Chapter

A Rhizobox-Study Elucidating Biogas-Digestate Fertilization and Soil Compaction Effects on Juvenile Maize Growth and Rhizosphere pH

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Abstract

Biogas digestate (BD) contains nitrogen (N), phosphorus (P), and potassium (K) and is easily and largely available in Germany and other countries in Europe. Nevertheless, few studies compare BD to mineral NPK fertilizer, particularly under soil compaction. The characteristics of these fertilizers and soil compaction may affect rhizosphere pH and root development, thereby affecting nutrient uptake by plants. This 18-day rhizobox study evaluated initial maize growth and root architecture responses to BD (derived from maize silage+chicken manure), mineral NPK, and BD + NPK fertilization under compacted (0–25 cm compacted; 25–55 cm loose) and loose (0–55 cm) substrates. Treatments promoted similar shoot biomass, specific leaf area, and sufficient shoot N, P, and K nutrition. Shoot P content in BD + NPK and NPK was 29–33% higher compared to BD. Root P content in NPK was 26% higher than in BD, independent of compaction, likely favoring root proliferation and explaining the higher root:shoot ratio in NPK. In loose soil, the main root length in NPK was 49% higher compared to BD, but rooting was deeper in BD. Rhizosphere alkalinization measured non-invasively suggested preferential maize N absorption as nitrate. Combining BD with fast-soluble P sources may provide maize performance comparable to mineral NPK.

Keywords: mineral fertilizer, rhizosphere alkalinization, non-invasive rhizosphere pH, root architecture, shoot nutrition

1. Introduction

Plants face spatial and temporal variability of biological, chemical, and physical soil properties and show the ability to adjust to such heterogeneity through their morphological and physiological plasticity [1, 2]. Typical responses of a plant subjected to soil compaction may include restriction of downward penetration of the main root axes, decrease of root elongation rate and total root length [3], increase in

shoot-to-root biomass ratio [4], and, lastly, reduction of crop yield [5]. Furthermore, soil compaction affects plant water use and nutrient uptake [6–8]. Interestingly, studies reported that plants differ in the extent of compensatory root growth responses to heterogeneous soil strength [9–12].

The arable soil layer (0–25 cm) is also the main rooting zone, and its compaction has been reported as one of the most limiting plant performance factors [13–15]. Measurements such as soil bulk density and soil penetration resistance are widely used to determine soil compactness [7, 16]. It should be mentioned that critical limits for soil bulk density and soil penetration resistance vary depending mainly on soil texture, soil water content, and plant species [17]. Organic substrates, commonly used as a growth medium in greenhouse experiments, normally present low bulk density [11]. Moreover, organic substrates under compaction usually present penetration resistance values lower than the critical thresholds determined for soils, but the effects of compaction on root and shoot are presumably reproducible in organic substrate [18, 19]. In addition, a slight degree of soil compaction may be favorable for the adhesion of the soil to roots and subsequent root exploration for water and nutrients [20, 21].

Changes in rhizosphere pH are considered a critical parameter that influences the bioavailability of nutrients and toxic elements for plant uptake [22]. Roots can induce an increase or decrease of rhizosphere pH by OH^- or H^+ release, respectively, changing the equilibrium between cations and anions at the root-soil interface [23]. In general, when more cations than anions are absorbed by the roots, plants release proportionally more protons in the rhizosphere, thereby acidifying the rhizosphere pH [23]. On the other side, when more anions than cations are absorbed, either H^+ is taken up, or OH^- or HCO_3^- is excreted, hence leading to the alkalinization of the rhizosphere [22, 23]. Alkalinization or acidification of the rhizosphere and its intensity depend mainly on plant species and soil factors, such as initial bulk soil pH, soil water content, and fertilization. Nitrogen (N) uptake as nitrate (NO_3^-) or ammonium (NH_4^+) generally leads to rhizosphere alkalinization and acidification, respectively, in non-legume species such as maize (*Zea mays* L.) [24]. In this way, monitoring the rhizosphere pH allows us to improve our understanding of how plants use the nutrients released from mineral or organic fertilizers in the soil.

The elucidation of root phenotypic traits and biogeochemical processes is strongly dependent on the availability of non-invasive analytical methods [25]. In this sense, plant experiments performed in transparent rhizoboxes enable the performance of repeated measurements of the same roots with a high temporal resolution to quantify static and dynamic characteristics and correlate it to the plant growth performance [18]. Likewise, planar optodes are state-of-the-art technology that can non-invasively measure spatial and temporal dynamics of pH, oxygen (O_2), and carbon (C) dioxide (CO_2) in the soil and in the root-soil interface, which are among the main drivers of processes occurring in the rhizosphere [26–28]. In this scenario, maize has been used as a model plant to investigate rhizosphere processes [29–31].

Within the biogas production scenario, maize has been considered an important energy crop for anaerobic digestion [32]. Conceptually, anaerobic digestion for biogas production consists of the natural breakdown of organic materials by microorganisms in the absence of O_2 to produce biogas as an energy carrier, while the residual by-product is known as digestate [33, 34]. Biogas is a mixture of high-energy methane (CH_4) and CO_2 and is a highly useful source of renewable energy [33–35]. This might be of particular importance in recent times of energy shortages and crises. A wide range of feedstock can be used for biogas production, such as industrial and

municipal waste, energy crops, animal manures, and crop residues [36]. Thus, the chemical composition of digestates varies according to the feedstock characteristics and to the digestion conditions as well [33, 34].

Biogas digestate (BD) is a valuable organic fertilizer containing considerable amounts of N, mainly as NH_4^+, among other plant macronutrients such as potassium (K) and phosphorous (P) and micronutrients [37–39]. Therefore, BD has emerged as an alternative to reduce the demand for mineral fertilization, which is associated with a high environmental footprint [40, 41]. In addition, the application of BD in agricultural lands may be considered a sustainable practice of closing the energy and nutrient cycles for environmentally friendly biomass and food production [42]. In the last decades, technological innovation and governmental policies boosted the biogas production sector in Europe [32, 43]. In fact, the number of biogas plants in Europe has tremendously increased from 6227 in 2009 to 17,783 in 2017, with Germany, Italy, and the United Kingdom considered the leaders of biogas production and responsible for consuming 50, 11, and 10%, respectively, of the final gross biogas consumption in the continent [44].

Increasing biogas production inevitably results in increasing BD amounts. Recent estimations suggest that nearly 180 million Mg of BD are produced annually in Europe, where Germany contributes to 48% of this amount [45]. In Germany, most of the biogas produced in 2017 had agricultural residues (44%) and energy crops (48%), mainly maize, as feedstock [44]. Moreover, closely 100% of the so-called agricultural BD, which is composed of a mixture of manure and plants, particularly energy crops, produced in Germany is directly used as fertilizer [45]. Usually, energy crops are co-digested with other materials, such as manure [33]. Although manure alone may result in low biogas yield, it contains great amounts of N, P, and K due to animal excretions, enriching the BD product as fertilizer and concomitantly avoiding the improper disposal of manure in the environment [33]. Yet, as an organic product, BD can contribute to increasing soil organic matter content, water holding capacity, and cation exchange capacity [46], which may enhance soil biological, chemical, and physical attributes [41].

Recently, many studies have been interested in closing the nutrient cycle by adding BD to fields as a renewable form of organic fertilizer [46–50]. Nevertheless, few studies acknowledge that plants are frequently exposed to certain levels of soil compaction in the field, as arable layer compaction, and that mineral and organic fertilizers may undergo distinct dynamics in these circumstances. Furthermore, the interaction between fertilization type and soil compaction may alter root architecture and thus plant performance [11]. Through the investigation of rhizosphere pH dynamics, the uptake of nutrients by plants subjected to such fertilization and soil compaction conditions may be elucidated, allowing insights for the optimization of fertilizers.

We hypothesized that partially replacing regular mineral NPK fertilization with BD fertilization has no drawbacks for the shoot and root parameters of maize seedlings compared to regular mineral NPK fertilization, regardless of the compaction levels tested. In this context, the main purpose of our study was to improve our knowledge regarding sustainable agricultural practices for maize fertilization with BD. Therefore, we investigated through non-invasive techniques (rhizoboxes and planar pH optodes) the performance and root-induced rhizosphere pH variation of juvenile maize in response to ordinary fertilization in Germany with maize silage+chicken manure-derived BD, mineral NPK, and the combination of the two, under arable layer (0–25 cm) compacted substrate and loose substrate. For that, we compared the effects of fertilizer type and substrate compaction condition with

respect to: (a) maize shoot development parameters, (b) nutrient content in maize and substrate; (c) maize root architecture; and, (d) maize root-induced rhizosphere pH variation.

2. Materials and methods

2.1 Experimental set-up

A rhizobox experiment was established under greenhouse conditions at IBG-2: Plant Sciences, Forschungszentrum Jülich, in Germany (50.89942° N, 6.39211° E). The experiment was performed in a completely randomized design, considering fertilizer type (Biogas Digestate - BD, mineral NPK fertilizer - NPK, and a mixture of BD and NPK - BD + NPK) and substrate compaction condition (layered compacted substrate and loose substrate) as experimental factors and five replicates, totaling 30 experimental units.

