

Article

Maize Silage Digestate Application Affecting Germination and Early Growth of Maize Modulated by Soil Type

Ana A. Robles-Aguilar ^{1,2}, Vicky M. Temperton ³ and Nicolai D. Jablonowski ^{1,*} 

¹ Forschungszentrum Jülich GmbH, Institute für Bio- und Geowissenschaften, IBG-2: Plant Sciences, 52425 Jülich, Germany

² Faculty of Bioscience Engineering, Ghent University, Coupure links 653, 9000 Ghent, Belgium

³ Institute of Ecology, Faculty of Sustainability, Leuphana University Lüneburg, Universitätsallee 1, 21335 Lüneburg, Germany

* Correspondence: n.d.jablonowski@fz-juelich.de; Tel.: +49-2461-61-8682

Received: 11 July 2019; Accepted: 14 August 2019; Published: 20 August 2019



Abstract: During biogas production anaerobic digestion of plant material produces a nutrient-rich residue called digestate. The application of the nutrients present in the digestate should improve soil fertility, particularly in nutrient poor soils, and thus crop yield, promoting the closure of the nutrient cycle. This study evaluated the effect of digestate application on the germination and early stages of plant development since these are the first steps to be considered when studying the benefits on plant growth in low fertility substrates. A greenhouse experiment was conducted to evaluate the effects of three substrates of different texture and fertility (field loam, field sand, sand), as well as type and amount of fertilizer (pure maize digestate vs. inorganic nitrogen/phosphorus/potassium (NPK) fertilizer) on both germination and early plant performance of maize (*Zea mays* L. subsp. *mays*). While digestate and NPK fertilizer applications had no significant effect on germination in the two field soils, digestate applications significantly decreased the germination rate in sand (36–82% reduction) due to an increase of surface water repellency. In contrast, for aboveground biomass yield, the most positive fertilization effects of digestate application were found on sand (up to 3.5 times the biomass of the unamended control) followed by field sand (1.5 times), compared to no effect for field loam. Our findings suggest that digestate application have positive fertilization effects in low-fertility substrates, similar to NPK, even though digestate application may have a negative impact on the permeability in sandy substrates that could interfere with germination.

Keywords: biogas-residues; fertilizer; biomass; soils; permeability; nutrients

1. Introduction

To promote a sustainable biomass production within a growing bio-based economy, the use of organic residues warrants consideration as fertilizers and soil conditioners. The use of residues from biogas production as bio-fertilizers should be seen as a highly valuable resource [1]. Besides providing a viable option for disposal, digestate represents a strategy to sustain or increase soil fertility and to foster plant biomass yields within the long-term goal to substitute mineral fertilizers [2,3].

The amount of digestates resulting from biogas production has increased rapidly in the last 10 years due to the growing number of biogas facilities. In Germany, the number of biogas plants increased from 1600 in 2002 to more than 7000 in 2011 [4] due to the German Act launched in 2002 that made renewable energy sources a high priority.

The application of digestates to field soils is already a common practice. During anaerobic digestion, dry matter is partially degraded, resulting in a volume reduction and concentration of

nutrients in the digestates. Anaerobic digestion produces digestates having a higher ammonium:total nitrogen ratio, a decreased organic dry matter, an elevated pH value and a smaller C:N ratio than the undigested substrates [5]. Several field studies have previously reported as positive these changes in the composition of the digestate compared with non-fermented organic fertilizers, which are relevant for the plant availability of macro- and micronutrients that lead to increased biomass yields. These studies are mostly related to manure-based digestates [3,6–8], or from co-digestion with additional plant material [9–11]. However, little research has been conducted to evaluate if these positive effects on plant performance can also be observed when digestates are generated only from plant material [12,13] are used.

The use of digestate as a fertility enhancer in nutrient-poor sandy soils is scarcely studied even though a growing demand for agricultural land may promote the use of low fertility soils for plant biomass production [14]. In this scenario, digestate application to these soils would alleviate the fuel-versus-fuel controversy generated by the use of rich agricultural soils for energy plant production [15], allowing an improved plant biomass cultivation on marginal sandy soils [16–18].

The addition of digestate as a fertilizer and soil conditioner would be expected to have an overall beneficial effect on plant growth and biomass production. However, the application amount and certain components of the digestate may have negative effects either on the plant [5] or on the soil system due to a reduction of wettability and a promotion of soil dispersion [19], and may also cause plant mortality due to acidification of the soils, as reported by Albuquerque and co-workers [20].

The objective of our study was to evaluate a commercial digestate derived from maize-based biogas production for their effects on maize germination and early growth in field soils and silica sand under greenhouse conditions. We chose maize-based digestate since this plant species is the most commonly used in biogas plants in central Europe and testing effects of this type of digestate will improve our options for helping to close the nutrient cycle within energy plant production.

2. Materials and Methods

2.1. Experimental Setup and Treatments

The pot experiment was conducted in a research greenhouse (located in Jülich, Germany, 50.89942° N 6.39211° E) covered with low-iron float glass with a predominant diffuse light transmission. Additional assimilation lighting (SON-T AGRO 400, Philips, Koninklijke Philips N.V., Netherlands) was used whenever natural light intensity was below $400 \mu\text{mol s}^{-1} \text{m}^{-2}$, providing a total daily light period of 16 h. Average temperature during the course of the experiment was 19 °C during the day and 17 °C at night, with 60% relative humidity during the day, and 50% at night as the standard controlled operation temperatures for day and night of the greenhouses at IBG-2: Plant Sciences, Forschungszentrum Jülich GmbH, Germany.

Our experiment followed a factorial completely-randomized design: The first factor being substrate type and the second factor being fertilizer addition. The substrate type had three levels: Two soils excavated from the ploughing layer (0–30 cm depth) in the region around Jülich: (i) An arable field soil (Endogleyic Stagnosol) developed from loess, with a silty-loamy texture (hereafter called field loam) considered as a high-nutrient content agricultural soil, (ii) a Gleyic Cambisol from the field site at Kaldenkirchen—Hulst (51°18′40″ N, 6°12′10″ E) near the Forschungszentrum Jülich, Germany, considered as a marginal, low-nutrient content agricultural soil (hereafter called field sand); and (iii) a nutrient-free silica sand (silica particle size 0.71–1.4 mm). Information on substrate properties before treatment applications are given in Table 1. The fertilizers used were (i) digestate and (ii) a mineral NPK fertilizer; both were compared to no-fertilizer addition as an unamended control. Digestate is the by-product obtained during biogas production after the anaerobic digestion of pure maize silage and originated from a commercially operating, thermophilic biogas facility (NaturPower GmbH and Co. KG, Titz-Ameln, Germany) with a fermenter volume of 2500 m³ and a hydraulic retention time of the biomass of 72 days.

Table 1. Chemical properties of the substrates used in the experiment. TOC, total organic carbon; TC, total carbon; TN, total nitrogen; WHC, water-holding capacity. Mean variation $n = 3$: For concentration $>1\% = \pm 3\%$; for concentration $<1\%$ and $>0.1\% = \pm 10\%$, and for concentration $<0.1\% = \pm 20\%$.

