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Assessing the Effects of Digestates and Combinations of Digestates and Fertilizer on Yield and Nutrient Use of *Brassica juncea* (Kai Choy)

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Abstract: Anaerobic digestion of organic wastes produces solid residues known as digestates, which have potential as a fertilizer and soil amendment. The majority of research on digestate focuses on their fertilizer value. However, there is a lack of information about additional effects they may have on plant growth, both positive and negative. Understanding the effects of digestate on plant growth is essential to optimizing their use in agriculture and helping close the loop of material and energy balances. This greenhouse study evaluated the effects of two different digestates, a food waste digestate (FWD) and a lignocellulosic biomass digestate (LBD); a liquid fertilizer; and various combinations of fertilizer and digestates on plant growth, nutrient uptake and nutrient use efficiency (NUE) of *Brassica juncea* (kai choy) plants. It also evaluated potential negative attributes of the digestates, including salinity and possible biohazards. Combinations of LBD and fertilizer performed as well or slightly better than the fertilizer control for most parameters, including aboveground biomass and root length. These same combinations had significantly higher nitrogen use efficiency than the fertilizer control. Inhibitory effects were observed in 100% LBD treatments, likely due to the high electrical conductivity of the media from digestate application. Based on this research, LBD could partially replace mineral fertilizers for kai choy at up to 50% of the target nitrogen rate and may lead to increased plant growth beyond mineral fertilizers. FWD could replace up to 100% of the target nitrogen application, without causing significant negative effects on plant growth. Increasing the use of digestates in agriculture will provide additional incentives for the anaerobic digestion process, as it produces two valuable products: biogas for energy and digestate for fertilizer.

Keywords: anaerobic digestion; digestate; biofertilizer; nutrient use efficiency

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1. Introduction

With rapid urbanization and population growth, significant amounts of waste, particularly organic waste, are generated. More than a quarter of the waste produced in the United States consists of organic materials, much of which is landfilled or incinerated, leading to a multitude of environmental problems [1]. In addition, global dependence on fossil energy has created energy insecurity in much of the world, along with significant environmental costs, including production of greenhouse gases and degradation of air quality.

Anaerobic digestion (AD) is a promising technology with the capability of turning a variety of organic materials, including organic waste, into two potentially useful products: renewable bioenergy in the form of biogas and the digested solids, hereafter referred to as "digestate", as a potential fertilizer and soil amendment [2]. Various feedstocks are used in AD, ranging from waste products (e.g., animal manures, agricultural wastes/residues, food waste, etc.) to dedicated energy crops, including various grasses and grains. The use of waste products in AD provides additional benefits including net energy production rather than consumption and mitigation of the need for costly waste disposal [3], while also producing a valuable soil amendment [4]. While often seen as a waste or by-product of the anaerobic digestion process, digestate must be considered as another valuable resource: fertilizer.

Digestate helps recycle valuable nutrients back into agricultural systems, often in a more plant-available form than the raw materials [5]. Digestate is particularly rich in ammonium nitrogen (NH_4^+-N), a form of nitrogen that is readily available for uptake by plants. The organic matter that makes up the bulk of digestates has multiple benefits in agriculture, including increasing the microbial activity and nutrient cycling [6]. In addition, bioactive substances such as phytohormones and hormone-like compounds that may further improve plant growth have been found in digestates [7–10].

Much of the research on digestates has focused on its value as a nitrogen fertilizer [11], but digestates are a complex mixture that contain nutrients, as well as various organic compounds, including phytohormones and other bioactive molecules that can affect root growth and morphology [9,10,12]. Roots are integral for plant growth and nutrient acquisition and changes in root morphology can, in turn, have significant effects on nutrient use efficiency (NUE) and yield. The limited research on the effects of digestates on root growth shows varying and somewhat contradictory effects on overall root biomass, with some studies showing increased root biomass under digestate treatment [13,14], some showing decreased root biomass with digestates [15,16] and another showing no difference in root biomass with digestate, as compared with mineral fertilizer [17]. However, digestate addition may induce important changes in root growth rate and root morphology that affect NUE. These effects may not be captured by biomass measurements alone and may have significant effects on plant yield. Specifically, early root growth and root hairs can have important implications for NUE, plant establishment, and overall plant growth and yield [18,19].

While digestates have multiple beneficial attributes for plant growth, there are important and potential negative aspects of using digestates as fertilizers. Specifically, the high electrical conductivity (EC) associated with salinity of many digestates can make them unsuitable as sole fertilizers and may instead be better used in combination with other fertilizers, particularly with more sensitive plants [6,20]. Maunuksela et al. [21] found increased or similar growth with barley (*Hordeum vulgare*) when using digestates, as compared with mineral fertilizers, but decreased Chinese cabbage (*Brassica pekinensis*) growth, suggesting that digestate alone may be too saline for less salt-tolerant plants. However, two studies have found that replacing some fertilizer with digestate can produce similar or greater plant growth than fertilizer alone [20,22]. There is a lack of information on how digestates affect plant growth and nutrient status when combined with fertilizers, and the only information available on the combination is either in grassland or hydroponic systems, both quite different from annual vegetable systems [20,23].

The objectives of this study were to (1) evaluate the effects of two different digestates, both alone and in combination with an organic fertilizer, on plant growth (*Brassica juncea*, kai choy) parameters, including aboveground biomass and root growth; (2) evaluate the effects of the digestates on nutrient uptake and use efficiency; and (3) evaluate potential negative attributes of the digestates, including salinity and possible biohazards.

2. Materials and Methods

2.1. Digestate and Fertilizer Characterization

The digestates for this study were produced in Khanal's laboratory at the University of Hawaii at Manoa. The lignocellulosic biomass digestate (LBD) was produced from Napier grass (*Pennisetum purpureum*, a typical energy crop grown specifically to produce bioenergy) via semi-continuous anaerobic digestion. The food-waste digestate (FWD) was produced from food waste collected at the University of Hawaii cafeteria and produced via semi-batch anaerobic digestion. The food waste used in the FWD was collected over a one-week period to account for the effects of slight menu changes and frozen after each collection. The final mixture consisted of approximately 40–50% starch (rice and bread), 20–30% meat and 20–30% fruits and vegetables. The food waste was blended until homogenized, and a portion of this composite sample was used as the feedstock for anaerobic digestion to produce FWD. The liquid fertilizer used in this experiment was GrowBig® (6-4-4, with micronutrients) from FoxFarm (Samoa, CA, USA). Relevant chemical properties of the feedstocks, digestates and fertilizer are shown in Table 1. Digestate and feedstock measurements were taken in duplicate, unless otherwise noted, and the mean and standard deviation are reported in Table 1.