In this study, we aimed to test explicitly the fertilizer effect of BD under the given experimental soil conditions. Therefore, the organic fertilizer (BD) was compared to the mineral NPK fertilizer (control) with reference doses of fertilizer application of 40 and 1.3 Mg ha^{-1}, respectively. The applied doses of BD and NPK fertilizer are in accordance with an ordinary agricultural field application of fresh BD as received from the biogas plant and based on an ordinary agricultural application of 200 kg N ha^{-1}, respectively, which are suitable for maize fertilization [49]. The BD + NPK fertilization consisted of a half BD dose (20 Mg ha^{-1}) + half NPK dose (0.67 Mg ha^{-1}). The BD used in this experiment was a product of the co-digestion of maize silage as the major feedstock and minor amounts of chicken manure, provided by a commercially operating biogas facility (ADRW NaturPower GmbH & Co. Kg Titz-Ameln, Germany). The BD contained: 7.2% dry matter, 0.53% total N, 0.32% NH_4^+, 0.14% P, 0.68% K, 0.037% Mg, 0.16% Ca, 0.03% S, 5.3% organic matter, C:N ratio 6, and pH in $CaCl_2$ 8.2 (all values referring to fresh weight). The mineral NPK fertilizer (Compo Rasendünger, Compo GmbH, Münster, Germany) contained: 15% N (1% NO_3^-, 9.5% ammonia, 4.5% isobutylidenediurea), 5% P, 8% K, and 3% Mg. The amounts of N, P, and K present in the applied doses of BD, NPK, and BD + NPK fertilizer were calculated in kg ha^{-1} and are described in **Table 1**. Application of NPK and BD resulted in similar N and P doses (**Table 1**). The higher K dose derived from BD compared to NPK was considered for the experiment since this type of BD (maize

Fertilizer	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})	N:P:K
BD[1]	212	56	272	1.00:0.26:1.28
BD + NPK	206	61	189	1.00:0.30:0.92
NPK[2]	200	67	107	1.00:0.33:0.53

[1]N, P and K content in the full BD fertilizer applied dose was calculated based on 0.53% total N, 0.14% P, and 0.68% K in accordance with an agricultural field application of 40 t ha^{-1} of digestate.
[2]N, P and K content in the full NPK fertilizer applied dose was calculated based on 15% total N, 5% P, and 8% K in accordance with an agricultural field application of 200 kg N ha^{-1}.

Table 1.
Nitrogen (N), phosphorus (P) and potassium (K) content and the N:P:K ratio in the applied doses of Biogas Digestate (BD), Biogas Digestate+mineral NPK fertilizer (BD + NPK) and mineral NPK fertilizer (NPK).

silage and chicken manure feedstocks) is ordinarily applied in place of mineral NPK fertilizer in Germany [44, 45].

Maize plants were grown in a mixture of 50% (v/v) arable field soil (Endogleyic Stagnosol, with a silty-loamy texture containing 1.1% C; 0.1% N; 0.1% P; 1.6% K; pH in $CaCl_2$: 6.3) collected from 0 to 30 cm depth near Jülich municipality, and 50% (v/v) black peat potting substrate (Einheitserde Null; containing 19.3% C; <0.01% N; 0.7% P; <0.01% K; pH in $CaCl_2$: 6.0). Therefore, the growth medium was called substrate. The substrate was manually sieved to a maximal aggregate size of 0.5 cm. The utilization of dark peat improves the visual contrast between plant roots and the growth medium in the rhizoboxes, enhancing the detection of roots during the non-invasive root growth analysis.

The fertilizers were homogeneously mixed into the substrate using an end-over-end mixer for 15 min. The rhizoboxes (dimensions: 60 cm height x 30 cm width x 2 cm depth) were filled with the substrate-fertilizer mixture, and thereafter the compaction protocol (see layered compacted and loose substrate rhizoboxes section) was followed. Subsequently, two maize seeds were sown in the top center of the rhizoboxes at a depth of 2 cm in relation to the substrate surface. One day after seedling emergence, the smallest seedling was manually removed from the rhizobox. Plants were watered with 100 mL of deionized water three times per week to maintain a minimum of 30% volumetric water content in the substrate during the experiment. During cultivation, the rhizoboxes were kept at an inclination angle of 45° to force the roots to grow along the transparent plate of the rhizobox [51]. The transparent plates were facing downwards on the horizontal plane and were covered with lightproof material (black plate) throughout the experiment to avoid light disturbances to root growth and to planar pH optodes signal (see pH measurements in Section 2.3). Plants were grown for 18 days after germination (DAG) under controlled conditions (16 h light per day, day/night temperature of 22/17°C, and 60% relative humidity).

2.2 Layered, compacted, and loose substrate rhizoboxes

A protocol described by Nagel et al. [18] was adapted to obtain rhizoboxes with different levels of compaction, named layered compacted substrate (C/L) and loose substrate (L/L), as illustrated in **Figure 1**. The C/L rhizoboxes contained a top layer (0–25 cm depth) of compacted substrate followed by a bottom layer (25–55 cm depth) of loose substrate. The bottom layer of the loose substrate was obtained by pouring gradually 1 L portions of the substrate into the rhizobox. Subsequently, the compacted top layer of 25 cm depth was obtained by pouring gradually 1 L portions of the substrate into the rhizobox, and then each 1 L portion was compressed by using a custom-built compaction frame. The compression was applied with a manual pallet forklift by lifting individual rhizoboxes against the frame while pressure was applied to the substrate surface using a wooden plank. Each compressed 1 L portion of substrate received an equivalent pressure of 1.2 kg cm^{-2}. The L/L rhizoboxes contained a continuous loose layer of 55 cm depth that was obtained by pouring gradually 1 L portions of substrate into the rhizobox. Two drainage holes (diameter of 0.8 cm) at the bottom of the rhizoboxes allowed sufficient substrate drainage.

Three additional replicates of C/L and L/L rhizoboxes were destined to determine the substrate dry bulk density and penetration resistance. To determine the substrate bulk density, the frontal rhizobox plates (transparent) were opened (with minimal disturbance) to collect substrate samples at 10 and 50 cm depth using sampling

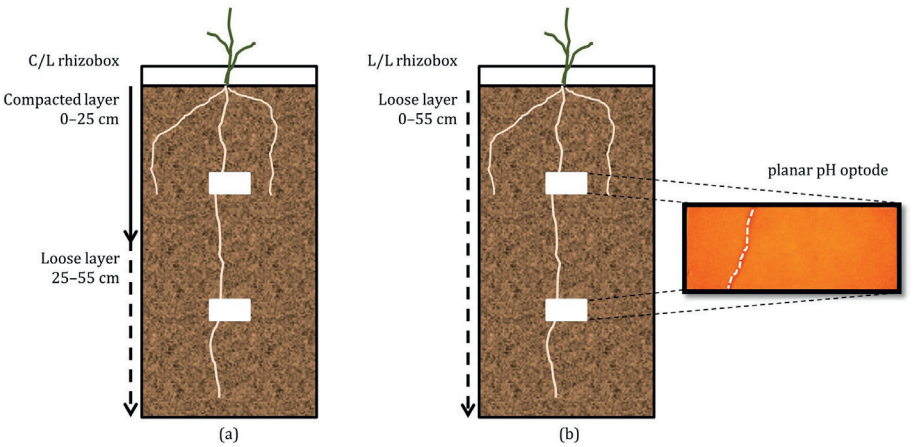

Figure 1.
Drawings illustrate: (a) layered compacted (C/L) rhizobox: top compacted layer (0–25 cm depth; 1.0 MPa, 0.84 g cm^{-3} at 10 cm depth) followed by loose layer (25–55 cm depth; 0.66 g cm^{-3} at 50 cm depth), and (b) loose (L/L) rhizobox: continuous loose layer (0–55 cm depth; 0.04 MPa, 0.62 and 0.61 g cm^{-3} at 10 and 50 cm depth). White rectangles represent planar pH optodes positioned at 15 and 35 cm depth.

cylinders (volume: 10.6 mL). The samples were dried for 48 h at 105°C and weighed. The substrate dry bulk density was obtained by dividing the dry substrate mass by the volume of the sampling cylinder. The substrate penetration resistance was measured with a hand penetrometer for the top layers (Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands). The penetrometer had a 30° cone angle, an 8 mm maximum cone diameter, and a penetration depth of 10 cm and was fitted with 50-N steel springs. The C/L substrate presented densities of 0.84 ± 0.04 and 0.66 ± 0.05 g cm^{-3} at 10 cm and 50 cm depths respectively, and penetration resistance of 1.0 MPa at 10 cm depth. The L/L substrate presented bulk densities of 0.62 ± 0.04 and 0.61 ± 0.04 g cm^{-3} at 10 cm and 50 cm depths respectively, and penetration resistance of 0.04 MPa at 10 cm depth.