Analysis	Field Loam	Field Sand	Sand
TOC (%)	1.09	0.92	<0.005
TC (%)	1.12	0.95	<0.005
TN (%)	0.12	0.09	<0.01
K (%)	1.64	0.78	0.047
P (%)	0.085	0.087	<0.05
Al (%)	4.05	1.57	0.062
Ca (%)	0.470	0.210	0.042
Fe (%)	1.79	1.00	0.016
Mg (%)	0.30	0.11	<0.05
Na (%)	0.65	0.24	<0.01
Mn (%)	0.069	0.039	<0.01
S (%)	<0.05	<0.05	<0.05
Zn (%)	<0.01	<0.01	<0.01
Mo (%)	<0.01	<0.01	<0.01
pH	7.12	6.18	7.00
WHC (%)	48.4	42.0	40.0

The three different substrate types were treated with 7 different combinations of fertilizer type and fertilizer application amount as follows: digestate high (DG high), digestate regular (DG regular), digestate low (DG low); positive control: NPK high, NPK regular, NPK low; negative control (control): no fertilizer application. The digestate was obtained from the digestate storage tank at the biogas plant and was analyzed by the agricultural research laboratory of North-Rhine Westphalia, Germany, in accordance with analytical standards (LUFÄ NRW). All values given in the following refer to the fresh digestate as received, without previous solid–liquid separation. The digestate contained 0.53% total nitrogen (including 0.32% ammonium-N), 0.19% phosphate (P_2O_5), and 0.71% potassium (K_2O). Considering the true elemental P and K content and not their oxidized forms, the NPK ratio for 0.53% N, 0.08% P, 0.58% K is 1.0:0.15:1.11, respectively. The digestate contained 6.9% dry matter (consisting of 41.1% total carbon), and had a pH value of 7.9 ($CaCl_2$). In addition, 0.14% CaO and 0.06% MgO were determined in the fresh digestate. To compare the nutrient availability and fertilizing effects of the digestate with the application of easily plant-available nutrients, a commercial NPK fertilizer consisting of 16% N (composed of 6.3% NH_4 ; 9.7% NO_3), 8% P_2O_5 , 22% K_2O , and 3% MgO (with a NPK ratio of 1.0:0.22:1.14, when considering the elemental values accounting for 16% N, 3.44% P, 18.26% K, respectively) was used. The specific composition of the commercial fertilizer used had the most similar NPK ratio compared with the digestate among 17 other commonly-available mineral fertilizers.

Three different application amounts (high, regular, and low) of digestate and NPK fertilizer were used in the experiment. The applied amounts of digestate were calculated in accordance with a regular agricultural field application of 40 t ha^{-1} of fresh digestate as received from the biogas plant. This was considered in the experiment as a regular application, as was also found a suitable dosage for the fertilization of the non-food energy crop *Sida hermaphrodita* (L.) Rusby in a sandy marginal substrate [21]. The regular application amount of NPK fertilizer used in the experiment was chosen based on an agricultural application of 200 kg N ha^{-1} . Double the amount of the regular application amount of digestate or NPK fertilizer was considered a high application and half the amount of the regular amount applied was considered a low application. The amounts of N, P, and K applied in the experiment (calculated in kg ha^{-1}) are given in Table 2 and were adjusted to the pot surface areas ($11 \times 11\text{ cm}$) used.

Table 2. Quantity of N, P, and K present in the digestate and the mineral NPK fertilizer used as a reference at low, regular and high application rates.

Reference Quantities			
		Digestate (Kg ha ⁻¹) ¹	NPK-Fertilizer (Kg ha ⁻¹) ²
Low application	N	106	100
	P	16.4	21.5
	K	116	114
Regular application	N	212	200
	P	32.8	43
	K	232	228
High application	N	424	400
	P	65.6	86
	K	464	456.5

¹ Amount of N, P, and K present in the total digestate fertilizer applied was calculated according to 0.53% total N, 0.19% P₂O₅ (0.082% P), and 0.71% K₂O (0.58% K) in reference of an agricultural field application of 40t ha⁻¹ of digestate. ² Amount of N, P, and K present in the total NPK fertilizer applied was calculated according to 16% total N, 8% P₂O₅ (3.44% P) and 22% K₂O (18.26% K) in reference of an agricultural field application of 200 Kg N ha⁻¹.

2.2. Soil Treatment and Cultivation Practices

The different amounts of digestate and NPK fertilizer were applied to each individual substrate, and were thoroughly mixed in an end-over-end mixer for 10 min to allow an even distribution of the fertilizers. The fertilized substrates were left undisturbed in closed containers for a period of one week prior to experimental application for equilibration and nutrient distribution. Subsequently, each pot (11 × 11 × 11 cm; *n* = 7) received 1 L of the field loam (equaling 1.1 kg), field sand (1.3 kg), or silica sand (1.5 kg) with one specific fertilizer application of digestate or NPK fertilizer. For each substrate, unamended controls (*n* = 7) were also established for comparison. All pots were kept at 50% water-holding capacity using an automated watering system.

Pre-tests of the maize seeds used (*Zea mays* L. subsp. *mays*, Hybrid LG 30.222LG, Limagrain, Deutschland GmbH, Edemissen, Germany) without any fertilizer treatment showed a germination rate of 98% after seven days. To determine the effects of the digestate or NPK fertilizer application on the germination in our experiment, four maize seeds were sown in each pot at a depth of 2 cm. To minimize potential local abiotic effects, all pots were re-randomized in position weekly throughout the experiment.

During the first ten days after sowing, germination of the four seeds sown in each pot was counted. Subsequently, the oldest plant was kept per pot (while the others were entirely removed) giving one plant per pot in order to focus on the fertilizer effects on single plant performance.

The experiment was terminated when the plants reached 45 days after germination, and plants were cut at ground level, weighed for fresh weight, and dried at 70 °C until constant weight. Root material was washed manually, and was dried at 70 °C until constant weight.

2.3. Additional Experiments Related to Germination

A pre-experiment, using ten replicates, was conducted to evaluate the effects of a single amount of surface-applied digestate (corresponding to 40 t ha⁻¹ digestates) and NPK fertilizer on the germination and performance of juvenile maize plants in the different substrates and treatments. This approach was necessary since surface application of the digestate led to clogging of the substrates, and a resulting reduction of hydraulic conductivity and germination. This pre-experiment, thus, provided the necessary information on the best way to apply the fertilizers in the main experiment.

A complementary germination experiment was conducted three months later than the main experiment, under the same greenhouse conditions, using two lower amounts of digestate in field sand and silica sand, in order to define if the phenomenon of water repellency previously observed in

the silica sand could also be observed with lower amounts of the digestate. To do so, five replicates of each application for each substrate with ten seeds per pot were used. The treatments were as follows: (1) High application of digestate (half amount of the low application used in the main experiment; i.e., equivalent to 10 t ha⁻¹); (2) low application of digestate (half amount of the above mentioned high application; i.e., equivalent to 5 t ha⁻¹), and (3) unamended control.

2.4. Data Collection on Plant Performance

The germination rate for maize in all treatments was calculated by counting the number of emerged seedlings per pot during the first ten days after sowing and calculating percentage germination.

As an indicator of growth and development, the greenness of the leaves was measured at regular intervals using a soil plant analysis development chlorophyll meter (SPAD-502, Konika-Minolta, Marunouchi, Japan), as the green color of the leaves is an indicator of the chlorophyll content and SPAD values thus provide indirect information on plant nitrogen status [22]. In addition, plant maximum height in cm was measured 10, 16, 25, 30, 35, and 45 days after emergence. We measured SPAD weekly from all emerged leaves of each remaining plant and height of plants in order to follow changings in growth and chlorophyll dynamics over time, as these parameters often vary over time.