Table 1. Selected chemical characteristics of the feedstocks, digestates and fertilizer used in this study. Feedstock and digestate parameters were measured in duplicate, and both measurements are included, while the values for the commercial FoxFarm product came from the manufacturer's label. Nutrient values for the feedstocks are on a dry-weight basis, and measurements for digestates are on a wet-weight basis.

Parameter	Lignocellulosic-Biomass Feedstock	Food-Waste Feedstock	
Total Solids (%)	91.9, 92.1	29.6, 31.8	
Total Nitrogen (mg kg^{-1})	7840, 7680	30,080, 29,920	
Phosphorus (mg kg^{-1})	2300, 2200	3600, 3700	
Potassium (mg kg^{-1})	14,400, 16,000	6000, 6400	
Calcium (mg kg^{-1})	1700, 1600	1300, 1300	
Magnesium (mg kg^{-1})	1800, 2100	600, 600	
Sulfur (mg kg^{-1})	1200, 1100	2400, 2600	
Sodium (mg kg^{-1})	180, 150	6990, 7470	
Iron (mg kg^{-1})	395, 450	32, 33	
Zinc (mg kg^{-1})	17, 16	19, 19	
Copper (mg kg^{-1})	3, 4	3, 3	
Manganese (mg kg^{-1})	27, 24	12, 12	
	Lignocellulosic-Biomass Digestate (LBD) *	**Food-Waste Digestate (FWD) ***	**FoxFarm GrowBig®**
Total Solids (%)	2.93, 2.81	1.21, 1.24	0
Electrical Conductivity (EC, mS cm^{-1}) †	8.34 ± 1.26	10.99 ± 0.030	**
pH †	8.4 ± 0.102	8.6 ± 0.015	3.24
Total Nitrogen (mg kg^{-1})	740, 680	1770, 1800	60,000
Ammonium Nitrogen (mg kg^{-1})	280, 260	1150, 1140	29,000
Organic Nitrogen (mg kg^{-1})	460, 420	620, 660	0
Nitrate Nitrogen (mg kg^{-1})	0, 0	0, 0	31,000
Phosphorus (mg kg^{-1})	120, 110	460, 100	17,500
Potassium (mg kg^{-1})	910, 920	380, 310	33,200
Calcium (mg kg^{-1})	90, 90	250, 60	0
Magnesium (mg kg^{-1})	110, 110	130, 10	6000
Sulfur (mg kg^{-1})	70, 60	410, 40	**
Sodium (mg kg^{-1})	890, 920	930, 850	230
Chloride (mg kg^{-1})	2500, 2500	1300, 1200	0
Iron (mg kg^{-1})	31, 27	496, 24	1000
Zinc (mg kg^{-1})	1.0, 0.9	13.1, 0.9	500
Copper (mg kg^{-1})	<1, <1	3.7, <1	500
Manganese (mg kg^{-1})	1, 1	1, <1	500

* Indicates full strength or concentrated form; ** indicates not measured; † n = 5 with error reported as standard deviation.

2.2. Experimental Growing Conditions

The experiment was conducted in the Pope Greenhouse facility at the University of Hawaii at Manoa, from 14 February to 25 March 2019, for a total of 40 days of growth. Kai choy (*Brassica juncea*, var. Hirayama) was used as the test crop due to its rapid growth and relative heat tolerance. The experiment was conducted as a randomized complete block design and plants were grown in a series of rhizoboxes on a single greenhouse bench. Each rhizobox consisted of an opaque plastic back and a clear plexiglass front that was covered by foil at all times except for imaging to limit UV exposure to roots. A single piece of black felt was placed along the clear plane to increase contrast for imaging. The boxes were filled with potting medium behind the felt. Rhizoboxes were kept at a 45° angle with the clear plane facing downwards throughout the growing period to allow root growth along the clear plane.

Sunshine Mix # 1 (Sun Gro Horticulture, Agawam, MA, USA) was pre-mixed with water, to reach 80% of field capacity, and calcium sulfate ($CaSO_4 \cdot 2H_2O$) was added at a rate of 2 g/kg, to ensure adequate calcium for plant growth in all treatments. Rhizoboxes were filled with this mixture at a rate of 177 g of oven-dried medium equivalent per rhizobox. Five kai choy seeds were sown directly into each rhizobox container and thinned to a single plant after 5 days of growth. Digestates and/or fertilizer were mixed to achieve the target treatment levels with water to ensure a consistent volume of liquid was added to each plant regardless of treatment. The first treatment solutions were added immediately after seeding. In order to simulate application by fertigation, treatments were subsequently applied every 10 days throughout the growing period for a total of 4 applications. Mature plants were harvested 40 days after seeding, corresponding to a typical harvest timeline for this vegetable. At harvest, roots had occupied the full volume of the rhizoboxes in the high performing treatments, but they did not appear to be compromised by the rhizobox size.

2.3. Experimental Growing Conditions

The application rate for treatments was normalized based on the amount of mineral nitrogen (N) applied. Fertilization was based on nitrogen recommendations of 56 kg N ha^{-1} for leafy greens grown in organic soils [24]. This amount was then doubled to ensure adequate nutrition in the constrained volume of the rhizoboxes for the treatments receiving 100% of the recommended fertilizer N. Therefore, the 100% nutrient rate was established at 518 mg N kg^{-1} based on a depth of 15 cm and a bulk density of 0.144 g cm^{-3}. Ammonium N was considered as the only nitrogen source in digestates since shorter growing periods have been shown to produce little to no mineralization of organic N in previous studies with digestates [14].

Treatments consisted of varying combinations of each digestate and fertilizer to reach the appropriate level of nitrogen. Two control treatments were included in this study: an unamended control (CTRL) receiving no fertilizer or digestate was used for nutrient use efficiency calculations, and a fertilizer control (FCTRL) was used for comparison with digestate treatments receiving 100% of the target N in the form of fertilizer. Plants receiving 100% of the nutrient level were fertilized with 91.7 mg N per pot containing 177 g of oven-dried media equivalent. Increasing amounts of each digestate (at rates of 10%, 50% and 100% digestate) were supplemented with fertilizer to reach the target N level.

Percentages recorded indicate the percent of the 100% rate of nitrogen applied with each amendment and are specified in Table 2. There were 8 treatments and 5 blocks (5 replicates), for a total of 40 experimental units.