2.3 pH measurements via planar optode technique

The planar pH optode is a sensor foil with embedded fluorescent molecules that emit a characteristic pattern of fluorescence after excitation with light, which allows measurements of pH *in vivo* and *in situ* [52]. The pattern of fluorescence depends on the concentration of the analyte. In the present study, protons were the analyte, and planar pH optodes were used to monitor non-invasively and quantitatively the spatial and temporal dynamics of rhizosphere pH over the experimental period of 18 days. The relatively short experimental period can provide relevant information on maize rhizosphere pH changes and associated nutrition as younger maize plants have considerably higher root exudation activity than older ones [53], being assumingly more prone to detection by the used optodes. As previously described by Blossfeld et al. [52], optode measurements use the ratiometric approach, which is based on the change of the emission spectrum of the planar optodes depending on the analyte concentration. In our study, the ratio of red to green in the emitted fluorescence response was recorded in the respective color channels of an RGB (red, green, blue) chip. The RGB chip of the camera can capture the fluorescence signals in one single image, and the subsequent data analysis by the software creates the ratio of the red

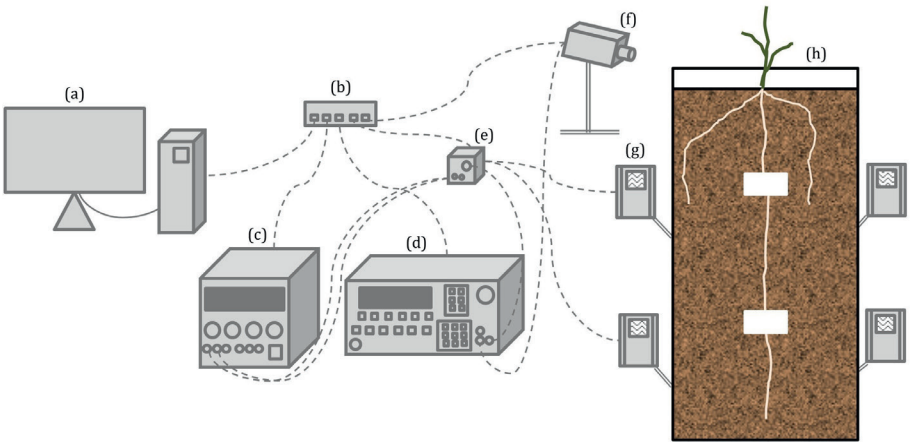

Figure 2.
Schematic drawing of the setup of VisiSens TD1 system, including: (a) computer system, (b) switch, (c) power supply, (d) function generator, (e) strobe controller, (f) color camera including objective, (g) 4 light sources, (h) rhizobox with upper (15 cm depth) and lower (35 cm depth) planar pH optodes. Dotted lines represent the electric, trigger, and network cables.

and the green channel (i.e., R-value), creating a two-dimensional map of the quantity of the measured parameter.

The VisiSens TD1 system (PreSens GmbH, Regensburg, Germany) was used to process the pH data. The system relies on a camera device, a power supply, a function generator, a strobe controller, a light source for pH measurements, and a software to identify pH changes in the root zone through the pH-sensitive planar optodes (product code SF-HP5-OIW; PreSens GmbH) (**Figure 2**). In this study, since maize sowing, one image per day of the optode sensors was taken using a distance of 12.5 cm between the transparent plate of the rhizobox where the planar pH optode was positioned and the camera, resulting in a pixel resolution of 1292 x 964. Every pixel carries discrete quantitative analyte information. The software ImageJ was then used to compute the quantitative maps from the raw sensor response images. Detailed information on high-resolution color radiometric planar optode imaging approaches was previously described by Larsen et al. [54].

Calibration of the pH planar optodes was performed prior to and at the end of the experimental phase in accordance with Blossfeld and Gansert [26]. For that, two stock solutions of phosphate buffers with the same ionic strength (solution A: 0.1 mol L^{-1} of K_2HPO_4 and solution B: 0.1 mol L^{-1} of KH_2PO_4 + 0.2 mol L^{-1} of NaCl) were used to produce seven calibration buffer solutions with different pH values (4.6, 5.0, 5.5, 6.0, 6.5, 7.0, and 7.5) by mixing different volumes of the stock solutions. A replicate of the planar pH optode used in the rhizoboxes was installed in the calibration chamber. The calibration chamber was then subsequently filled with each of the pH buffer solutions, and the change of the R-values of the planar pH optode was monitored from outside the system every minute over a period of 10 min, at which time the R-value was constant. The resulting R-values were used as input data for a Boltzmann fitting curve. The resulting fitting curve was then used to calculate the pH value.

For all images obtained with the same sensor throughout the experimental period, a fixed bulk substrate area (without roots) and a fixed root area (rhizosphere) were selected and analyzed using the software ImageJ to create temporal and quantitative maps of pH. Rhizosphere pH variation promoted by maize roots was based on

rhizosphere pH and bulk substrate pH (Δ rhizosphere pH) obtained in each planar pH optode sensor and was calculated as described in Eq. (1):

$$\Delta \text{ rhizosphere } pH = bulk\ substrate\ pH - rhizosphere\ pH \quad (1)$$

Rhizosphere pH variation over the experimental period, shown as alkalization and acidification pH units, are presented as positive and negative values, respectively.

Prior to filling the rhizoboxes with substrate, the planar pH optode (dimensions: 3 cm height x 8 cm width) was fixed to the inner surface of the front (transparent) plate of the rhizobox at 15 and 35 cm depth (in relation to substrate surface level) using a thin layer of silicon grease as an adhesive (**Figure 1**). The pre-installation of a planar optode in a fixed area of the rhizobox may increase the chances of roots growing outside the installation area. However, pre-installed planar optodes are expected to reduce overall disturbances to the system as compared to opening the rhizobox after roots have grown attached to the front plate of the rhizobox.

2.4 Root system development measurements

Images of the root systems were firstly acquired at 4 DAG and afterward, every 2 to 4 days during the experimental period using a single-lens reflex camera (SLR, 10 Mpx, with a 28 mm electro-focus lens EFS, EOS digital 400D; Canon USA, Inc., Melville, NY, USA). Images were acquired within a custom-made photo box to avoid light interferences in the images. The resolution of the acquired images was adequate for the detection of main and lateral roots. The image analysis software GROWSCREEN-Root [18] was used to manually draw the visible roots growing attached to the transparent plate of the rhizobox and to quantify the visible main roots length, total lateral root length, root system width, and rooting depth.

2.5 Maize development and substrate parameters

At 18 DAG, the photosynthetic assimilation was measured on the third youngest leaf of all individual plants using a soil plant analysis development chlorophyll meter (SPAD-502, Konika-Minolta, Marunouchi, Japan). At harvest, maize plants had four leaves, and the investigated leaf was fully expanded. Subsequently, the shoot (stem and leaves) was harvested at substrate level, and leaf area measurements were determined using a leaf area meter (LI-3100 Area meter, LI-COR Inc., Lincoln, NE, USA) to calculate the specific leaf area (SLA), as described in Eq. (2):

$$SLA = leaf\ \ area\left(m^2\right) \div leaf\ \ dry\ weight\left(kg^{-1}\right) \quad (2)$$

After harvesting, the rhizoboxes were opened and substrate samples (100 g) were taken from all replicates at 20 and 45 cm depth. Thereafter, roots were separated from the substrate by carefully flushing with water. Shoot and root biomass was dried at 70°C and substrate samples were dried at 30°C until constant weight was reached. Maize root:shoot ratio was calculated based on the biomass dry weight. Shoot, root, and soil samples were milled using a swing mill (Retsch MM200, Retsch GmbH,

Haan, Germany), homogenized and analyzed for C and N content using an elemental analyzer (vario Max CNS, Elementar GmbH, Langenselbold, Germany) and for P and K content using the inductively coupled plasma-optical emission spectrometer (ICP-OES, iCAP7600, Thermo Scientific, Dreieich, Germany).

2.6 Statistical analysis

Normality of data was tested using the Shapiro-Wilk test, which revealed a normal distribution of the data. In order to test treatment effects on response variables, the data were submitted to analysis of variance (ANOVA). A post-hoc Tukey test was applied to compare means of treatments when ANOVA indicated significant effects of experimental factors (fertilizer type, substrate compaction condition, and the interaction between the two). Statistical significance was set as $p < 0.05$.

3. Results

The p values of ANOVA testing the effect of fertilizer type, substrate compaction condition, and interaction between the two on the response variables at harvest (18 DAG) are shown in **Table 2**.

3.1 Maize shoot and root dry weight

Maize germination occurred similarly across treatments 3 days after sowing. Means of maize shoot and root dry weight at harvest (18 DAG) are shown in **Figure 3**. Maize plants presented shoot dry weight ranging from 416 (NPK L/L) to 550 mg plant^{-1} (NPK C/L). ANOVA revealed neither significant interaction between fertilizer type and compaction condition nor significant effect of the main factors for shoot dry weight (**Table 2**). These results indicate that BD fertilization promoted similar maize shoot biomass compared to NPK and BD + NPK fertilization, regardless of compaction condition.