After harvesting, fresh and dry weights of stems and roots were determined. Since the pre-experiment revealed a plant biomass distribution of 80% stem and 20% leaves for all treatments and substrates tested, leaves and stem biomass were not separately measured. The hydraulic conductivity (Ks) of both field substrates at both treatments was measured to consider potential effects of digestate application on the permeability of the substrates. The substrate permeability was measured in digestate-treated and unamended silica sand using the water drop penetration time (WDPT) method [23,24], since water repellency was observed in the silica sand treated with digestate.

2.5. Statistical Analysis

Statistical analyses were performed using the statistical program R.2.12.2 (R: A Language and Environment for Statistical Computing (2012) <http://www.R-project.org/>). Biomass yield, germination rate, height, and SPAD measurements were compared with two-way analysis of variance (ANOVA), using substrate type and fertilizer treatment as factors.

The factor substrate type had three levels (field loam, field sand, and silica sand), and the fertilizer factor had seven levels (digestate high, digestate regular, digestate low, NPK high, NPK regular, NPK low, and unamended control). Any data that did not conform to homogeneity of variance were log-10 transformed before analysis. Note that figures show non-transformed data. All time-series measurements (SPAD, plant height, germination rate) were also analyzed using repeated measures ANOVA (RMANOVA) with a greenhouse-Geisser correction, using SPSS (IBM, NY, USA) to take into account that time intervals were not always equal. We used Tukey's honestly significant difference (HSD) *posthoc* test after ANOVAs at a significance level of $p < 0.05$, to see which level of a factor differs from one another. Data were calculated as arithmetic means $\pm$ standard error of the mean of seven replicates.

3. Results

3.1. Germination

Germination measurements during the first eight days after sowing (Figure 1) showed that substrate type ($p < 0.0001$) and fertilizer treatment ($p = 0.005$), as well as the interaction between the two ($p = 0.032$), had significant effects on germination (using repeated measures ANOVA). Posthoc tests showed that the significant effect of substrate type was due to differences between silica sand and the field substrates ($p < 0.0001$). Germination in field loam and field sand was significantly higher than in silica sand ($p < 0.0001$). The effects of substrate type and fertilizer treatment on germination

changed over time ($p < 0.001$ and $p < 0.0001$, respectively), as well as the interaction effect between substrate type and fertilizer ($p = 0.035$).

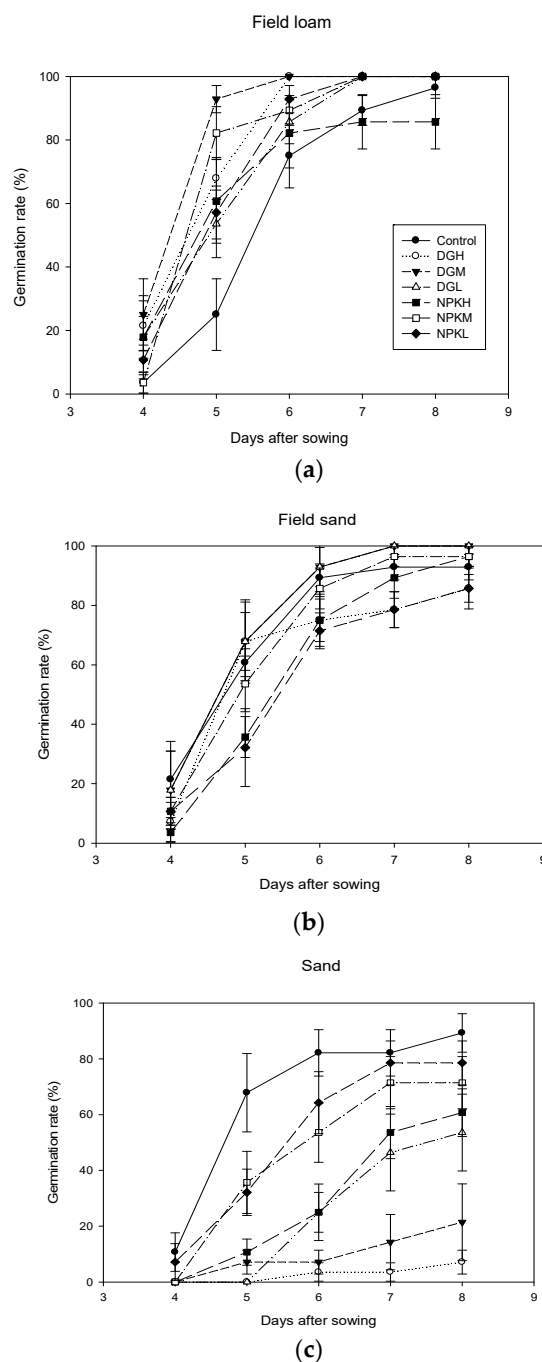


Figure 1. Germination rate of maize in field loam (a), field sand (b), and sand (c) with the application of maize digestate (DG) or NPK inorganic fertilizer/positive control (NPK) at different levels: no-fertilizer/negative control (C), high application (H), regular application (M), low application (L). Values are means $\pm$ standard error of the mean ($n = 28$).

Comparing fertilizer effects on germination by substrate over time (RMANOVA with different substrate types analyzed separately) showed that for the field loam, fertilizer effects were initially positive ($p = 0.028$), and this was found to be due to differences between the unamended control and regular digestate application. At day five after sowing, for example, see Figure 1a, the germination in field loam was more than 90% in regular application of digestate ($p = 0.001$ for ANOVA of all fertilizer

treatments on day five) compared to 20% for unamended control. At day eight, ANOVA tests of fertilizer effects on germination (separated by field soil type) no longer showed any significant effects on germination ($p > 0.05$) in the field loam. In contrast, in the field sand there was no overall significant effect of fertilizer treatment on germination over the whole timespan (RMANOVA, $p = 0.203$).

For the silica sand, fertilizer effects on germination were found to be very significant between the fertilizer treatments ($p < 0.0001$; posthoc tests showed that all treatments were significantly lower than the unamended control except for NPK low and regular; see Figure 1c for details). In contrast to the positive effect of digestate application on germination in the field soils, larger amounts of digestate in silica sand resulted in a significant ($p < 0.0001$) reduction in germination, accounting for -82% compared with the unamended control.

3.2. Complementary Germination Experiments

Germination in the post-experiment was measured ten times over the first 15 days after sowing in the field sand and silica sand, testing the lower end of the digestate application gradient. Data showed that substrate type ($p < 0.0001$) and fertilizer treatment ($p < 0.0001$), as well as the interaction between the two ($p < 0.0001$), had a significant effect on germination. The effect of fertilizer treatment was analyzed in both substrates daily from day five until day 15.

In the field loam, all the treatments reached $>90\%$ of germination after six days; however, in the silica sand the differences between the treatments were very significant. Five days after sowing the percentage of germination under low digestate application (94%) to the silica sand was significantly higher ($p < 0.05$) than for the high application (10%) or the control (24%). By one week after sowing, the low application already presented 100% germination, which was significantly higher than the control (with 62%; $p < 0.05$) or the high application (with 32%; $p < 0.05$). By 11 days after sowing, the germination rate of the low application was similar to that of the control ($p > 0.05$) whereas the high application of digestate in the silica sand was still significantly lower than low application and control. Overall, this showed that the low digestate application was below the threshold of the negative substrate permeability effect.