Table 2. Treatment specifications including proportion of N added per treatment, proportion coming from digestate addition and proportion from organic fertilizer.

Treatment	Description	% N	% N from Digestate	% N from Fertilizer
CTRL	No amendments	0	0	0
FCTRL	100% fertilizer control	100	0	100
FWD10	10% FWD, 90% fertilizer	100	10	90
FWD50	50% FWD, 50% fertilizer	100	50	50
FWD100	100% FWD	100	100	0
LBD10	10% LBD, 90% fertilizer	100	10	90
LBD50	50% LBD, 50% fertilizer	100	50	50
LBD100	100% LBD	100	100	0

CTRL, unamended control; FCTRL, fertilizer control; FWD, food-waste digestate; LBD, lignocellulosic-biomass digestate.

2.4. Plant Growth and Root Length Analyses

At harvest, plants were cut just above soil level to separate shoots from roots. Shoots were immediately weighed for fresh weight and roots were cleaned thoroughly and blotted dry before weighing for fresh weight. Both roots and shoots were oven-dried at 65 °C, until they maintained a constant weight to obtain dry weight.

In order to assess differences in root growth, digital photographs of the clear plane of the rhizoboxes were taken every 3 to 4 days during the active growth period for a total of 7 photographic events. A Nikon D7500 digital camera (Nikon USA, Melville, NY, USA) mounted on a tripod was used to capture images. Photographs were then standardized by cropping each image to a 15 × 15 cm square in the center of each rhizobox in order to minimize edge effects. Image analysis on roots was started 18 days after seeding. The 225 cm^2 area was then analyzed at each of 7 time points during the growing period for total root length, using ImageJ [25]. The grid intersect method [26] was used to estimate root length based on the number of intersections between roots and the lines of a randomly oriented 1 cm^2 grid. Briefly, all intersections between roots and lines of the grid were counted and converted into root length, using Equation (1):

$$R = \frac{\pi N A}{2H} \tag{1}$$

where R is total root length (cm), N is number of intersections between the root and straight lines of the grid, A is the area of the grid (cm^2) and H is the total length of straight lines within the grid (cm).

2.5. Plant Nutrient Analyses

All aboveground parts were ground and sieved to 250 µm after oven drying. Samples were weighed on a microbalance before N analysis. Following dry ashing and acid digestion, they were run on a Carlo Erba NC 2500 Elemental Combustion System/Pneumatic Autosampler (CE Elantech, Inc., Lakewood, NJ, USA) for determining weight percent nitrogen. All other macro- and micronutrients were measured by DairyOne forage laboratory (Ithaca, NY, USA). Following dry ashing and acid digestion, samples were analyzed, using a Thermo iCAP 6300 Inductively Coupled Plasma Radial Spectrometer (Thermofisher Scientific, Waltham, MA, USA). The amount of nutrient applied was calculated, using the mean of the duplicate samples for the LBD and using the first value reported for FWD in Table 1. For the FWD, the analytical results from the two samples showed large differences for certain nutrients, most notably phosphorus. To address this, we utilized the replicate with the higher values because it was most similar to digestates made from food-waste streams in other studies [27–29]. These data were then used to calculate the apparent recovery efficiency (*ARE*) for nitrogen, phosphorus and potassium, using Equation (2) [30]:

$$ARE\ (\%) = (U - U_0)/F \tag{2}$$

where U is nutrient uptake in aboveground biomass (concentration X dry weight, mg) with nutrient applied, U_0 is nutrient uptake in aboveground biomass with no nutrient applied (mg) and F is amount of nutrient applied (mg). Recovery efficiency indicates how much of the nutrient applied the plants took up.

2.6. Electrical Conductivity and Biohazard Analysis

Electrical conductivity (EC) was measured, using the 1:2 (*v*:*v*) soil:water extract method (Dellavalle, 1992). We added 20 cm^3 of potting media from the selected treatments and 40 mL of distilled water to a falcon tube. The tubes were shaken for 30 s every hour for four hours and then allowed to equilibrate for 24 h. After 24 h, EC was measured with an Orion DuraProbe 4-electrode conductivity cell (Thermo Scientific, Waltham, MA, USA) for each 1:2 extract (*EC*1:2). The *EC*1:2 values were converted to saturated paste values (*ECSS*) in order to evaluate phytotoxicity. We used the EQN 3 given below, adapted from Sonneveld and Voogt [31] and omitted the term for SO_4 because of the negligible amount present in the media (Equation (3)):

$$ECSS = 0.908dEC1:2 - 0.89d + 0.68 \tag{3}$$

where *d* is the dilution factor, a ratio between the water content of the 1:2 suspension and the water content of field moist soil that was determined, in a previous experiment, to be 2.52.

Fecal coliforms were quantified both in the fresh digestates and in media samples collected from each pot at harvest. Fecal coliforms were enumerated, using the multiple tube fermentation technique [32].

2.7. Statistical Analysis

Statistical analyses were performed in both R (R Core Team, 2013) and Minitab 18 (State College, PA, USA). For all analyses, a *p*-value was considered significant at the 0.05 level. Blocks were treated as replicates and the blocking effect was accounted for in all models. Models were tested for assumptions of normality and equal variance. For all parameters described, we conducted a one-way analysis of variance (ANOVA) followed by Tukey's Honest Significant Difference test (HSD) at the $\alpha = 0.05$ level of significance in Minitab. Regression analysis with a General Linear Model in Minitab was used to analyze response of growth parameters to increasing amounts of digestate at the 100% fertilization level. In addition, we analyzed root-growth data over time, using repeated measures analysis for the 100% nutrient levels in R. There was no significant difference between the model with mixed effects vs. fixed effects ($p = 0.627$), and the data were further analyzed by using the fixed-effects model. We compared the least squares means (LS means) between treatments, over time, using Tukey's HSD. Treatments at individual time points of interest were then further analyzed, using ANOVA and Tukey's HSD in Minitab. The raw data used to generate all figures are contained within the supplementary materials.

3. Results

3.1. Digestate Effects on Plant Growth

The unamended control treatment produced the lowest amount of biomass and was included only for nutrient use efficiency calculations. The treatments containing low to moderate amounts of lignocellulosic-biomass digestate mixed with fertilizer (LBD10 and LBD50) produced the highest amount of biomass of all the treatments, although not significantly different from the fertilizer control (Figure 1). The 100% LBD treatment, on the other hand, produced the lowest amount of biomass, which was significantly lower ($p = 0.028$) than the LBD mixtures, but not lower than the fertilizer control. All digestate treatments produced similar amount of biomass to the fertilizer control.

