = 4$, Fig. 5A). Total phenolic content was higher in plant tissue fertilized with potent digestate solutions (1:5, 1:5P) than the OS+ control group (Fig. 5B). Addition of phosphorus benefitted antioxidant capacity of kale plants fed a potent digestate solution but did not increase antioxidant capacity for dilute digestate test groups or in the phenolics assay (Fig. 5A and B). AD digestate has been previously been utilized as an organic fertilizer on farms (Mostafazadeh-Fard *et al*., 2019). Our observations are supported by studies that show organic fertilizer regiments producing more nutritious vegetables when compared with conventional fertilizer systems (Bimova and Pokluda, 2009; Raigón *et al*., 2010; Aminifard *et al*., 2013; Ibrahim *et al*., 2013; Moreno-Reséndez *et al*., 2016) for certain test species and experimental conditions.

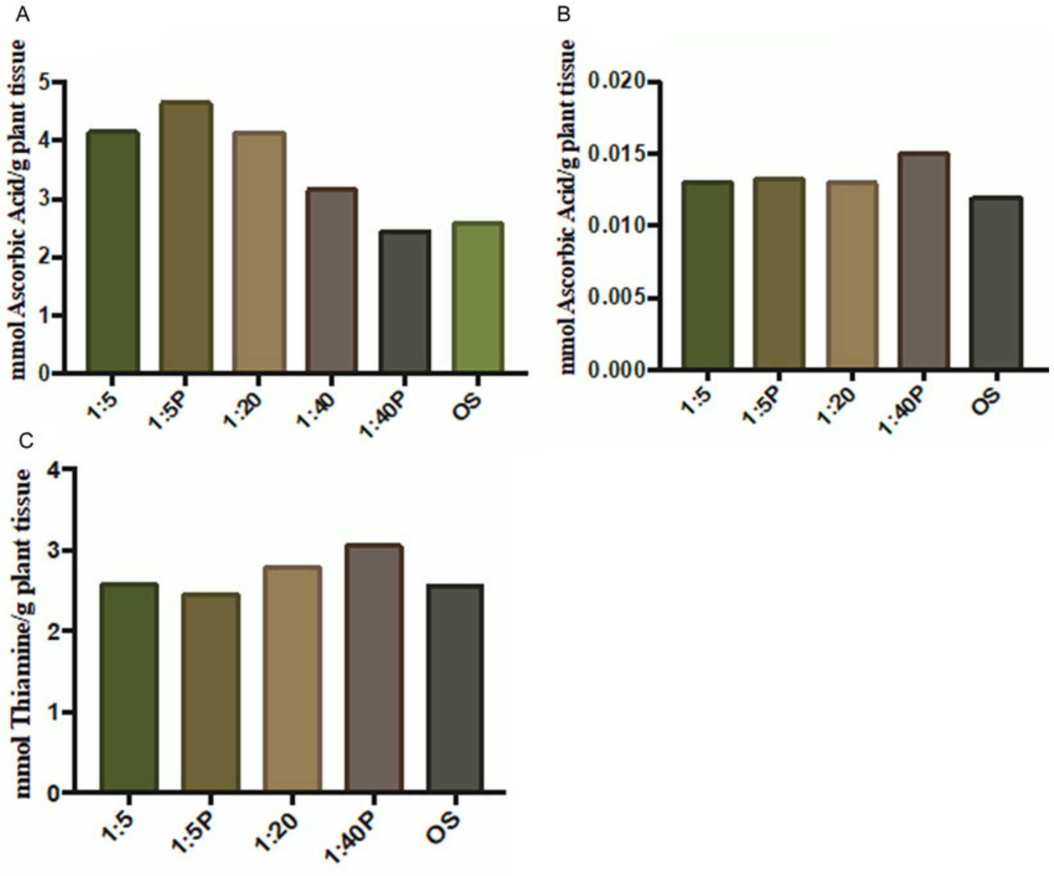

Fig. 4. Ascorbic acid content in curly kale (A) and bush bean (B) and thiamine content in bush bean (C) grown in different fertilizer treatments. Test group ratios indicate parts digestate:H_2O in fertilizer with OS+ as a control fertilizer. Increasing digestate potency increased ascorbic acid content in curly kale compared to dilute treatments and the control fertilizer.