Means of root dry weight obtained from 0 to 25 cm and 25 to 55 cm depth ranged from 78 (BD C/L) to 107 (BD + NPK C/L) mg plant^{-1} and from 14 (BD + NPK L/L) to 31 (NPK C/L) mg plant^{-1}, respectively (**Figure 3**), and were affected neither by the interaction between fertilizer type and compaction condition nor by the main factors (**Table 2**). Nevertheless, when considering total root dry weight (0–55 cm depth), significant interaction between fertilizer type and compaction condition occurred (**Table 2**). Under C/L, total root dry weight varied from 96 (BD) to 133 mg plant^{-1} (NPK) (**Figure 3**). In this case, the total root dry weight observed in NPK and NPK + BD treatment did not differ from each other, and it was about 38 and 39% higher than that in BD, respectively (**Figure 3**). Under this compaction condition, the proportion of the total root dry weight (0–55 cm depth) found at 0–25 and 25–55 cm depths slightly ranged from 77 to 81% and 19 to 23%, respectively, across fertilizer types (**Figure 3**). Under L/L, total root dry weight did not differ between fertilizer types and ranged from 105 (NPK) to 109 mg plant^{-1} (BD) (**Figure 3**), indicating that root dry weight promoted by BD was equivalent to that promoted by NPK and NPK + BD. Under this compaction condition, the proportion of the total root dry weight (0–55 cm depth) found at 0–25 and 25–55 cm depth varied from 80 to 86% and from 13 to 20%, respectively (**Figure 3**).

Variables	F	C	F*C
Shoot parameters			
Dry weight	> 0.05	> 0.05	> 0.05
Number of leaves	> 0.05	> 0.05	> 0.05
Specific leaf area	> 0.05	> 0.05	> 0.05
SPAD readings	> 0.05	> 0.05	> 0.05
root:shoot ratio	**0.037**	> 0.05	> 0.05
C content	> 0.05	> 0.05	> 0.05
N content	> 0.05	> 0.05	> 0.05
P content	**<0.001**	**<0.001**	> 0.05
K content	> 0.05	> 0.05	> 0.05
Root parameters			
Dry weight (0–25 cm)	> 0.05	> 0.05	> 0.05
Dry weight (25–55 cm)	> 0.05	> 0.05	> 0.05
Total dry weight (0–55 cm)	> 0.05	> 0.05	**0.017**
C content	> 0.05	**0.036**	> 0.05
N content	> 0.05	> 0.05	**0.013**
P content	**<0.001**	**<0.001**	> 0.05
K content	**0.029**	**0.007**	**<0.001**
Visible main roots length	> 0.05	> 0.05	**0.001**
Visible total lateral root length	**<0.001**	> 0.05	> 0.05
Visible root system width	> 0.05	> 0.05	> 0.05
Visible rooting depth	> 0.05	> 0.05	**0.046**
Soil parameters			
C content (0–25 cm)	> 0.05	> 0.05	> 0.05
N content (0–25 cm)	**<0.001**	> 0.05	> 0.05
P content (0–25 cm)	> 0.05	> 0.05	> 0.05
K content (0–25 cm)	**0.022**	**0.013**	> 0.05
C content (25–55 cm)	**0.033**	> 0.05	> 0.05
N content (25–55 cm)	**0.002**	> 0.05	> 0.05
P content (25–55 cm)	> 0.05	> 0.05	> 0.05
K content (25–55 cm)	**<0.001**	> 0.05	> 0.05

Table 2.
*ANOVA (2 x 3 factorial) results indicating effects of fertilizer type (F), substrate compaction condition (C) and their interaction (F*C) on shoot, root and soil response variables at harvest. p < 0.05 is written in bold.*

3.2 Maize development parameters

Means of maize development parameters such as number of leaves, SLA, SPAD readings, and root:shoot ratio at harvest (18 DAG) are presented in **Table 3**. Maize plants at harvest presented four fully expanded leaves in all treatments. The SLA

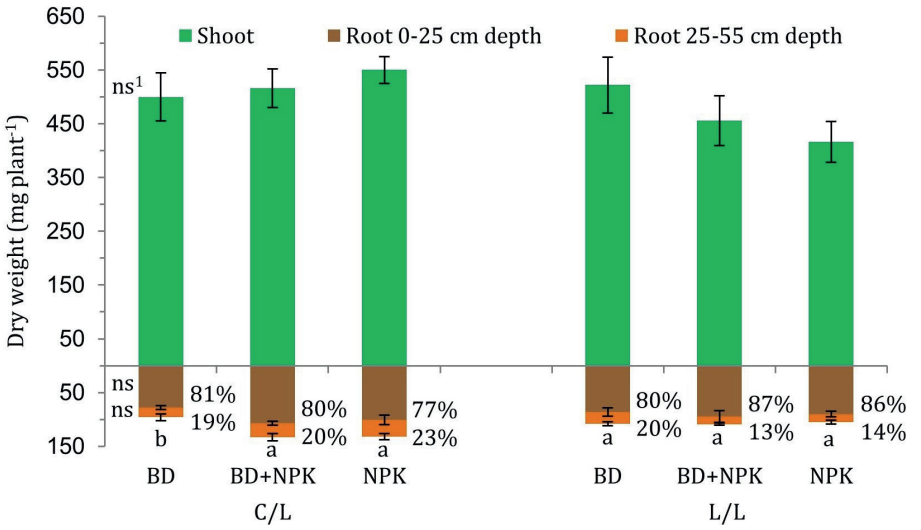

Figure 3.
Dry weight of maize shoot and root at harvest as affected by fertilizer type (Biogas Digestate - BD, Biogas Digestate+mineral NPK fertilizer - BD + NPK, mineral NPK fertilizer - NPK) and compaction condition (layered compacted substrate - C/L, loose substrate - L/L). Data are mean (n = 5) and error bars are standard error. Means of total root dry weight (0–55 cm depth) with different letter within compaction condition (C/L, L/L) are statistically different according to Tukey's test at p < 0.05. [1]ns = mean of shoot weight and root weight at 0–25 and 25–55 cm depth did not differ statistically according to Tukey's test at p < 0.05. Percentage numbers are the proportion of the total root dry weight (0–55 cm depth) at 0–25 and 25–55 cm depth.

Fertilizer	C/L	L/L	Mean[1]	C/L	L/L	Mean[1]
	Number of leaves			SPAD readings		
BD	4(0.0)	4(0.0)	4	44.5(1.3)	45.1(1.7)	44.8
BD + NPK	4(0.0)	4(0.0)	4	44.1(0.9)	40.6(0.8)	42.4
NPK	4(0.2)	4(0.0)	4	43.2(1.2)	40.9(0.7)	42.1
Mean[2]	4	4		44.0	42.2	
	Specific leaf area (m^2 kg^{-1})			Root:shoot ratio		
BD	37.5(2.0)	39.5(3.8)	38.5	0.198(0.038)	0.211(0.030)	0.204 B
BD + NPK	37.5(2.4)	39.7(1.4)	38.6	0.261(0.035)	0.239(0.030)	0.250 AB
NPK	35.5(2.0)	37.7(1.2)	36.6	0.243(0.034)	0.264(0.079)	0.253 A
Mean[2]	36.9	39.9		0.234	0.238	

[1]*Mean of fertilizer type (n = 10).*
[2]*Mean of compaction condition (n = 15).*

Table 3.
Maize number of leaves, specific leaf area, SPAD readings and root:shoot ratio at harvest as affected by fertilizer type (Biogas Digestate - BD, Biogas Digestate+mineral NPK fertilizer - BD + NPK, mineral NPK fertilizer - NPK) and substrate compaction condition (layered compacted substrate - C/L, loose substrate - L/L. Data are mean followed by standard error within parentheses. Means with the same lowercase letter for fertilizer type within compaction condition (n = 5) or same uppercase letter for compaction condition mean (n = 15) and fertilizer type mean (n = 10) are not statistically different according to Tukey's test at p < 0.05.

means ranged from 35.5 (NPK C/L) to 39.7 m^2 kg^{-1} (BD + NPK L/L), and SPAD reading means varied from 40.6 (BD + NPK L/L) to 45.1 (BD L/L) (**Table 3**). ANOVA revealed neither a significant effect of the interaction between fertilizer type and compaction conditions nor significant effects of the main factors for number of leaves, SLA, and SPAD readings (**Table 2**), indicating that BD fertilization enabled similar maize development up to 18 days compared to NPK and BD + NPK with respect to such parameters, regardless of compaction condition.

Regarding root:shoot ratio, ANOVA showed neither significant effects of the interaction between fertilizer type and compaction condition nor significant effects of compaction condition main factor, whereas significant effects of fertilizer type occurred (**Table 2**). The root:shoot ratio fertilizer mean (n = 10) was significantly higher in NPK (0.253) than in BD (0.204), while the root:shoot ratio in BD + NPK (0.250) did not differ from the other two fertilizers (**Table 3**).