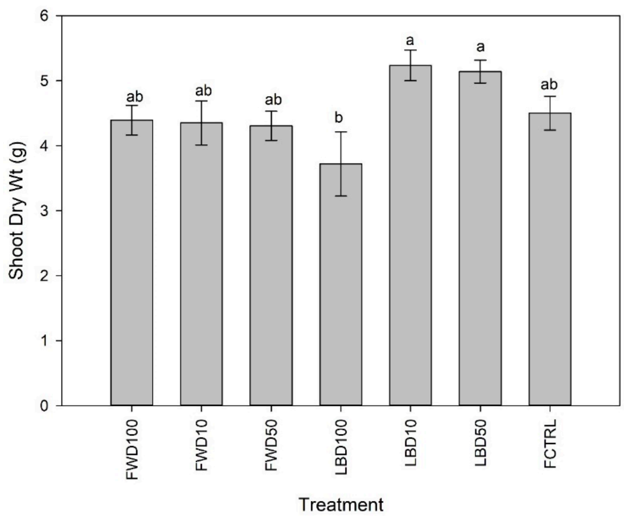

Figure 1. Digestate and fertilizer effects on kai choy shoot dry weight in a controlled greenhouse experiment. Error bars are the standard error of the mean ($n = 5$), and means that do not share a letter are not significantly different ($p < 0.05$).

Regression analysis for treatments at 100% N with increasing amounts of LBD showed a significant quadratic relationship between the amount of digestate added and shoot dry weight ($R^2 = 0.42$, $p = 0.01$). Shoot dry weight increased with increasing amounts of digestate and then showed a tendency to decrease above 50% digestate (Figure 2). For increasing additions of FWD at the 100% N level, there was no significant effect on shoot dry weight.

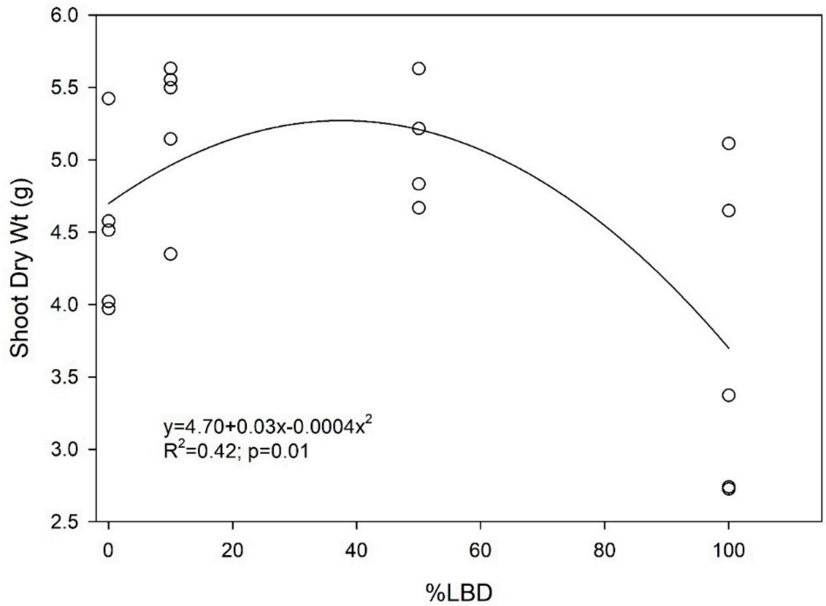

Figure 2. Regression curve for greenhouse-grown kai choy shoot dry weight with increasing LBD at the 100% N level.

3.2. Root Growth

There was a significant effect of treatment ($p < 0.0001$), time point ($p < 0.0001$), and the interaction of treatment and time point on root length ($p = 0.0301$). The increase in root length over time with different digestate treatments is shown in Figure 3. For FWD treatments (Figure 3a), there was a divergence beginning with the first measurement at 18 days after planting, with FCTRL and FWD10 producing significantly greater root growth than the other treatments ($p = 0.003$). The FCTRL continued to produce significantly greater root growth than the digestate treatments up to 30 days after planting. At harvest, FWD root length was not different from the FCTRL, except for a significant decrease in FWD50. For LBD treatments (Figure 3b), there was a divergence as early as 18 days as well, with the LBD100 significantly decreasing root growth, compared with the rest of the treatments ($p < 0.005$). From day 30 to day 38, the LBD10 treatment had the greatest root lengths, which were significantly greater than the LBD100 treatment, but not different from the FCTRL. By harvest time, there were no significant treatment effects on root length.

Least squared means (LSmeans) were significantly different between treatments averaged over time points (Figure 4). The greatest root lengths were in the combined LBD and fertilizer treatments (LBD10 and LBD50) and FCTRL. All FWD and the LBD100 treatment had significantly lower root lengths than the FCTRL.