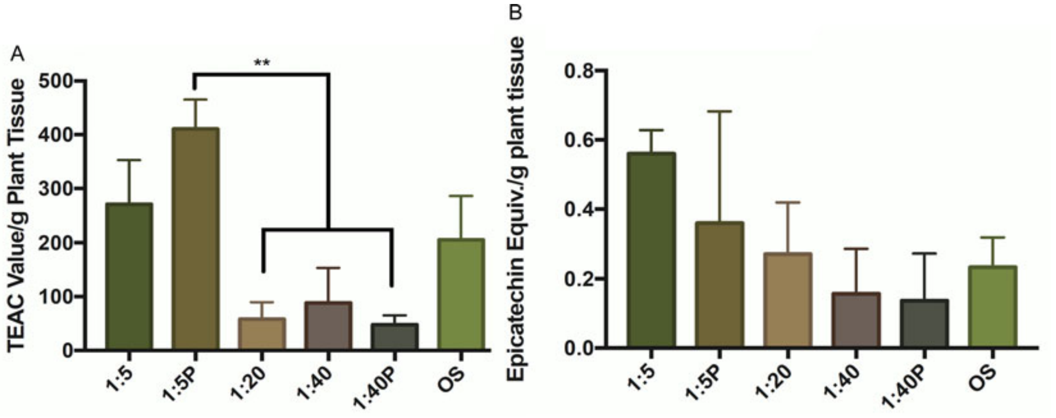

Fig. 5. Antioxidant capacity (A) and total phenolic content (B) in curly kale grown with different fertilizer treatments. Test group ratios indicate parts digestate:H_2O in fertilizer with OS+ as a control fertilizer. Increasing digestate potency increased antioxidant capacity in curly kale compared to dilute digestate treatments and the control fertilizer. Phenolic content trended upward with digestate potency.

Conclusion

Growth and fruit production are nutrient-intensive processes that require substantial inputs. Increased growth and nutrition for kale test groups fertilized with potent digestate solutions indicate that AD digestate can function as a nutrient-rich soil amendment. Our work agrees with prior investigations that concluded biogas digestate has the potential to be utilized as a viable NPK fertilizer to benefit crop growth (Loria *et al.*, 2007; Nishikawa *et al.*, 2012; Vanegas and Bartlett, 2015) and, for certain study species, crop nutrition (Liu *et al.*, 2009), reducing demand for chemical fertilizers (Saigusa *et al.*, 2018). Proper application of organic or renewable fertilizers such as AD digestate has the potential to offer a lower-cost and environmentally-friendly alternative to conventional fertilizers, avoiding the pervasive effects associated with conventional mineral fertilizers on agroecosystems, including eutrophication and heavy metal accumulation (Yin *et al.*, 2007; Teglia *et al.*, 2011; Savci, 2012). Biogas digestate contains fewer harmful pathogens (Furukawa and Hasegawa, 2006) and lower levels of heavy metals (Mukhuba *et al.*, 2018) compared with raw cattle manure, indicating its viability as a substitute for manure. There are many ways to test (Walsh *et al.*, 1991; Young *et al.*, 2012) and treat (Drennan and DiStefano, 2010; Ji *et al.*, 2017; Zhang *et al.*, 2018; Xu *et al.*, 2019) digestate before application to fields, lessening concerns about toxicities to ecosystems. Future investigations should explore how fortification of AD digestate with a micronutrient source benefits crop growth and vegetable production. Investigating how more potent digestate solutions impact crop production would allow comparison with findings that dilutions containing >1:1 digestate:water ratio negatively impact crop production (Kaparaju *et al.*, 2012).

Diverting food waste from compost operations to AD and feeding the resulting digestate to compost piles with biochar does not negatively impact compost production. Instead, this process provides an alternative method for compost nutrient fortification in addition to generation of an energy-rich gas. AD systems are applicable to agricultural communities that lack stable supplies of cooking fuel, fertilizer or water sanitation mechanisms (Noyola *et al.*, 2006). Expanding biogas production on farms offers benefits ranging from increased energy generation to lowered water contamination to renewable fertilizer production that increases crop nutrition and plant health. These results are applicable to communities that could repurpose livestock waste to produce fuel and a viable soil amendment, including many impoverished agrarian areas both domestically and globally.

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