3.3. Hydraulic Conductivity and Permeability Analyses in the Substrates

High water repellency in silica sand treated with digestate was found. In addition, soil permeability analysis with brilliant blue solution gave evidence of preferential water flow in digestate-treated silica sand, resulting in limited wetting of the bulk sand and the seeds.

The hydraulic conductivity (K_s) was analyzed for both soil treatments (Table 3). According to the standard values of K_s digestate treatment increased the permeability in the loamy field soil, changing its classification, from low to medium permeable soil. In field sand, samples treated with digestate had a higher permeability than the respective control, considering the soil in all conditions medium permeable. Digestate treatment decreased the K_s values in field loam and field sand, resulting in an increased permeability in these soils. Due to the high permeability, the K_s value could not be determined for the silica sand.

Table 3. Hydraulic conductivity values for field loam and field sand under all the fertilizer conditions. C, control; NPK, commercial NPK fertilizer; D, digestate.

	K_s (cm/sec)	K_s (cm/h)
Field loam C	2.27×10^{-4}	8.17×10^{-1}
Field loam NPK	3.45×10^{-4}	1.24
Field loam D	7.72×10^{-3}	2.78×10^1
Field sand C	2.06×10^{-3}	7.41
Field sand NPK	3.10×10^{-3}	1.11×10^1
Field sand D	9.84×10^{-3}	3.54×10^1

3.4. Plant Growth and SPAD Measurements

With decreasing nutrient levels in the experimental substrates, the benefits of digestate application for plant performance were more pronounced. Significant differences on plant height over the whole experimental period (tested using RMANOVA) showed that substrate type ($p < 0.0001$) and fertilizer treatment ($p = 0.0001$), as well as their interaction ($p = 0.0001$), had significant effects on plant growth. *Posthoc* tests showed that the significant effect of substrate type was again due to differences between silica sand and the field soils ($p < 0.0001$). The effect of fertilizer treatment and substrate type on plant height changed over time ($p < 0.0001$), as well as the interaction effect between substrate type and fertilizer ($p < 0.0001$).

The effect of fertilizer treatment on height was analyzed for each substrate at specific time points. We only found significantly lower heights of digestate-treated plants compared with the mineral NPK fertilizer in the field soils 25 days after germination ($p < 0.001$, see Table 4). In field loam, considered as the most fertile substrate, equal effects on plant growth were only observed for DG low compared to NPK low treatments ($p < 0.05$), whereas the effects of NPK high and NPK regular were more pronounced (not significantly for the high amount) compared to the DG high and DG regular treatment. In these cases, plant heights reached 90% to 80%, respectively, compared to the plants treated with the NPK fertilizer.

Table 4. Height and SPAD measurements of plants grown in the field loam, field sand, and sand after the application of maize digestate and NPK fertilizer at various amounts: No-fertilizer/unamended control (C), digestate (DG), NPK inorganic fertilizer (NPK), low application (L), regular application (M), high application (H). Different superscript letters above numbers indicate significant differences ($p < 0.05$) between treatments within the same substrate. All values are means $\pm$ standard error of the mean ($n = 7$). Since a time-series of measurements were made on all plants in all three substrates for both height and SPAD parameters, all data were analyzed using repeated measures ANOVA. The presented data only show measurement days for each substrates and each parameter where significant differences between the treatments were found.

		Field Loam (Measurements at Day 25)	Field Sand (Measurements at Day 35)	Sand (Measurements at Day 45)
Height (cm)	C	72.9 $\pm$ 2.6 ^{ab}	91.3 $\pm$ 1.7 ^b	47.9 $\pm$ 0.8 ^b
	DG L	74.4 $\pm$ 2.4 ^{ab}	89.5 $\pm$ 4.6 ^b	70.0 $\pm$ 6.2 ^a
	DG M	69.5 $\pm$ 1.2 ^b	101.2 $\pm$ 1.9 ^{ab}	66.9 $\pm$ 2.3 ^a
	DG H	77.4 $\pm$ 2.1 ^{ab}	99.0 $\pm$ 2.5 ^b	74.7 $\pm$ 2.4 ^a
	NPK L	76.6 $\pm$ 1.8 ^{ab}	97.1 $\pm$ 3.7 ^b	63.0 $\pm$ 2.5 ^a
	NPK M	80.9 $\pm$ 2.1 ^a	99.9 $\pm$ 3.3 ^b	69.3 $\pm$ 1.9 ^a
	NPK H	81.0 $\pm$ 2.7 ^a	116.0 $\pm$ 4.8 ^a	72.8 $\pm$ 2.6 ^a
		Field Loam (Measurements at Day 45)	Field Sand (Measurements at Day 45)	Sand (Measurements at Day 28)
SPAD	C	19.3 $\pm$ 1.1 ^b	15.5 $\pm$ 0.7 ^{bc}	20.4 $\pm$ 1.0 ^b
	DG L	16.2 $\pm$ 0.7 ^b	13.6 $\pm$ 1.0 ^c	26.9 $\pm$ 0.8 ^{ab}
	DG M	17.0 $\pm$ 1.0 ^b	18.3 $\pm$ 0.9 ^{bc}	29.8 $\pm$ 3.8 ^a
	DG H	20.2 $\pm$ 1.2 ^b	17.5 $\pm$ 0.9 ^{bc}	34.0 $\pm$ 1.2 ^a
	NPK L	18.7 $\pm$ 1.0 ^b	19.3 $\pm$ 0.9 ^{bc}	26.8 $\pm$ 0.9 ^{ab}
	NPK M	26.8 $\pm$ 0.9 ^a	20.6 $\pm$ 2.8 ^b	29.0 $\pm$ 1.8 ^a
	NPK H	29.5 $\pm$ 2.2 ^a	30.3 $\pm$ 2.5 ^a	31.8 $\pm$ 1.1 ^a

However, in field sand, considered as a low-nutrient content soil, the fertilizing effect of the applied digestate on plant height was more pronounced compared to the NPK fertilizer treatments. In field sand, DG regular and DG low showed the same effects on plant performance as the easily plant-available NPK regular and NPK low fertilizer applications. In contrast, the fertilizing effect of NPK high treatments was pronounced compared to DG high treatments ($p < 0.05$). Significant differences on plant maximum height between the two fertilizers were observed only after 35 days, when plants treated with high amount of digestate reached 85% of the plants treated with NPK fertilizer.

In silica sand, plants with high and low digestate treatments tended to be taller compared to plants with NPK treatments (although not necessarily significantly), and all treatments were significantly ($p < 0.05$) higher compared to the unamended control, accounting for an increase of 46%, 40%, and 56% at DG low, DG regular, and DG high treatments, respectively.

SPAD analyses also indicated the beneficial use of digestate for plant performance in low fertility substrates, and were in accordance with the findings described above for the height. SPAD data obtained over the entire duration of the experiment showed that substrate type (RMANOVA $p < 0.0001$) and fertilizer treatment ($p = 0.0001$), as well as the interaction between the two ($p = 0.0001$), had significant effects on SPAD values of the leaves (see Table 4 for statistically significant results at specific time points). Posthoc test showed that the SPAD values obtained for plants with the three substrates were significantly different from one another ($p < 0.01$), compared to height data where the field soils were different from the silica sand.