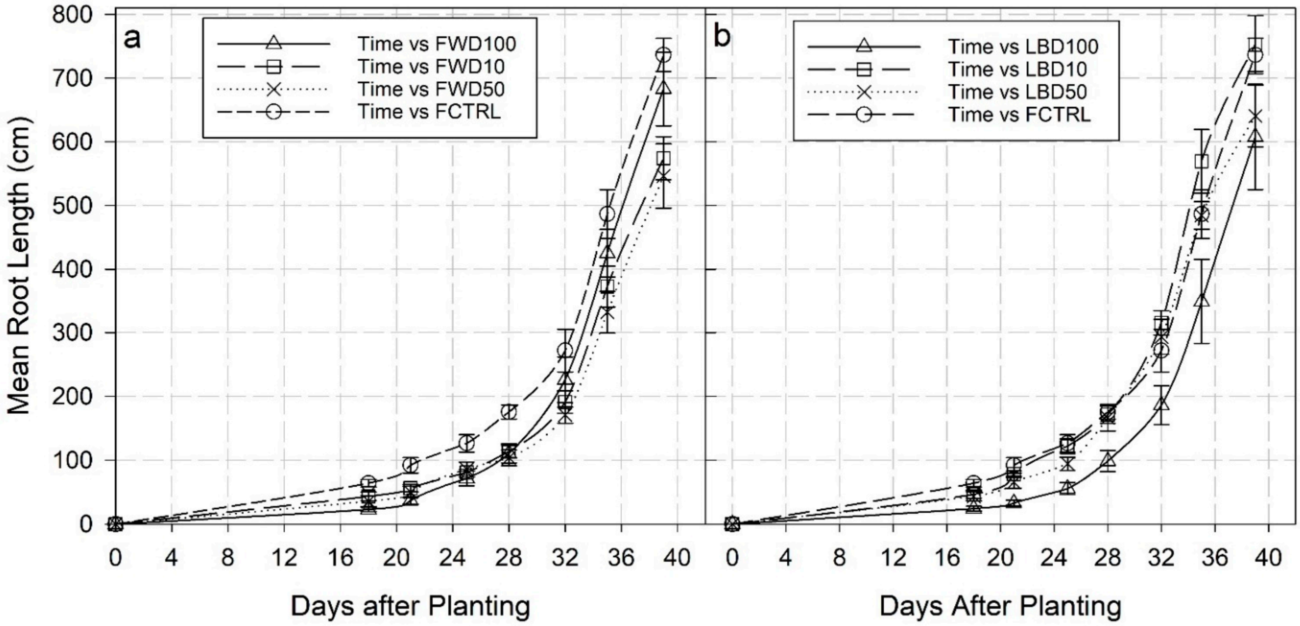

Figure 3. Mean root growth dynamics of kai choy plants during the 40-day greenhouse experiment, as affected by FWD (**a**) and LBD (**b**). Error bars represent the standard error of the mean ($n = 5$).

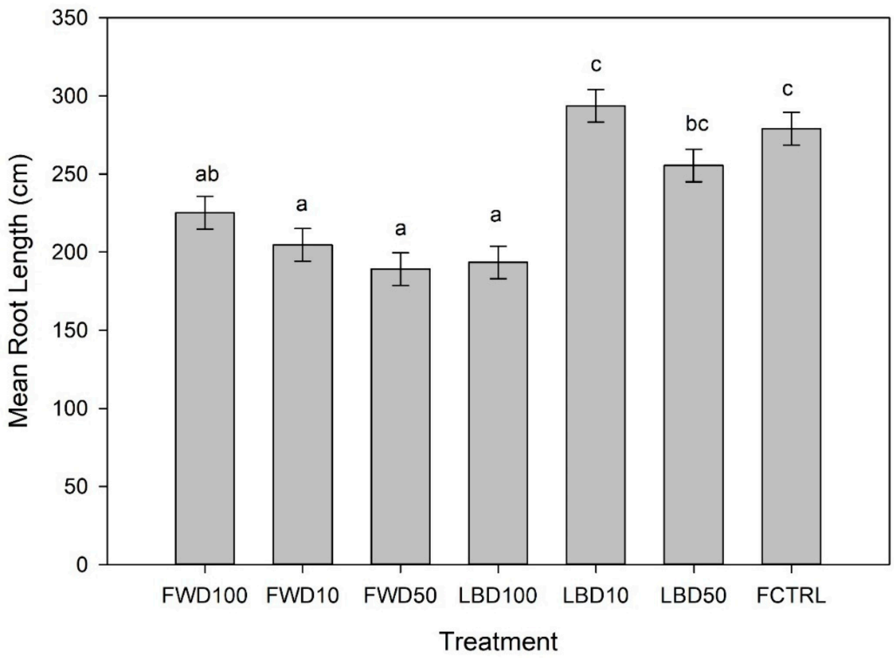

Figure 4. Treatment effects on least squared means (Lsmeans) for root length averaged over all time points. Error bars are the standard error of the mean ($n = 5$), and means that do not share a letter are significantly different ($p < 0.05$).

3.3. Nutrient Uptake and Use Efficiency

Apparent recovery efficiency (RE) for nitrogen was significantly different due to treatment ($p = 0.0032$). RE was highest for the LBD and fertilizer combinations (LBD10 and LBD50) and lowest for the FCTRL (Figure 5a). RE for phosphorus was significantly different due to treatment ($p < 0.001$). Phosphorus RE was highest for LBD 10, LBD50, FWD10 and FCTRL (Figure 5c,d). Again in the case of P, the mixtures of LBD and fertilizer performed amongst the best, and the 100% digestate treatments both had the lowest RE of any treatments. RE for potassium was significantly different due to treatment ($p < 0.001$). RE was highest for FWD100 and FWD50 and lowest for LBD50 and LBD100 (Figure 5e,f).