3.3 Shoot and root chemical analyses

Means of C, N, P, and K content in maize shoot and root at harvest (18 DAG) are presented in **Table 4**. Maize shoot C content ranged from 389.5 (NPK L/L) to 396.0 g kg^{-1} (NPK C/L), N content from 45.9 (BD L/L) to 49.2 g kg^{-1} (BD + NPK

	Shoot			Root		
Fertilizer	**C/L**	**L/L**	**Mean[1]**	**C/L**	**L/L**	**Mean[1]**
BD	395.1(1.6)	394.8(2.5)	395.0	386.6(6.2)	393.2(1.5)	389.9
BD + NPK	390.5(0.4)	391.7(1.2)	391.1	380.2(0.9)	385.0(3.3)	382.6
NPK	396.0(3.7)	389.5(1.3)	395.0	381.2(8.9)	396.0(2.1)	388.6
Mean[2]	393.9	392.0		382.7 B	391.4 A	
BD	49.0(0.4)	45.9(2.6)	47.5	29.6(0.6) a	29.0(0.1) b	29.3
BD + NPK	49.2(0.5)	47.9(0.6)	48.6	29.1(0.4) a	32.5(1.2) a	30.8
NPK	46.6(2.1)	47.7(0.8)	47.2	30.0(1.3) a	28.6(0.2) b	29.3
Mean[2]	48.3	47.2		29.6	30.0	
BD	5.2(0.2)	6.3(0.2)	5.8 B	3.1(0.1)	3.7(0.1)	3.4 B
BD + NPK	6.7(0.4)	8.2(0.5)	7.5 A	3.1(0.2)	4.0(0.1)	3.6 B
NPK	7.9(0.7)	9.2(0.4)	8.6 A	4.2(0.3)	4.4(0.1)	4.3 A
Mean[2]	6.6 B	7.9 A		3.6 B	4.1 A	
BD	65.2(1.2)	65.0(1.2)	65.1	16.4(0.5) b	31.0(4.0) a	23.7
BD + NPK	65.7(0.7)	66.2(1.6)	66.0	16.1(0.5) b	22.0(1.4) ab	19.1
NPK	64.7(1.6)	63.6(0.8)	64.2	27.2(1.8) a	20.9(0.7) b	24.0
Mean[2]	65.2	65.0		19.9	24.6	

[1]Mean of fertilizer type (n = 10).
[2]Mean of compaction condition (n = 15).

Table 4.
Carbon (C), nitrogen (N), phosphorous (P) and potasium (K) content in maize shoot and root at harvest as affected by fertilizer type (Biogas Digestate - BD, Biogas Digestate+mineral NPK fertilizer - BD + NPK, mineral NPK fertilizer - NPK) and compaction condition (layered compacted substrate - C/L, loose substrate - L/L). Data are mean followed by standard error within parentheses. Means with the same lowercase letter for fertilizer type within compaction condition (n = 5) or same uppercase letter for compaction condition mean (n = 15) and fertilizer type mean (n = 10) are not statistically different according to Tukey's test at $p < 0.05$.

C/L), P content from 5.2 (BD C/L) to 9.2 g kg^{-1} (NPK L/L), and K content from 63.6 (NPK L/L) to 66.2 g kg^{-1} (BD + NPK L/L) across the different treatments (**Table 4**). ANOVA revealed neither significant interaction between fertilizer type and compaction condition nor significant effect of the main factors for shoot C, N, and K contents (**Table 2**).

Maize shoot P content was significantly affected by both factors fertilizer type and compaction condition, but not by the interaction of both (**Table 2**). Regardless of compaction condition, shoot P content fertilizer mean (n = 10) promoted by BD + NPK (7.5 g kg^{-1}) and NPK (8.6 g kg^{-1}) did not differ statistically from each other, but it was 29 and 33% higher than that of BD (5.8 g kg^{-1}) (**Table 4**). These results point out that P nutrition of juvenile maize shoot promoted by BD alone was reduced in relation to the fertilization sources containing mineral fertilizer (NPK). Independently of fertilizer type, shoot P content compaction condition mean (n = 15) in L/L (7.9 g kg^{-1}) was 20% higher than in C/L (6.6 g kg^{-1}) (**Table 4**).

Maize root C content ranged from 380.2 (BD + NPK C/L) to 396.0 g kg^{-1} (NPK L/L), N content from 28.6 (NPK L/L) to 32.5 g kg^{-1} (BD + NPK L/L), P content from 3.1 (BD and BD + NPK C/L) to 4.4 g kg^{-1} (NPK L/L), and K content from 16.1 (BD + NPK C/L) to 31.0 g kg^{-1} (BD L/L) across the different treatments. In general, treatments apparently affected more remarkably the elemental content in maize roots than in shoots. For instance, ANOVA revealed that N and K content in maize roots were affected by the interaction between fertilizer type and compaction condition, that C and P content in maize roots were affected by compaction condition, and that P content in maize roots was also affected by fertilizer type (**Table 2**).

With respect to root C content, mean of compaction condition (n = 15) was slightly (2.3%) higher in L/L (391.4 g kg^{-1}) than in C/L (382.7 g kg^{-1}) (**Table 4**). Regarding root N content, the fertilizer type did not differ from each other within C/L condition, while within L/L condition, root N content promoted by BD + NPK (32.5 g kg^{-1}) was 12 and 14% higher than that promoted by BD (29.0 g kg^{-1}) and NPK (28.6 g kg^{-1}), respectively, which did not differ from each other (**Table 4**). Overall, these results demonstrate that BD promoted similar root N content in relation to NPK, regardless of compaction condition, and yet that under L/L conditions, the mixture of BD with NPK (BD + NPK) incremented the root N content in relation to regular NPK fertilization.

Independently on compaction condition, root P content fertilizer mean (n = 10) resulting from NPK application (4.3 g kg^{-1}) was 27 and 19% higher compared to that conferred by BD (3.4 g kg^{-1}) and BD + NPK (3.6 g kg^{-1}), respectively, which did not differ from each other (**Table 4**). Still, regarding root P content, the mean of compaction condition (n = 15) was 12% higher in L/L (4.1 g kg^{-1}) than in C/L (3.6 g kg^{-1}) (**Table 4**). The pattern of root P content in response to the sources of variation, particularly to fertilizer type, is conformable to that observed for shoot P content. Together, such patterns suggest that maize fertilization with the BD type tested in this study should be complemented by other P sources, preferably highly soluble P fertilizers, in order to avoid possible juvenile maize P deficiency in relation to regular mineral fertilization.

Root K content was responsive to the interaction between fertilizer type and compaction condition. Within C/L condition, root K content in response to NPK fertilization (27.2 g kg^{-1}) was 66 and 70% higher compared to that promoted by BD (16.4 g kg^{-1}) and BD + NPK (16.1 g kg^{-1}), respectively, which did not differ from each other (**Table 4**). Contrastingly, within L/L condition, root K content promoted by BD application (31.0 g kg^{-1}) was 48% higher than that resulting from NPK (20.9 g kg^{-1})

application, and root K content due to BD + NPK amendment (22.0 g kg^{-1}) did not differ from the other fertilizers (**Table 4**).

3.4 Substrate chemical analyses at harvest

Means of C, N, P, and K content in substrate samples collected at 0–25 and 25–55 cm depths at maize harvest are shown in **Table 5**. Substrate C content at 0–25 cm depth ranged from 60.5 (NPK L/L) to 65.0 g kg^{-1} (BD + NPK L/L), and at 25–55 cm from 56.6 (NPK L/L) to 65.4 g kg^{-1} (BD L/L) (**Table 5**). In general, substrate C content tended to be lower in NPK treatments compared to that with BD. However, ANOVA revealed that such a tendency was statistically significant only at 25–55 cm depth, where substrate C content was significantly affected by fertilizer type (**Table 2**). At this depth, C content fertilizer type mean (n = 10) in BD (63.9 g kg^{-1}) was 9% higher compared to NPK (58.9 g kg^{-1}), whereas BD + NPK mean (62.4 g kg^{-1}) did not differ from the other two fertilization types (**Table 5**).

Substrate N content at 0–25 cm depth varied from 2.1 (NPK L/L) to 2.6 g kg^{-1} (BD + NPK C/L and L/L), and at 25–55 cm depth it ranged from 2.3 (NPK L/L) to

	0–25 cm depth			25–55 cm depth		
Fertilizer	**C/L**	**L/L**	**Mean[1]**	**C/L**	**L/L**	**Mean[1]**
BD	64.4(1.9)	62.2(2.9)	63.3	62.4(1.2)	65.4(3.0)	63.9 A
BD + NPK	64.7(2.4)	65.0(1.6)	64.8	61.2(1.0)	63.6(2.1)	62.4 AB
NPK	64.0(1.8)	60.5(1.0)	62.3	61.1(1.6)	56.6(1.2)	58.9 B
Mean[2]	64.4	62.6		61.6	61.9	
BD	2.4(0.1)	2.4(0.1)	2.4 AB	2.4(0.0)	2.4(0.1)	2.4 A
BD + NPK	2.6(0.1)	2.6(0.1)	2.6 A	2.4(0.0)	2.5(0.0)	2.5 A
NPK	2.4(0.0)	2.1(0.1)	2.3 B	2.3(0.1)	2.3(0.0)	2.3 B
Mean[2]	2.5	2.4		2.4	2.4	
BD	815.9(26.1)	790.7(10.5)	803.3	840.9(12.9)	778.2(7.9)	809.6
BD + NPK	825.5(14.5)	813.7(20.1)	819.6	817.6(11.9)	835.8(20.6)	826.7
NPK	841.8(26.2)	835.1(22.2)	838.4	819.6(21.5)	820.2(13.7)	819.9
Mean[2]	827.7	813.2		826.0	811.4	
BD	13.0(0.3)	15.0(0.7)	14.0 AB	14.0(0.2)	14.2(0.1)	14.1 B
BD + NPK	14.8(0.4)	15.0(0.2)	14.9 A	14.8(0.1)	15.4(0.2)	15.1 A
NPK	13.1(0.2)	14.0(0.7)	13.6 B	14.9(0.2)	14.8(0.3)	14.6 A
Mean[2]	13.7 B	14.7 A		14.6	14.8	

[1]Mean of fertilizer type (n = 10).
[2]Mean of compaction condition (n = 15).