In field loam, differences in SPAD values ($p < 0.05$ for field loam analyzed alone at each time point) between digestate and NPK fertilizer treatments increased during the experiment until the final harvest, when high and regular application of digestate reached 60% to 70% of the greenness measured in the leaves of the NPK treated plants. Low amounts between the fertilizers did not show significant differences.

In the field sand, SPAD values with high DG treatments reached 60% of the SPAD values of the high NPK treatment at the end of the experiment, and were 10% higher than the unamended control.

In silica sand, digestate treatments improved SPAD values significantly compared with the unamended control at the beginning of the experiment, with no significant differences between NPK treatments ($p < 0.05$). However, 28 days after sowing, SPAD values of the digestate-treated plants were higher compared to both the unamended control and the NPK treatment. In this substrate SPAD values were equal at termination of the experiments for all treatments.

3.5. Biomass Yield

Shoot and root biomass yields were measured at the end of the experiment 45 days after sowing (Figure 2). The beneficial effects of digestate application to low fertility substrates (as described above with regard to plant height and chlorophyll content) were even more pronounced regarding the biomass yield.

Data showed that substrate type ($p < 0.0001$) and fertilizer treatment ($p = 0.0001$) as well as the interaction between the two ($p = 0.0001$) had significant effects on shoot biomass yield. Posthoc tests showed that the significant effect of substrate type was due to differences between silica sand and the field soils ($p < 0.0001$).

Comparing fertilizer effects on shoot biomass by substrate showed that NPK treatment was only better at fertilizing in the higher application levels (regular and high treatments) in field substrates ($p < 0.05$).

In field loam, no significant differences between digestate treatments and unamended control were observed. However, in the field sand the positive effect of digestate on shoot biomass yield was observed up to a factor of 1.5 compared with the unamended control. The NPK treatment was only better than the unamended control at high application amounts.

In silica sand, digestate treatments increased shoot biomass yield by a factor up to 3.5 compared to the unamended control, and showed no significant differences compared to the biomass yield in the NPK treatment.

Root biomass distribution between different treatments was similar to the above ground biomass distribution. Shoot:root biomass ratios were high in both field soils due to an increase of shoot biomass (Figure 2) (4.1:1 in field loam, 4.9:1 in field sand), while in the silica sand the ratio was less pronounced (2.2:1) due to increased root biomass yield.

Shoot biomass yield was compared with the maximum height of plants with a linear regression. Values for both field substrates showed a high correlation ($p < 0.05$) between plant height and biomass

(field loam: $r = 0.99$, field sand: $r = 0.96$). The correlation was less pronounced for the silica sand ($r = 0.76$) since digestate application increased biomass yield in a range of 2.2 to 3.5 compared with the unamended control, while the height increased in a range from 1.1 to 1.5.

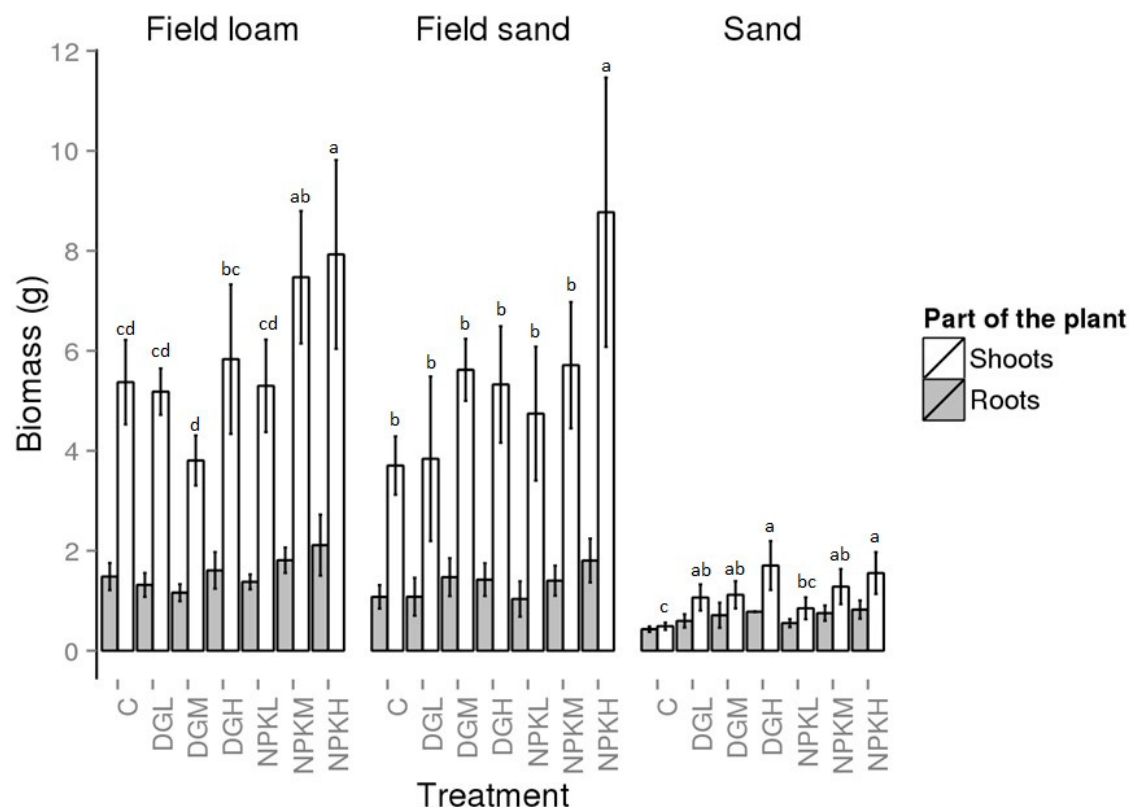


Figure 2. Maize shoot and root biomass production in field loam, field sand, and sand after the application of maize digestate and NPK fertilizer at various amounts: No-fertilizer/negative control (C), digestate (DG), NPK inorganic fertilizer (NPK)/positive control, low application (L), regular application (M), high application (H). Different letters above shoot bars indicate significant differences ($p < 0.05$) between treatments within the same substrate for the whole plant. All values are means $\pm$ standard error of the mean ($n = 7$).