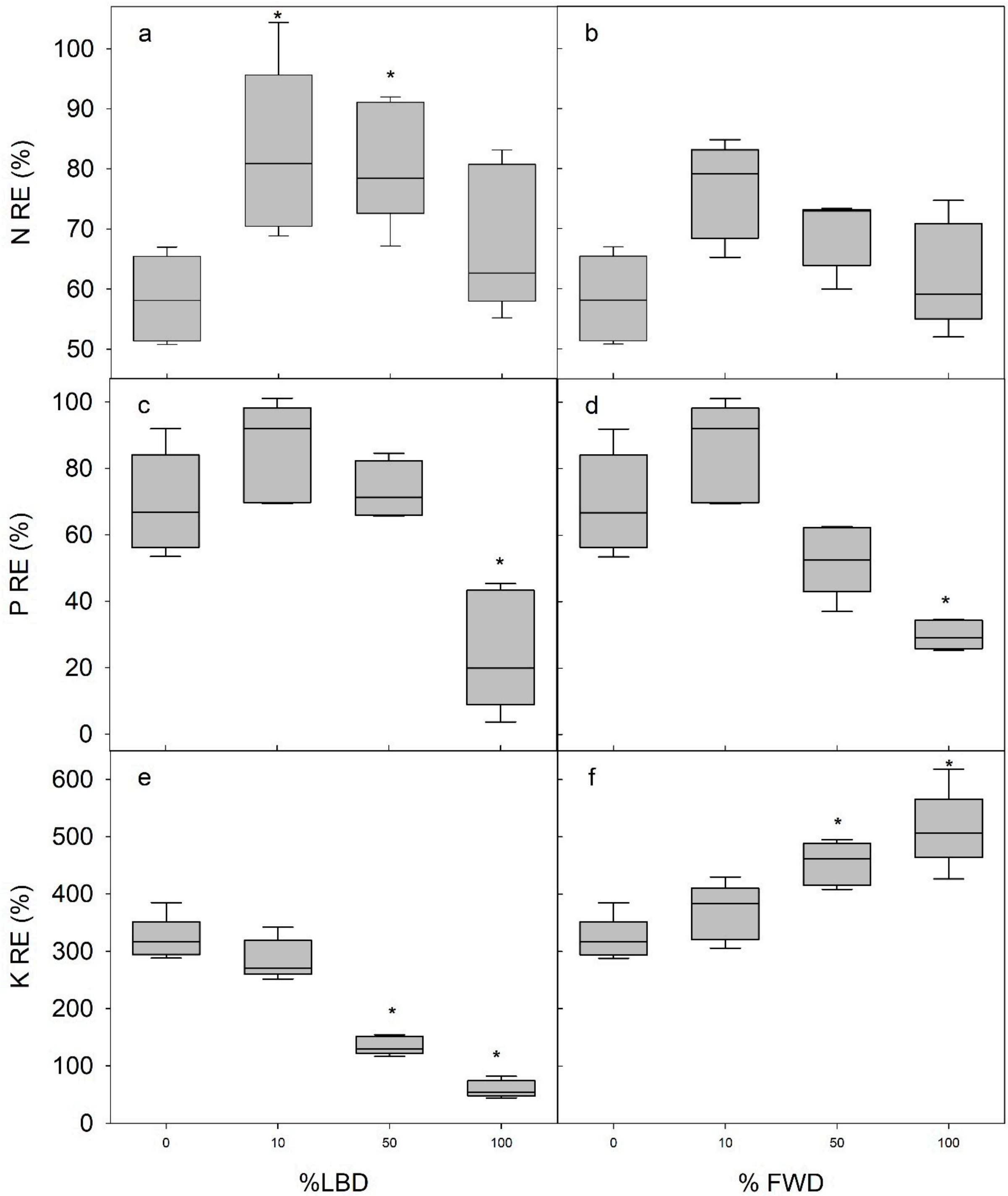

Figure 5. Digestate treatment effects on nitrogen recovery efficiency (RE) for LBD (**a**) and FWD (**b**), phosphorus RE for LBD (**c**) and FWD (**d**) and potassium RE for LBD (**e**) and FWD (**f**). Treatments with an asterisk are significantly different from the fertilizer control ($p < 0.05$). Error bars represent the standard error of the mean ($n = 5$).

3.4. Salinity and Fecal Coliforms

There was a significant effect of treatment on media EC values (Figure 6a, $p = 0.001$). The media from the treatments receiving LBD alone had the highest EC values at the end of the growing period (4.460 ± 0.576 mS cm^{-1}), followed by LBD50 (3.170 ± 0.320). An

EC value of 3.2 mS cm^{-1} is considered the threshold above which yield loss is expected in Chinese cabbage [33]. The lowest values were found in treatments with the smallest amounts of digestate applied, including FWD10, LBD10, FWD50 and FCTRL. There was also a significant treatment effect on tissue Na^+ concentration (Figure 6b, $p < 0.001$). Plants treated with LBD alone had the highest tissue Na^+ concentrations, which was above reported values associated with sodium toxicity [34]. The high tissue concentrations likely were the cause of reduced growth in this treatment. This is supported by a significant negative correlation between tissue Na^+ and shoot dry weight ($p = 0.017$, $r = -0.498$) (see Supplemental Materials).

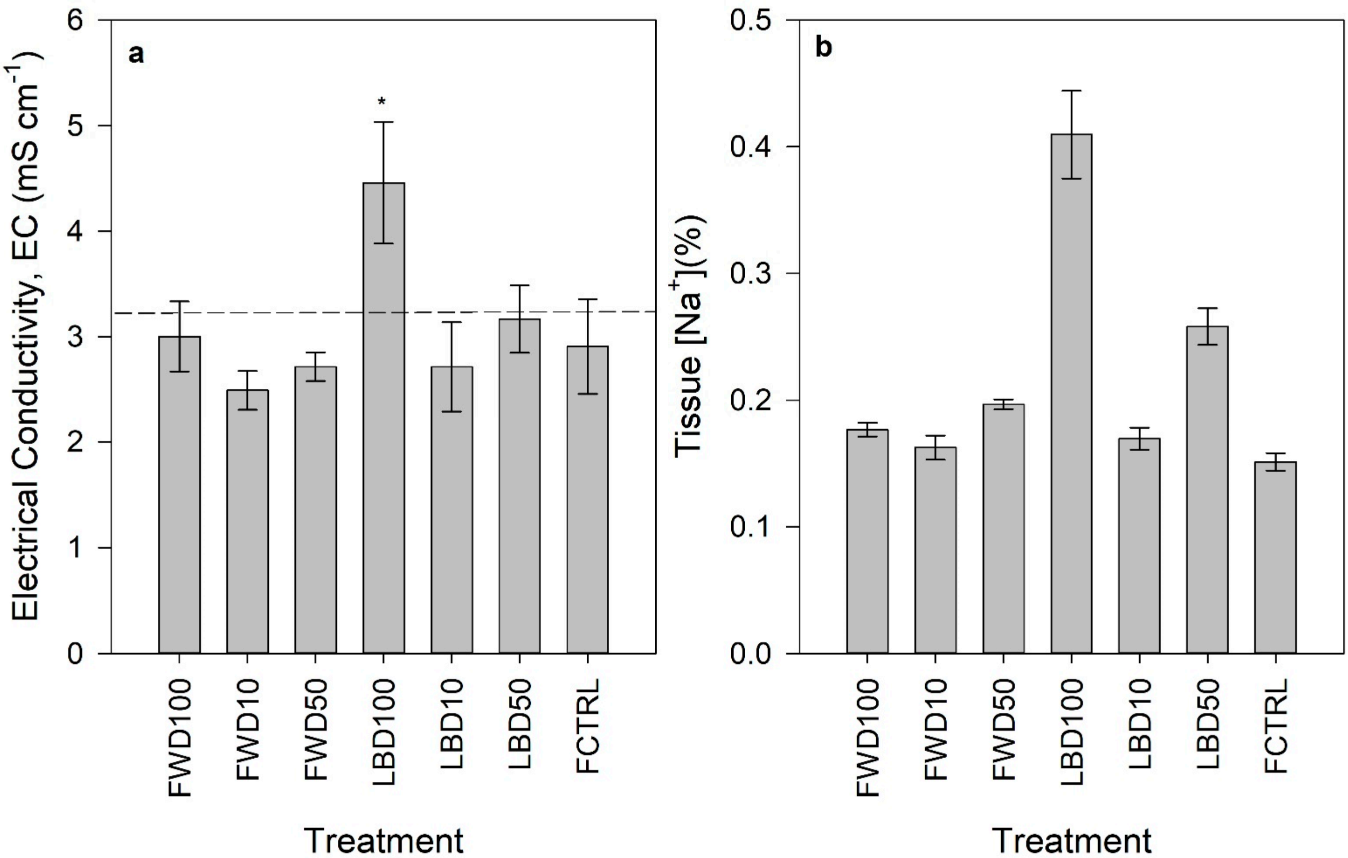

Figure 6. Digestate treatment effects on planting media electrical conductivity (EC) (**a**) and kai choy tissue [Na+] (**b**). Error bars represent the standard error of the mean ($n = 5$). The dash line in panel (a) represents the EC threshold of 3.2 mS cm^{-1} for kai choy, and the asterisk shows a significant difference ($p < 0.05$) between mean EC in the LBD100 treatment, as compared with the fertilizer control. Means that share the same letter in panel (b) are not significantly different from each other ($p < 0.05$).