Table 5.
Carbon (C), nitrogen (N), phosphorous (P) and potassium (K) content in the substrate at 0–25 and 25–55 cm depth at harvest as affected by fertilizer type (Biogas Digestate - BD, Biogas Digestate+mineral NPK fertilizer - BD + NPK, mineral NPK fertilizer - NPK) and compaction condition (layered compacted substrate - C/L, loose substrate - L/L). Data are mean followed by standard error within parentheses. Means with the same lowercase letter for fertilizer type within compaction condition (n = 5) or same uppercase letter for compaction condition mean (n = 15) and fertilizer type mean (n = 10) are not statistically different according to Tukey's test at p < 0.05.

2.5 g kg^{-1} (BD + NPK L/L) (**Table 5**). For both depths, ANOVA revealed that substrate N content was significantly affected only by fertilizer type (**Table 2**). At 0–25 cm depth, substrate N content fertilizer mean (n = 10) in BD + NPK (2.6 g kg^{-1}) was 13% higher than that in NPK (2.3 g kg^{-1}), and BD mean (2.4 g kg^{-1}) was indistinct from the others (**Table 5**). Similarly, at 25–55 cm depth, substrate N content fertilizer mean (n = 10) decreased as BD + NPK = BD > NPK (**Table 5**).

Means of substrate P content at 0–25 cm depth varied from 790.7 (BD L/L) to 841.8 mg kg^{-1} (NPK C/L), while at 25–55 cm depth the range was 778.2 (BD L/L) and 840.9 mg kg^{-1} (BD C/L) (**Table 5**). For both depths, ANOVA revealed that substrate P content was affected neither by the interaction between compaction condition and fertilizer type nor by these two main factors (**Table 2**).

Means of substrate K content at 0–25 cm depth ranged from 13.0 (BD C/L) to 15.0 g kg^{-1} (BD and BD + NPK L/L), and at 25–55 cm depth it varied from 14.0 (BD C/L) to 15.4 g kg^{-1} (BD + NPK L/L) (**Table 5**). For both depths, ANOVA revealed that substrate K content was significantly affected by fertilizer type (**Table 2**). Yet, at 0–25 cm depth, compaction condition also significantly affected substrate K content (**Table 2**). Contrasting patterns of fertilizer mean (n = 10) were observed among substrate depths. At 0–25 cm depth, substrate K content fertilizer mean (n = 10) in BD + NPK was 10% higher than that in NPK, and BD (14.0 g kg^{-1}) did not differ from the other two treatments (**Table 5**). Still at this depth, K content compaction condition mean (n = 15) in L/L was 7% higher in comparison to C/L (**Table 5**). At 25–55 cm depth, substrate K content fertilizer mean (n = 10) in BD + NPK and NPK was slightly higher (approx. 5%) than in BD (14.1 g kg^{-1}), respectively (**Table 5**).

3.5 Root system development

Means of visible main roots length, total lateral root length, root system width, and rooting depth over the experimental period, as well as statistical significance between treatments for these parameters at harvest (18 DAG), are shown in **Figure 4**. ANOVA revealed that visible main root length and rooting depth were significantly affected by the interaction between fertilizer type and compaction condition, while visible total lateral root length was significantly affected only by fertilizer type, and visible root system width was affected neither by the interaction between compaction condition and fertilizer type nor by the two main factors (**Table 2**).

Means of visible main roots length under C/L condition for BD, BD + NPK and NPK were 108.5, 104.3, and 84.2 cm, respectively, and did not differ from each other (**Figure 4a**). Differently, within L/L condition, the visible main roots length mean in NPK (107.6 cm) was significantly higher (49%) than in BD (72.2 cm), whereas BD + NPK mean (93.1 cm) did not differ from the others (**Figure 4b**). Regarding visible total lateral root length, the mean values (n = 10) of NPK (198.1 cm) and BD (187.0 cm) did not differ from one another and were 60% and 51% higher in comparison with BD + NPK (124.2 cm), respectively (see **Figure 4d** and its statistical footnote). As a general trend for visible main root length and total lateral root length of contrasting fertilizer treatments (BD versus NPK), it was verified that BD application resulted in a significant reduction of main root length (in L/L) (**Figure 4b**) and a similar total lateral root length in relation to NPK (**Figure 4d**).

Treatments did not affect the width of maize root system either under C/L (**Figure 5a**) or under L/L (**Figure 5b**). Maize visible rooting depth under C/L condition reached depths of 50.1, 51.3, and 54.2 cm in NPK, BD + NPK and BD treatments, respectively, and means were not statistically different (**Figure 5c**). Whereas, within

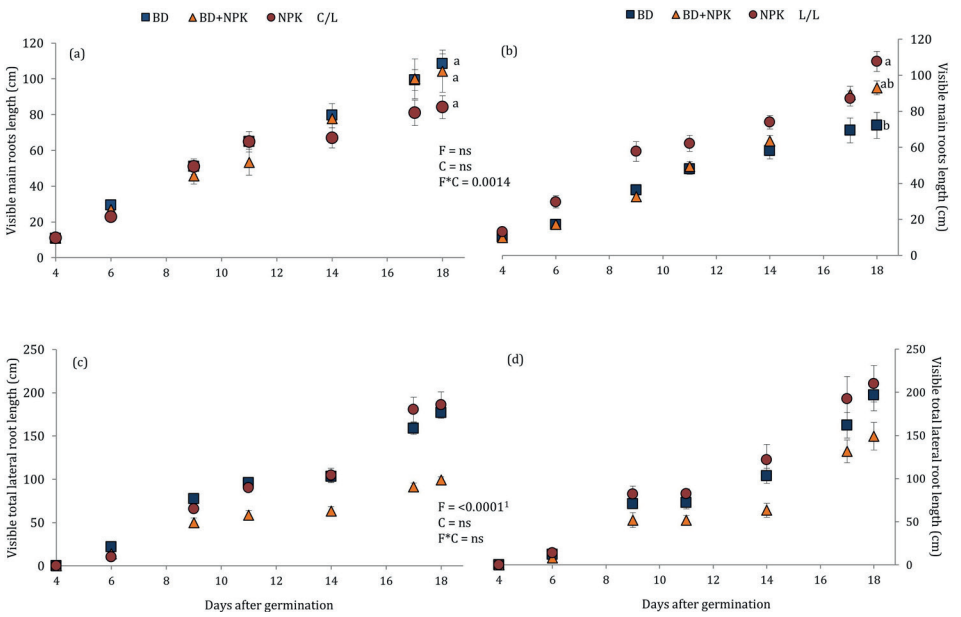

Figure 4.
*Effect of fertilizer type (Biogas Digestate - BD, Biogas Digestate+mineral NPK fertilizer - BD + NPK, mineral NPK fertilizer - NPK) and compaction condition (layered compacted substrate - C/L, loose substrate - L/L) on maize visible main roots length (a, b) and total lateral root length (c, d) over days after germination. Data are mean (n = 5) and error bars are standard error. Means with the same letter within compaction condition (C/L, L/L) at 18 days after germination are not statistically different according to Tukey's test at p < 0.05. F = effect of fertilizer; C = effect of compaction; F*C = interaction between fertilizer and compaction; ns = not significant.*
[1]The effect of fertilizer type (n = 10) was statistically significant (p < 0.0001) on visible total lateral root length (cm) as it follows: NPK (198.1 a), BD (187.0 a), BD + NPK (124.2 b).

L/L condition, roots grew 23% deeper in NPK (54 cm) than in BD + NPK (44 cm), and that in BD (48.1 cm) did not differ from the other two treatments (**Figure 5d**). Together these results indicate that juvenile maize root system exploration in depth and width promoted by BD was equivalent to that promoted by NPK, irrespective of compaction condition.

3.6 Rhizosphere and bulk substrate pH over days after germination

Three of the five replicates of each treatment contained planar pH optodes sensors positioned at 15 and at 35 cm depth. From the total of 36 planar pH optodes sensors (18 at 15 cm and 18 at 35 cm depth) placed within the 18 rhizoboxes, maize roots crossed 12 and six of the sensors placed at 15 and 35 cm depth, respectively. The contact among roots and the planar pH optodes sensors can be reduced due to root curvature, especially for optodes placed in deeper depths [31]. In general, root tips reached the sensors at 15 cm depth at 4 DAG and the sensors at 35 cm depth at 8 DAG, except for BD + NPK L/L, where root tips reached the lower sensor at 11 DAG. Roots crossed the entire surface of the sensors within 24 h.