4. Discussion

4.1. Fertility of the Substrate after Digestate Application

The addition of digestate is expected to increase the fertility and quality of poor substrates [2,25,26]. A number of previous studies have tested the possible beneficial effects of digestate on plants, but all these studies included digestates from co-digestion rather than from pure plant material, as used in our experiment [1,9,10,27]. We hypothesized that adding pure maize digestate from biogas production would have a fertilizing effect on plant performance broadly comparable to that of NPK fertilizer addition and that the effect would be modulated by the original fertility of the substrate used, observing in the lower fertility substrates the more significant effect of the digestate. In our study, although the ratio of P and K was slightly lower in the digestate than in the mineral fertilizer, the fertilizing effect of maize digestate was surprisingly high overall. Indeed, the digestate was equivalent in its fertilizing effect in the field substrates and performed better than mineral NPK on the silica sand, where the initial nutrient content was close to zero. We assume that organic matter added with the digestate not only provides nutrients for crops, but also increased the water-holding capacity [28], facilitating the maintenance of structure, drainage, and aeration, leading to a beneficial environment for nutrient availability and plant growth [29]. The organic matter mineralization process increases

the amount of ammonium and nitrate in the soil; however, the $\text{NO}_3\text{-N}$ is minimally adsorbed by the soil particles because it is very mobile and is susceptible to losses [30]. Therefore, the application of digestates may reduce nitrogen leaching compared to the NPK fertilization, as digestates contain a high amount of mineralized nitrogen mainly as ammonium [27], but, in contrast, in mineral NPK fertilizer (as employed in our study) nitrogen is mostly conserved as nitrate. The higher mobility of nitrate compared to ammonium (due to its negatively charged ions that are not attracted to soil particles) could move the N from NPK fertilizers easily to the root zone, making it more available than the N in the digestate. That could be the reason why we found a better plant performance with high levels of NPK fertilizer in the substrates with higher nutrient content and higher cation exchange capacity during the first weeks after application. This finding is not observable in long term experiments, with slow and constant mineralization in digestates, often without raising the nitrate leaching to groundwater, as observed in another study on ryegrass over a longer time scale (>6 months), where no differences between NPK and digestate application treatments on final biomass yield were observed [12].

Our findings support previous studies where a pronounced fertilizing effect of digestate has been observed for substrates with structural or fertility problems [31]. As shown, the digestate increased the fertility of the poor sandy substrate providing sufficient nutrients for early maize growth, assuming that also the physical structure, water-holding capacity, and cation-exchange capacity of the substrate were also improved [28]. Giusquini and co-workers [32] also found that similar digestates increased the total porosity of the substrates, and that was correlated with an increase in the water-holding capacity. Overall, the highest nutrient substrate (field loam) required a high application of digestate to produce a significantly better fertilizing effect on the plants compared with NPK, whereas in lower nutrient content substrates (field sand and silica sand) lower amounts of digestate were enough to significantly increase plant growth (Figure 2). The NPK-mineral fertilizer showed similar or better effects than the digestate when applied to soils with a naturally given nutrient content. However, the digestate improved the general condition of the soil better in the very low fertility soils compared to NPK.

Our results suggest the potential for digestate to increase the fertility of soils with low nutrient content. This effect of the maize digestate suggests that not only the easily available nutrients are important for plant growth, but also the contribution of digestate to improve physical properties or to increase organic content in the soils. Organic compounds present in the sand will increase the cation-exchange capacity making ammonium attached to the soil more available, rather than the nitrate from the NPK that leaches easily.

4.2. Dose Effect of the Digestate

We secondly hypothesized that the fertilizing effect of the digestate would be modulated by the amount applied. This contribution of the digestate to improve soil physical properties in addition to the nutrient supply could explain why the effect on plant performance of different amounts of one fertilizer type (e.g., DG high versus DG regular and DG low) for one specific substrate was less pronounced compared to the effect within the same application amount across fertilizer types (e.g., DG high versus NPK high).

4.3. Effect of Digestate on SPAD

SPAD readings in plants growing in the silica sand, as a nitrogen deficient substrate, were lower compared to plants grown on the field soils. This not very surprising result is confirmed by other authors [33] who also found lower leaf chlorophyll content (determined via SPAD readings) in plants growing on nitrogen-deficient soils, whereas higher values were observed in more nitrogen-rich soils. The differences in the SPAD readings between digestate-treated plants and NPK-treated plants decreased as the fertility of the substrates decreased, which we also found for aboveground biomass, indicating also that the digestate increased the fertility of soils with low nutrient content. These results are supported by other studies reporting that digestate application increased plant height and SPAD measures [34,35]. In contrast, a previous investigation with digestates from animal manures added to

sand showed that N content of the plant (calculated by SPAD) decreased when applying digestates as an alternative to mineral fertilizers [36]. In the present study with silica sand, no difference was found among treatments in leaf nitrogen content (as SPAD measures) at the end of the experiment, as was also found in a previous study over longer time scales [37], indicating that maybe the organic materials were utilized in the short term period.

4.4. Effect of the Digestate on the Hydraulic Conductivity of the Soil, Germination, and Plant Performance

We also hypothesized that effects of digestate would differ depending on whether one focusses on germination and plant establishment or the plant biomass production. In silica sand, digestate had a negative effect on germination but clearly improved plant performance (biomass, plant height, and SPAD measures). In field sand, no clear effect of digestate application on either germination or plant biomass yield was observed. In field loam, however, the patterns were opposite to those found on silica sand: The digestate improved germination but did not have a clear effect on biomass yield. Possible reasons for these findings could include different hydraulic conductivity characteristics of the substrates used. The hydraulic conductivity (or permeability) can affect the hydrology and moisture condition of the substrates and hence germination. Confirming this, a recent study found that the effect of the addition of digestates on the soil-wetting behavior was mainly influenced by the soil texture and soil moisture content [19]. In our study, the silica sand treated with digestate showed a phenomenon of water repellency at the surface and a preferential water flow resulting in an inhomogeneous wetting of the substrate and, consequently, a decline in the germination rate. Similarly, a previous study [38] found that when water potential decreased, the time for germination of wheat was slow or halted. The increase in the degree of water repellency with higher digestate applications in the silica sand might be associated with an increased content of organic matter in this fertilizer, leading to a higher hydrophobicity, as has been previously reported [39,40], and to the fact that digestates contain large quantities of monovalent salts and long-chained fatty acids, that can consequently increase water repellency [19]. The decomposition of hydrophobic organic materials originating from digestates can coat soil particles preventing the infiltration of water into the soil profile [41,42].

Additional studies found an interaction between the texture of the soil and the hydrophobic substances present in the digestate [43,44]. Soils with a small surface area (e.g., silica sand) are more prone to water repellency compared to other substrates, such as silt or clay, after the application of digestates [45], due to the fact that individual small particles become more completely covered by hydrophobic substances.

In contrast, on field loam and field sand (finer textured soils) the increase of the permeability after digestate application (see K_s values in Table 3) was related to improved moisture status and this, in turn, positively affected germination. This is in line with other studies showing that digestate can be an enhancer of soil structure [25] and water-holding capacity [19]. In our study we found a relation in the increase of plant yield with the application of digestate and an improved hydraulic conductivity (Table 3) and moisture retention. This positive effect on the germination rate was also previously observed in field loam and field sand [46]. However, other studies have also hypothesized that application of manure-based digestates could have a negative effect on germination in these soils due to phytotoxicity effects, if the digestate is not completely digested [47]. Seemingly a toxic effect on germination using pure maize digestate did not occur in our study.

To our knowledge, no comparable data on the effect of pure maize digestate applied as a fertilizer for plant biomass production are available to date. Therefore, the findings of our study provide important additional information on the effect of maize digestate on plant performance, particularly on low fertility soils.

5. Conclusions

Digestate from plant material, which commonly contains large amounts of mineralized N in ammonium form and no heavy metals, presents a promising alternative to mineral fertilizers that

require substantial energy input for its production. Digestate addition to soils does not only provide nutrients for crops, but also supply organic matter to the soils that facilitates the maintenance of its structure and improves the water-holding capacity. Digestate application did not always affect soil properties in the same way, and it depended on the substrate used, showing a negative effect in the silica sand reducing the permeability and hence the germination of maize, not observable in the field substrates. These findings can contribute to further understanding of the economic and environmental benefits of the use of digestate as fertilizer and soil conditioner in low fertility soils. The extent to which digestate application of pure plant biomass origin can facilitate biomass production on nutrient-poor soils still needs scientific field-scale investigation.