All pre-plant digestate samples tested below 200 most probable number (MPN) of fecal coliform per 100 mL, the detectable limit of the assay. Based on total solids of 2.87% and 1.21% for LBD and FWD, respectively, this equates to less than 574 or 242 MPN per gram of total solids of LBD and FWD, respectively. This is well below the EPA limit of 1000 MPN per gram established for Class A Biosolids [35]. Of the treatments for which media samples were tested at the end of the growing period, only LBD50 had a fecal coliform count significantly different from zero (22.0 ± 8.35 MPN g^{-1}), indicating that digestate addition caused little to no increase of fecal coliform counts during the growing cycle.

4. Discussion

4.1. Plant Growth and Salinity

All digestate and combined digestate and fertilizer treatments produced more biomass and longer roots than the unamended control. When compared with the fertilized control, there were small but statistically non-significant yield improvements from the combined use of LBD and fertilizer (LBD10 and LBD50). Similar growth to the fertilized control was observed with FWD alone, and FWD and fertilizer mixtures. Both were highest in LBD mixtures and FCTRL, and lowest in LBD100. No digestate or digestate–fertilizer treatments were significantly different from the fertilizer control for shoot dry weight, indicating that digestates could serve as an effective substitute for mineral fertilizers. This is in agreement with other studies comparing plant-biomass production under digestate treatments vs. mineral fertilizers [6,36,37]. It is important to note that, in this study, the significant differences in biomass at the 100% nitrogen level were between the LBD mixtures and the LBD100 treatments, with the LBD100 treatments performing the worst. Wentzl et al. [17] found a non-linear relationship between the amount of NH_4-N applied as digestate and aboveground biomass similar to the findings in this study. In both cases, the benefits of application diminish and may begin to decrease with higher rates of application.

High salinity (Na^+ and Cl^-), resulting in high EC in the media treated with LBD alone, likely contributed to decreased plant growth in these treatments. Plants grown in these treatments also had the highest tissue Na^+. Along with nutrients, digestates also contribute soluble Na^+ and Cl^- that can have adverse impacts on the plant root environment. This harsh environment can cause osmotic stress and ultimately lower productivity of plants. A previous study investigating the use of the solid portion of digestates as an alternative to peat for greenhouse production found reduced plant growth in *Salvia oficianalis* grown in mixtures of digestate and peat, as compared with peat alone [38]. A more recent study found a high EC and high plant tissue content of Na^+ under digestate treatments, similar to findings from this study with the use of liquid digestates [39]. In this experiment, the LBD had a much lower nitrogen content than did the FWD, and thus more LBD digestate was needed to satisfy the nitrogen requirements. Treatments that received the highest amount of LBD digestate showed the highest EC values. Along with nitrogen, digestates added more Na and Cl, contributing to the greater observed EC in treatments with 100% and 50% of nitrogen satisfied by LBD. LBD100 treatments had media with EC measurements above the tolerance threshold of 3.2 mS cm^{-1} for Chinese cabbage [33]. Beyond this threshold, yield losses are expected at a rate of 10% per unit of EC, which may explain the decline in productivity in the LBD100. The LBD50 treatments were just below this threshold and still produced slightly, albeit insignificantly, more biomass than did the fertilized control treatment. This is similar to findings from Tsachidou et al. [23], where substituting chemical fertilizers with digestate up to 65% showed no reduction in biomass and Wang et al. [20], where substituting mineral fertilizer with poultry digestate up to 50% produced beneficial results. However, Maunuksela et al. [21] found increased or similar growth of barley with digestate addition, but an inhibition of growth with Chinese cabbage. Barley is one of the most salt tolerant major crops [40] and this highlights the fact that different plant species may exhibit different levels of tolerance to the possible negative effects of digestate addition with high Na^+ or Cl^-. Due to the effects of high amounts of digestate on EC, partial substitution of fertilizer up to 50% with digestates having relatively low nutrient value (i.e., LBD) or partial to full substitution of 50–100% for digestates having relatively high nutrient value (i.e., FWD) would be recommended for kai choy.

4.2. Root Growth

Much of the research on effects of digestates on root growth centers on root biomass. There are widely varied findings from a decrease in root biomass [16] to no difference [17] to an increase in root biomass with digestate, as compared to mineral fertilizer [14]. Our study quantified root growth and morphology throughout the growing cycle rather than a one-point root biomass at harvest, in order to better understand how the effects of digestate on roots may be affecting plant growth more broadly. We found no significant differences in mean root length between treatments at the end of the growing period. This is slightly different from results by Wang et al. [20] and Zhang et al. [22], who found increased root biomass and root length when combining synthetic nutrients with digestate in hydroponic lettuce production and paddy rice production, respectively. This could be due to the difference in production systems, digestates used and/or the different ratios of nutrients to digestate utilized in the studies.

Nonetheless, there were significant differences in root length during the rapid growth phase in the middle of the growing period. The grouping that occurred during this critical growth period corresponded with the aboveground biomass results, with LBD10, LBD50 and the fertilizer control performing best in both. This indicates that root length and/or biomass measurements taken only at the end of the growing period may not capture important differences that occur earlier in the plant life cycle, but affect yield in the longer term. Increased root growth at this critical stage can be important for plant establishment and growth and may have contributed to the higher yield of these treatments [19].

4.3. Nutrients and Nutrient Use Efficiency

Nutrient recovery efficiency (RE) is one of the most common metrics for comparing digestate treatments to controls. This measurement incorporates biomass yield and % nutrient in plant tissue and compares the nutrient uptake in treated plants with uptake in untreated plants based on the total amount of that nutrient applied. RE essentially standardizes the measurement based on the amount of nutrient applied, and thus provides a good comparison between treatments that are receiving different amounts of the nutrient, as was the case with all but nitrogen in this study.