Bulk substrate pH over the experimental period is shown in **Table 6**. Considering that the pH of the arable field soil and of the peat substrate used to prepare the maize growing medium were 6.3 and 6.0, respectively, as a general trend, an acidification of the substrate was observed after the application of the treatments. Within C/L condition, bulk substrate pH values at 15 cm depth for BD, BD + NPK and NPK

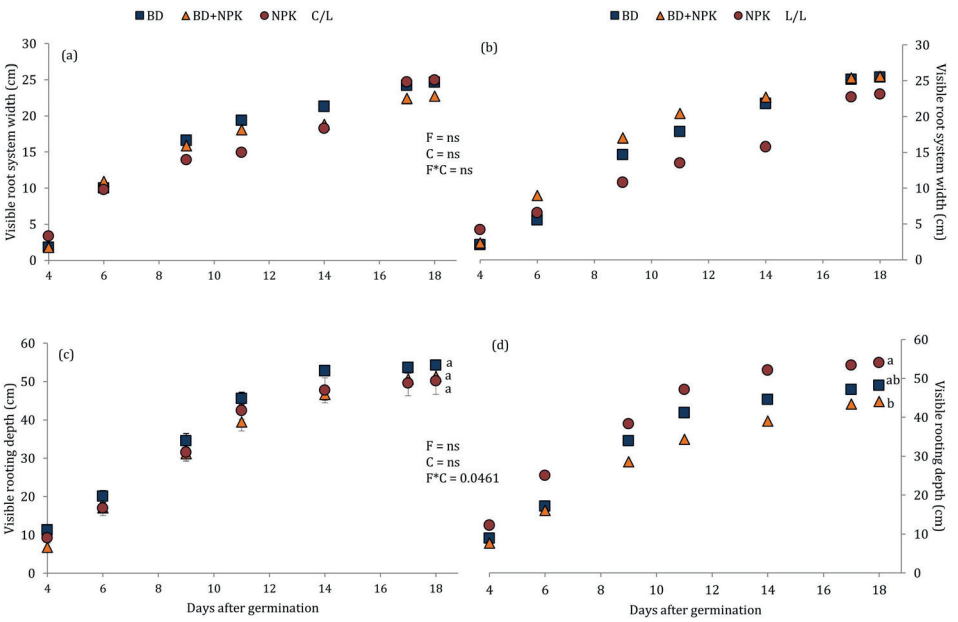

Figure 5.
*Effect of fertilizer type (Biogas Digestate - BD, Biogas Digestate+mineral NPK fertilizer - BD + NPK, mineral NPK fertilizer - NPK) and compaction condition (layered compacted substrate - C/L, loose substrate - L/L) on maize visible root system width (a, b) and visible rooting depth (c, d) over days after germination. Data are mean (n = 5) and error bars are standard error. Means with the same letter within compaction condition (C/L, L/L) at 18 days after germination are not statistically different according to Tukey's test at p < 0.05. F = effect of fertilizer; C = effect of compaction; F*C = interaction between fertilizer and compaction; ns = not significant.*

ranged from 4.3 to 5.2, 4.4 to 5.1, and 4.6 to 5.3, respectively, between 2 and 18 DAG (**Table 6**). At 35 cm depth, bulk substrate pH values ranged from 4.9 to 5.2, 4.6 to 5.2, and 4.8 to 5.4 for BD, BD + NPK and NPK, respectively (**Table 6**). Within L/L condition, bulk substrate pH values at 15 cm depth for BD, BD + NPK and NPK varied as follows: 4.6–5.2, 4.5–5.1, and 4.8–5.3, respectively (**Table 6**). Yet, in L/L condition, bulk substrate pH value ranges at 35 cm depth for BD, BD + NPK and NPK were 4.6–5.1, 4.9–5.3, and 4.9–5.3, respectively (**Table 6**). Differences between bulk substrate pH at 2 and 18 DAG within the same treatment were at maximum 0.4 pH units, which was observed for BD + NPK C/L at 15 cm (decrease of pH from 5.1 to 4.7) and at 35 cm (decrease of pH from 5.2 to 4.8) (**Table 6**).

Rhizosphere pH variation in relation to the bulk substrate over the experimental period is shown in **Figure 6**. Regarding the rhizosphere pH variation promoted by maize roots in relation to the bulk substrate pH, as a general trend, an alkalinization of rhizosphere pH was observed in all treatments at both 15 and 35 cm depth, except for BD + NPK 15 C/L and for BD 15 L/L, where an acidification of the rhizosphere pH up to −0.35 units was detected (**Figure 6**).

Under C/L condition, maximum rhizosphere pH alkalinization (+0.57 units) promoted by maize roots occurred in BD 15 at 8 DAG (**Figure 6a**). Similar alkalinization magnitude (+0.53 units) was also observed for BD 35 at 10 DAG (**Figure 6a**). Alkalinization promoted by NPK and BD + NPK application was in general less pronounced. The rhizosphere alkalinization was maintained up to maize harvest (18 DAG) with magnitudes of +0.43, +0.38, +0.23, and +0.16 pH units for BD 35, BD 15, NPK 15, and BD + NPK 35, respectively (**Figure 6a**). As previously mentioned,

DAG	BD 15	BD 35	BD + NPK 15	BD + NPK 35	NPK 15	NPK 35
			C/L			
2	5.0(0.0)	5.2(0.0)	5.1(0.1)	5.2(0.1)	4.7(0.1)	5.0(0.0)
4	4.3(0.1)	–[1]	4.7(0.2)	—	4.6(0.1)	—
5	4.7(0.0)	—	4.8(0.1)	—	4.9(0.1)	—
6	5.0(0.0)	—	5.0(0.1)	—	5.2(0.1)	—
8	5.0(0.1)	4.9(0.1)	4.8(0.2)	4.6(0.0)	5.1(0.1)	5.0(0.0)
9	5.2(0.0)	5.3(0.1)	5.0(0.2)	5.0(0.0)	5.2(0.1)	5.4(0.0)
10	4.6(0.0)	5.0(0.1)	4.4(0.2)	4.8(0.1)	4.8(0.1)	5.0(0.0)
11	4.9(0.1)	4.9(0.1)	4.7(0.1)	4.6(0.0)	5.0(0.1)	4.8(0.0)
12	5.2(0.0)	5.1(0.1)	5.0(0.2)	4.7(0.0)	5.3(0.1)	5.0(0.0)
13	4.9(0.0)	5.2(0.1)	4.8(0.1)	4.9(0.0)	5.0(0.1)	5.2(0.0)
17	5.1(0.0)	5.2(0.1)	5.0(0.1)	4.8(0.0)	5.3(0.1)	5.1(0.0)
18	5.0(0.1)	5.2(0.1)	4.7(0.2)	4.8(0.0)	4.9(0.1)	5.0(0.0)
			L/L			
2	5.1(0.1)	4.9(0.1)	5.0(0.1)	5.3(0.1)	4.8(0.1)	4.9(0.1)
4	4.7(0.1)	—	4.7(0.0)	—	4.7(0.0)	—
5	4.8(0.1)	—	4.9(0.0)	—	4.9(0.0)	—
6	5.0(0.1)	—	5.1(0.0)	—	5.2(0.0)	—
8	4.9(0.0)	4.7(0.1)	4.8(0.2)	5.0(0.1)	5.1(0.0)	5.0(0.1)
9	5.1(0.0)	5.1(0.0)	5.0(0.2)	5.3(0.1)	5.3(0.0)	5.3(0.0)
10	4.6(0.0)	4.8(0.0)	4.5(0.1)	4.9(0.1)	4.9(0.0)	5.0(0.1)
11	4.9(0.0)	4.6(0.1)	4.9(0.1)	4.9(0.1)	5.0(0.0)	4.9(0.1)
12	5.1(0.0)	4.9(0.0)	5.1(0.1)	5.1(0.1)	5.2(0.0)	5.1(0.0)
13	5.0(0.1)	5.0(0.1)	4.7(0.2)	5.2(0.0)	5.0(0.0)	5.2(0.0)
17	5.2(0.1)	5.0(0.1)	5.0(0.2)	5.1(0.1)	5.2(0.0)	5.1(0.1)
18	4.9(0.0)	4.9(0.0)	4.9(0.1)	5.1(0.0)	4.8(0.0)	5.0(0.1)

[1]*not determined.*

Table 6.
Bulk substrate pH over days after germination (DAG) of maize plants measured by optodes positioned at 15 and 35 cm depth in response to fertilizer type (Biogas Digestate - BD, Biogas Digestate+mineral NPK fertilizer - BD + NPK, mineral NPK fertilizer - NPK) and compaction condition (layered compacted substrate - C/L, loose substrate - L/L). Data are mean followed by standard error within parentheses.

within C/L condition rhizosphere pH acidification induced by roots occurred only in BD + NPK 15, where changes in rhizosphere pH oscillated between −0.35 (5 DAG) and +0.15 units (8 DAG) over the experimental period (**Figure 6a**).