Author Contributions: The conceptualization and methodology of the study was realized and approved by A.A.R.-A., V.M.T. and N.D.J.; investigation and formal data analysis, A.A.R.-A.; investigation, A.A.R.-A., V.M.T. and N.D.J.; writing—original draft preparation was done by A.A.R.-A., V.M.T., and N.D.J.; writing—review and editing was interactively realized by A.A.R.-A., V.M.T. and N.D.J.; visualization, A.A.R.-A.; supervision, V.M.T. and N.D.J.; project administration, N.D.J.; funding acquisition, N.D.J.

Funding: This research was funded by the European Community's Framework Programme (FP7/2007-2013), grant number 603744.

Acknowledgments: Laboratory and greenhouse assistance by Perrine Ratouis, Beate Uhlig, Marcel Schneider, Katja Herrmann and Andreas Linden is greatly appreciated. Special thanks to Johannes Max for constructive discussions of the experimental design and analysis. We thank Stefan Pätzold (Bonn University) for soil information and Lutz Weihermüller for water permeability analysis. Sincere thanks to Willy Kaulhausen and Andreas Dering, ADRW Nature Power GmbH, for providing the maize digestate and chemical analysis thereof. Funding for Ana Robles Aguilar was generously provided within the IAESTE program by the German Academic Exchange Service (DAAD). The work was partly conducted within ManureEcoMine project, funded by the European Community's Framework Programme (FP7/2007-2013) under Grant Agreement n° 603744.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Vaneeckhaute, C.; Meers, E.; Michels, E.; Ghekiere, G.; Accoe, F.; Tack, F.M.G. Closing the nutrient cycle by using bio-digestion waste derivatives as synthetic fertilizer substitutes: A field experiment. *Biomass Bioenergy* **2013**, *55*, 175–189. [[CrossRef](#)]
2. Holm-Nielsen, J.B.; Seadi, T.A.; Oleskowicz-Popiel, P. The future of anaerobic digestion and biogas utilization. *Bioresour. Technol.* **2009**, *100*, 5478–5484. [[CrossRef](#)] [[PubMed](#)]
3. Walsh, J.J.; Jones, D.L.; Edwards-Jones, G.; Williams, A.P. Replacing inorganic fertilizer with anaerobic digestate may maintain agricultural productivity at less environmental cost. *J. Plant Nutr. Soil Sci.* **2012**, *175*, 840–845. [[CrossRef](#)]
4. FNR. *Biogas*, 8th ed.; Fachagentur Nachwachsende Rohstoffe e.V.(FNR): Gülzow-Prüzen, Germany, 2012; p. 42.
5. Möller, K.; Müller, T. Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. *Eng. Life Sci.* **2012**, *12*, 242–257. [[CrossRef](#)]
6. Alburquerque, J.A.; de la Fuente, C.; Ferrer-Costa, A.; Carrasco, L.; Cegarra, J.; Abad, M.; Bernal, M.P. Assessment of the fertiliser potential of digestates from farm and agroindustrial residues. *Biomass Bioenergy* **2012**, *40*, 181–189. [[CrossRef](#)]
7. Botheju, D.; Svalheim, O.; Bakke, R. Digestate nitrification for nutrient recovery. *Open Waste Manag. J.* **2010**, *3*, 1–12. [[CrossRef](#)]
8. Sogbedji, J.M.; van Es, H.M.; Melkonian, J.J.; Schindelbeck, R.R. Evaluation of the PNM model for simulating drain flow nitrate-N concentration under manure-fertilized maize. *Plant Soil* **2006**, *282*, 343–360. [[CrossRef](#)]
9. Fouda, S.; von Tucher, S.; Lichti, F.; Schmidhalter, U. Nitrogen availability of various biogas residues applied to ryegrass. *J. Plant Nutr. Soil Sci.* **2013**, *176*, 572–584. [[CrossRef](#)]
10. Fuente, C.; Alburquerque, J.A.; Clemente, R.; Bernal, M.P. Soil C and N mineralisation and agricultural value of the products of an anaerobic digestion system. *Biol. Fertil. Soils* **2013**, *49*, 313–322. [[CrossRef](#)]
11. Vaneeckhaute, C.; Meers, E.; Michels, E.; Buysse, J.; Tack, F.M.G. Ecological and economic benefits of the application of bio-based mineral fertilizers in modern agriculture. *Biomass Bioenergy* **2013**, *49*, 239–248. [[CrossRef](#)]