Partial substitution of fertilizer with LBD for up to 50% of the N equivalent led to a significant increase in RE for nitrogen over the fertilizer control. Both the %N in tissue and N uptake in FCTRL were significantly lower than in all mixtures of digestate and fertilizer for both LBD and FWD. This, combined with the slightly greater biomass in the LBD mixtures contributed to the higher RE for nitrogen in mixed LBD–fertilizer treatments. Other treatments including digestates and mixtures of digestate and fertilizers had intermediate RE values. This is different from what is currently reported in the literature, where RE with digestate was found to be similar to or lower than RE with fertilizers [14,23,41,42]. Interestingly, Gunnarsson et al. [14] found lower dry mass production and NUE under digestate treatment than mineral fertilizers during early growth, and no difference at the end of a long-term (172-day) study with ryegrass. This indicates that different plants may respond differently to digestate treatment during early growth stages. However, these studies were also looking at digestates alone, which in this study produced biomass similar to or lower than fertilizer alone for both LBD and FWD. In addition, these were field-based studies in which the potential for loss of N through volatilization of NH_3 is likely higher. In this pot study, the high cation exchange capacity and low pH of the peat-based media likely limited volatilization of NH_3, allowing plants to utilize more of the applied N from digestates [14]. The increased RE of N in combined LBD and fertilizer treatments may have contributed to the slight increases in growth under these treatments.

Partial substitution of digestates for fertilizer did not significantly affect phosphorus RE in comparison with the fertilizer control treatments. However, it was significantly lower in treatments with digestate alone than in treatments with fertilizer. There are differing reports on the phytoavailability of P from digestates, but most such studies compare phytoavailability of P in digested vs. undigested materials. Such studies suggest similar or higher phytoavailability with digestates, compared to undigested materials [43] and one study found that digestates increased P uptake as much as mineral fertilizer [44]. However, results from this study suggest that P availability in the two digestates tested was relatively low, compared with mineral fertilizer. More P was added in both pure digestate treatments than in the fertilizer control treatment (36.8 mg per pot for FWD and 40.8 mg per pot for LBD vs. 26.8 mg per pot for fertilizer). However, a much smaller proportion of this phosphorus was actually taken up by the plants, as indicated by the lower RE in digestate-alone treatments, compared with mineral fertilizer treatments. Although there was a high amount of P in digestates, the pH of both FWD and LBD was high (>8), and thus may have decreased the solubility, and thus the phytoavailability, of phosphorus [11]. None of the findings with respect to NUE of phosphorus or potassium negate the observed effects of nitrogen.

4.4. Fecal Coliforms

All digestate samples tested well below the EPA limit for Class A Biosolids. Testing of media samples treated with digestate showed little to no increase in fecal coliforms at harvest, with only one sample showing fecal coliforms significantly different from zero. This is consistent with other studies that show a significant reduction in pathogens after anaerobic digestion [27,45]. Our results suggest that digestates made from lignocellulosic biomass and food waste may not pose a significant biohazard threat when applied at appropriate rates for crop growth. However, it is important to test any digestates for potential biohazards prior to land application, as their presence would likely be dependent on both the feedstock used and the conditions under which digestion took place.

4.5. Implications for Sustainability

While the results obtained in this greenhouse pot study support findings in the literature as to the usefulness of digestate, we recognize that results may not be directly extrapolated to field conditions. Nonetheless, anaerobic digestion itself is of great value to many developing countries for its ability to provide a renewable and inexpensive source of energy generated from a variety of organic wastes [46]. Biogas is an attractive alternative to conventional fuels, and its use can help reduce deforestation, indoor air pollution and the time spent collecting and using fuels like wood, while also reducing harmful methane emissions [47]. While anaerobic digestion has been shown as an attractive approach to addressing energy insecurity, it provides a co-benefit through the production of a highly valuable, nutrient-rich biofertilizer [6,11,17,48]. It is likely rural areas that would benefit most profoundly from the use of anaerobic digestion on farm as a way to achieve energy security while reducing methane emissions from agricultural wastes. In addition, as this and other studies show, digestates may provide an additional benefit, either by partial substitution for costly fertilizers, or, in cases where fertilizers are simply unavailable and nutrients are scarce, by providing a direct yield increase from the addition of digestate.

In this pot study, both food waste and lignocellulosic biomass proved to be good feedstocks for the production of methane (unpublished data), while also producing fertilizers that could provide up to half of the total nitrogen needs of kai choy plants. Our results provide further evidence that utilizing digestate as a biofertilizer has the potential to both reduce dependence on costly imported fertilizers and open up options for agricultural production in the face of nutrient scarcity. While these benefits need to be validated at the field scale, our results add to the growing body of research showing the value of both energy and fertilizer products from anaerobic digestion of various feedstocks.

5. Conclusions

We found that partial substitution of LBD for mineral fertilizer led to small, albeit not statistically significant, increases in plant biomass and that partial to full substitution with FWD produced similar biomass, when compared to the fertilized control. This was associated with a significantly higher nitrogen RE and slightly, though not significantly, higher AE with LBD–fertilizer mixtures and no difference for FWD treatments. LBD alone produced significantly lower biomass than did the other treatments at the same level of fertilization, likely due to the high EC of the media in these treatments. The agronomic efficiency was also lowest for LBD, alone, for N. All FWD treatments were produced intermediately. Overall, the results from this pot study suggest that digestates enriched in NH_4-N (i.e., FWD) can be applied at rates up to 100% of N required, without negatively affecting plant growth or nutrient use, as compared with mineral fertilizer. However, digestates lower in NH_4-N are better applied as mixtures of up to 50% digestate with fertilizer for optimum benefit due to possible negative impacts on EC associated with their higher rates of application. In this case, partial substitution of fertilizer with digestate may offer maximum benefit for plant yield, while also reducing commercial inputs. These strategies provide opportunities to reduce both costs and environmental impact, while at the same time increasing farm sustainability.

Supplementary Materials: Raw data used to generate tables and figures in the Results section are available online, at https://www.mdpi.com/2073-4395/11/3/509/s1. Supplemental Data Tables: Table S1: Digestate Treatment Effects on Shoot Dry Weight (Figures 1 and 2); Table S2: Digestate Tratment Effects on Root Length over Time (Figures 3 and 4); Table S3: Digestate Treatment Effects on Nitrogen, Phosphorus and Potassium RE (Figure 5); Table S4: Digestate Treatment Effects on Electrical Conductivity of Planting Media and Sodium in Plant Tissue (Figure 6); Figure S1: Pearson Correlation Plot for Sodium and Dry Weight.

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