12. Gunnarsson, A.; Bengtsson, F.; Caspersen, S. Use efficiency of nitrogen from biodigested plant material by ryegrass. *J. Plant Nutr. Soil Sci.* **2010**, *173*, 113–119. [[CrossRef](#)]
13. Möller, K.; Schulz, R.; Müller, T. Substrate inputs, nutrient flows and nitrogen loss of two centralized biogas plants in southern Germany. *Nutr. Cycl. Agroecosystems* **2010**, *87*, 307–325. [[CrossRef](#)]
14. Adediran, J.A.; De Baets, N.; Mkeni, P.N.S.; Kiekens, L.; Muyima, N.Y.O.; Thys, A. Organic Waste Materials for Soil Fertility Improvement in the Border Region of the Eastern Cape, South Africa. *Biol. Agric. Hortic.* **2003**, *20*, 283–300. [[CrossRef](#)]
15. FAO. *The State of Food Insecurity in the World Economic Crises—Impacts and Lessons Learned*; FAO: Rome, Italy, 2009.
16. Nabel, M.; Schrey, S.D.; Poorter, H.; Koller, R.; Nagel, K.A.; Temperton, V.M.; Dietrich, C.C.; Briese, C.; Jablonowski, N.D. Coming late for dinner: Localized digestate depot fertilization for extensive cultivation of marginal soil with *Sida hermaphrodita*. *Front. Plant Sci.* **2018**, *9*, 1095. [[CrossRef](#)] [[PubMed](#)]
17. Nabel, M.; Schrey, S.D.; Poorter, H.; Koller, R.; Jablonowski, N.D. Effects of digestate fertilization on *Sida hermaphrodita*: Boosting biomass yields on marginal soils by increasing soil fertility. *Biomass Bioenergy* **2017**, *107*, 207–213. [[CrossRef](#)]
18. Barbosa, D.B.P.; Nabel, M.; Jablonowski, N.D. Biogas-digestate as nutrient source for biomass production of *Sida hermaphrodita*, *Zea mays* L. and *Medicago sativa* L. *Energy Procedia* **2014**, *59*, 120–126. [[CrossRef](#)]
19. Voelkner, A.; Holthusen, D.; Horn, R. Influence of homogenized residues of anaerobic digestate on the physicochemical properties of differently textured soils. *J. Plant Nutr. Soil Sci.* **2015**, *178*, 261–269. [[CrossRef](#)]
20. Alburquerque, J.A.; de la Fuente, C.; Bernal, M.P. Chemical properties of anaerobic digestates affecting C and N dynamics in amended soils. *Agric. Ecosyst. Environ.* **2012**, *160*, 15–22. [[CrossRef](#)]
21. Nabel, M.; Barbosa, D.B.P.; Horsch, D.; Jablonowski, N.D. Energy crop (*Sida hermaphrodita*) fertilization using digestate under marginal soil conditions: A dose-response experiment. *Energy Procedia* **2014**, *59*, 127–133. [[CrossRef](#)]
22. Fox, R.H.; Piekielek, W.P.; Macneal, K.M. Using a chlorophyll meter to predict nitrogen fertilizer needs of winter wheat. *Commun. Soil Sci. Plant Anal.* **1994**, *25*, 171–181. [[CrossRef](#)]
23. Krammes, J.S.; Deban, L.F. Soil Wettability—A Neglected Factor in Watershed Management. *Water Resour. Res.* **1965**, *1*, 283–286. [[CrossRef](#)]
24. Miller, W.W.; Valoras, N.; Letey, J. Movement of 2 Nonionic Surfactants in Wettable and Water-Repellent Soils. *Soil Sci. Soc. Am. J.* **1975**, *39*, 11–16. [[CrossRef](#)]
25. Alburquerque, J.A.; de la Fuente, C.; Campoy, M.; Carrasco, L.; Najera, I.; Baixauli, C.; Caravaca, F.; Roldan, A.; Cegarra, J.; Bernal, M.P. Agricultural use of digestate for horticultural crop production and improvement of soil properties. *Eur. J. Agron.* **2012**, *43*, 119–128. [[CrossRef](#)]
26. Tiwari, V.; Tiwari, K.; Upadhyay, R. Effect of crop residues and biogas slurry incorporation in wheat on yield and soil fertility. *J. Indian Soc. Soil Sci.* **2000**, *48*, 515–520.
27. Galvez, A.; Sinicco, T.; Cayuela, M.L.; Mingorance, M.D.; Fornasier, F.; Mondini, C. Short term effects of bioenergy by-products on soil C and N dynamics, nutrient availability and biochemical properties. *Agric. Ecosyst. Environ.* **2012**, *160*, 3–14. [[CrossRef](#)]
28. Joshua, W.D.; Michalk, D.L.; Curtis, I.H.; Salt, M.; Osborne, G.J. The potential for contamination of soil and surface waters from sewage sludge (biosolids) in a sheep grazing study, Australia. *Geoderma* **1998**, *84*, 135–156. [[CrossRef](#)]
29. Six, J.; Elliott, E.; Paustian, K. Soil structure and soil organic matter II. A normalized stability index and the effect of mineralogy. *Soil Sci. Soc. Am. J.* **2000**, *64*, 1042–1049. [[CrossRef](#)]
30. Diacono, M.; Montemurro, F. Long-term effects of organic amendments on soil fertility. A review. *Agron. Sustain. Dev.* **2010**, *30*, 401–422. [[CrossRef](#)]
31. Tambone, F.; Genevini, P.; Adani, F. The effects of short-term compost application on soil chemical properties and on nutritional status of maize plant. *Compos. Sci. Util.* **2007**, *15*, 176–183. [[CrossRef](#)]
32. Giusquiani, P.L.; Pagliani, M.; Gigliotti, G.; Businelli, D.; Benetti, A. Urban Waste Compost: Effects on Physical, Chemical, and Biochemical Soil Properties. *J. Environ. Qual.* **1995**, *24*, 175–182. [[CrossRef](#)]
33. Rashid, M.T.P.V.; Parkin, G. Predicting nitrogen fertilizer requirements for corn by chlorophyll meter under different N availability conditions. *Can. J. Soil Sci.* **2005**, *85*, 149–159. [[CrossRef](#)]
34. Robinson, D.; Rorison, I.H. Plasticity in grass species in relation to nitrogen supply. *Funct. Ecol.* **1988**, *2*, 249–257. [[CrossRef](#)]

35. Schulz, D.G.; Fey, R.; Ruppenthal, V.; Malavasi, M.D.; Malavasi, U.C. Initial Growth of Physic Nut as a Function of Sources and Doses of Organic Fertilizers. *Cienc. Agrotec.* **2012**, *36*, 615–623. [[CrossRef](#)]
36. Liu, W.K.; Du, L.F.; Yang, Q.C. Biogas slurry added amino acids decreased nitrate concentrations of lettuce in sand culture. *Acta Agric. Scand. B Soil Plant Sci.* **2009**, *59*, 260–264. [[CrossRef](#)]
37. Montemurro, F.; Canali, S.; Convertini, G.; Ferri, D.; Tittarelli, F.; Vitti, C. Anaerobic digestates application on fodder crops: Effects on plant and soil. *Agrochimica* **2008**, *52*, 297–312.
38. Pawloski, M.C.; Shaykewi, C.F. Germination of Wheat as Affected by Soil-Water Stress. *Can. J. Plant Sci.* **1972**, *52*, 619–623. [[CrossRef](#)]
39. Ritsema, C.J.; Dekker, L.W. How Water Moves in a Water Repellent Sandy Soil: 2. Dynamics of Fingered Flow. *Water Resour. Res.* **1994**, *30*, 2519–2531. [[CrossRef](#)]
40. Horne, D.; McIntosh, J. Hydrophobic compounds in sands in New Zealand—extraction, characterisation and proposed mechanisms for repellency expression. *J. Hydrol.* **2000**, *231*, 35–46. [[CrossRef](#)]
41. Doerr, S.H.; Thomas, A.D. The role of soil moisture in controlling water repellency: New evidence from forest soils in Portugal. *J. Hydrol.* **2000**, *231*, 134–147. [[CrossRef](#)]
42. Moore, G.A. *Soilguide: A Handbook for Understanding and Managing Agricultural Soils*; Department of Agriculture, Western Australia Bulletin 4343: South Perth, Australia, 2001; Volume 4343, pp. 53–63.
43. Wallach, R.; Ben-Arie, O.; Graber, E. Soil water repellency induced by long-term irrigation with treated sewage effluent. *J. Environ. Qual.* **2005**, *34*, 1910–1920. [[CrossRef](#)]
44. Tarchitzky, J.; Lerner, O.; Shani, U.; Arye, G.; Lowengart-Aycicegi, A.; Brener, A.; Chen, Y. Water distribution pattern in treated wastewater irrigated soils: Hydrophobicity effect. *Eur. J. Soil Sci.* **2007**, *58*, 573–588. [[CrossRef](#)]
45. Woche, S.K.; Goebel, M.O.; Kirkham, M.B.; Horton, R.; Van der Ploeg, R.R.; Bachmann, J. Contact angle of soils as affected by depth, texture, and land management. *Eur. J. Soil Sci.* **2005**, *56*, 239–251. [[CrossRef](#)]
46. Garg, R.; Pathak, H.; Das, D.; Tomar, R. Use of flyash and biogas slurry for improving wheat yield and physical properties of soil. *Environ. Monit. Assess.* **2005**, *107*, 1–9. [[CrossRef](#)] [[PubMed](#)]
47. Huang, G.F.; Wu, Q.T.; Wong, J.W.C.; Nagar, B.B. Transformation of organic matter during co-composting of pig manure with sawdust. *Bioresour. Technol.* **2006**, *97*, 1834–1842. [[CrossRef](#)] [[PubMed](#)]



© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).