Rhizosphere alkalization promoted by maize roots among fertilizers under L/L was remarkably distinct from that observed under C/L. Under L/L, the greatest rhizosphere alkalinization was observed in BD + NPK 15 at 9 DAG (+0.65) (**Figure 6b**). Such alkalinization magnitude was followed by NPK 15 at 5 DAG

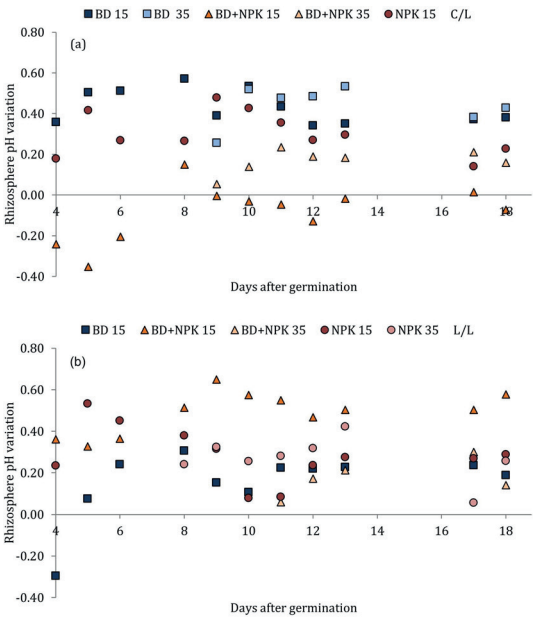

Figure 6.
Effect of fertilizer type (Biogas Digestate - BD, Biogas Digestate+mineral NPK fertilizer - BD + NPK, mineral NPK fertilizer - NPK) within layered compacted substrate (C/L) (a) and loose substrate (L/L) (b) condition on rhizosphere pH variation in relation to the bulk substrate pH. Rhizosphere and bulk substrate pH were measured by planar pH optodes positioned at 15 and 35 cm depth. Data are mean from different number of replicates, as it follows: under C/L - BD 15 (n = 3), BD 35 (n = 1), BD + NPK 15 (n = 1), BD + NPK 35 (n = 2), NPK 15 (n = 1), NPK 35 (n = 0); and, under L/L – BD 15 (n = 3), BD 35 (n = 0), BD + NPK 15 (n = 2), BD + NPK 35 (n = 1), NPK 15 (n = 2), NPK 35 (n = 2).

(+0.53) (**Figure 6b**), while a less pronounced alkalinization up to +0.31 units was observed in BD 15 at 8 DAG (**Figure 6b**). Rhizosphere alkalinization was predominant over acidification until maize harvest in all treatments and depths of pH measurement, when alkalinization magnitudes of +0.58, +0.29, +0.26, +0.19, and +0.14 units were observed in BD + NPK 15, NPK 15, NPK 35, BD 15 and BD + NPK 35, respectively (**Figure 6b**). In contrast to C/L condition, acidification of rhizosphere pH among treatments under L/L occurred only in BD 15 at 4 DAG (−0.29 units) (**Figure 6b**).

4. Discussion

4.1 Maize performance

Regarding substrate compaction condition, the low density of the peat substrate (0.21 g cm^{-3}) added to the field soil enabled the preparation of the loose layer in both C/L and L/L conditions. Maize roots subjected to C/L condition faced penetration resistance of 1.0 MPa and a dry bulk density of 0.84 g cm^{-3} (0–25 cm depth), which were 25- and 1.4-fold higher compared to L/L, respectively (0.04 MPa and 0.62 g cm^{-3}). It should be mentioned that substrate or soil bulk density and mechanical resistance may not have a linear relationship, which is in line with values

obtained in rhizoboxes filled with loose and compacted substrate by Pfeifer et al. [11]. Yet, significant positive correlation between visible root length obtained with GROWSCREEN-Root and total root length of maize determined destructively, both under two levels of soil compaction (0.07 and 0.16 MPa) in a 4-week rhizobox experiment, has previously been reported by Nagel et al. [18]. These findings indicate that compaction does not affect the proportion of the roots visible at the transparent face of the rhizobox, at least for early maize root growth. Therefore, in our study we assume that compaction did not affect the proportion of maize roots visible at the transparent face of the rhizobox, and thus differences in root architecture are attributed to treatment effects.

Interestingly, significant changes in root architecture of juvenile maize reported by Nagel et al. [18] were observed in plants subjected to an even narrower range of substrate compaction (0.07 and 0.16 MPa) compared to that of our study (0.04 and 1.0 MPa). Our findings showed a reduction of the total root biomass of juvenile maize plants receiving BD compared to NPK and BD + NPK fertilization under C/L condition (**Figure 3**). Plant root:shoot ratio is usually sensitive to nutrient deficiency, water stress, high plant density, shading, and soil compaction [55–57]. As reported by Correa et al. [2], maize root:shoot ratio at 42 days after planting decreased significantly when substrate density was increased by 12%. In our study, maize root:shoot ratio was not affected by substrate compaction condition, but root:shoot ratio mean of fertilizer types (n = 10) differed statistically (NPK > BD) (**Table 3**). According to Bhattacharya [58], adequate P supply is known to enhance overall crop development and to promote early root formation and proliferation. Thus, the greater P supply via NPK fertilizer in comparison with BD (**Table 1**) may explain the higher root:shoot ratio (regardless of compaction condition, n = 10) (**Table 3**), shoot P content (regardless of compaction condition, n = 10) (**Table 4**), and total root dry weight (C/L) of maize plants receiving NPK instead of BD fertilization (**Figure 3**). These findings may be preferentially assigned to P instead of N plant nutrition since N content in maize shoots and roots promoted by BD and NPK fertilization did not differ from each other (**Table 4**).

Concerning root system architecture, Thaler and Pagès [55] reported that primary roots may be more sensitive than laterals to an increase in soil compaction. Yet, Bingham and Bengough [10] reported a decrease of 65% and 47% of barley main axis length and lateral root length, respectively, with an increase in soil compaction (from 0.3 to 1 MPa). In our study, under L/L condition (0.04 MPa), plants fertilized with BD + NPK and BD were able to develop roots deeper compared to NPK at harvest (**Figure 5d**), while higher visible main root length was obtained in NPK treatment (**Figure 4b**). The visible main roots length between treatments at 4 DAG, ranging from 10.0 to 13.1 cm (**Figure 4a** and **b**), indicates that maize germination and initial rooting were not negatively affected by BD fertilization (BD and BD + NPK) compared to NPK. This is in line with a maize germination rhizobox experiment that included two field soils mixed with peat and application of either maize silage-derived digestate or mineral NPK as fertilizer [49]. In relation to visible total lateral root length, in our study it was first determined at 6 DAG, and at harvest it was only affected by fertilizer type, where BD fertilization effect was comparable to that of NPK, regardless of compaction condition (see **Figure 4d** and its statistical footnote). The formation of lateral roots enables plants to adjust to heterogeneous situations in the soil, especially under favorable conditions of water or nutrient availability [11]. Thus, we may assume that the substrate fertility condition promoted by BD and NPK affected juvenile maize lateral root length in similar magnitude.

In general, N, P, and K content of maize shoots (**Table 4**) of all treatments indicated that plants were sufficiently supplied with these macronutrients [59]. Despite the sufficient nutrition status of maize shoots in response to the different fertilizer types and substrate compaction conditions, maize plants responded sensitively to the different treatments regarding nutrient content in shoots (P) and roots (N, P, and K). Sufficient N supply to maize plants via BD could be expected since the ordinary BD application doses of 40 Mg ha^{-1} contained an N amount equivalent to that provided by NPK (**Table 1**), which in turn was equivalent to an ordinary NPK fertilization for maize [49]. Similarly, sufficient P and K maize nutrition via BD was expected because the BD used in this experiment presented high P and K content (**Table 1**). This is attributed to the co-digestion of maize silage with chicken manure, once both residues are rich in K, and because chicken manure is particularly expected to also add significant amounts of P to the BD due to animal excretions [33]. In fact, the maize silage+chicken manure-derived BD used in our study contained similar N but higher P and K content compared to another BD tested by Robles-Aguilar et al. [49] for maize fertilization, where the BD used by the authors was produced under similar conditions compared to our study but had exclusively maize silage as feedstock. Still with respect to sufficient N supply to maize via BD fertilization, two aspects should be mentioned. First, BD contains great amounts of readily available N forms (mainly NH_4^+). Second, the BD tested in our study presented a low C:N ratio of 6 that may have facilitated its fast degradation by the microbiota, consequently leading to a flush of available N in the growing medium, as previously reported by Andruschkewitsch et al. [40]. In this sense, we consider that BD mineralization, BD-N-released forms, and their transformation in soils should be addressed in further studies to enhance our understanding regarding nutrient cycling and timing between BD fertilization and crop sowing in BD-amended soils.

The SPAD readings were similar across all treatments (**Table 3**), indicating similar N leaf status of maize plants [60], and corroborating the similar shoot N content verified for the different treatments (**Table 4**). The SPAD readings observed in our study suggest that there was no N starvation during maize juvenile development (18 DAG) [61]. Furthermore, the SPAD values reported in our study were higher than that reported by Robles-Aguilar et al. [49] for maize plants (from 25 to 45 DAG) fertilized with 40 Mg ha^{-1} of pure maize silage-derived digestate. The dose of N application via BD (212 kg N ha^{-1}) along with the fact that N in the BD composition is predominantly in the form of NH_4^+, may explain the sufficient N nutrition of maize in our study. According to Lukehurst et al. [33], during anaerobic digestion part of the N in organic form is converted to NH_4^+, and this is the main component readily available to plants after fertilization with BD. In fact, substrate N content at 25–55 cm depth (regardless of compaction condition, n = 10) was higher in BD than in NPK (**Table 5**). Linking such results with the fact that maize shoot and root N content were not statistically different between BD and NPK, and that N amendments were similar between fertilizer type (**Table 1**), it may be suggested that N losses from the total substrate profile (0–55 cm) were possibly attenuated by the presence of BD. This hypothetical prevention of N leaching by BD may be tested in future studies.

Shoot K content of maize plants fertilized with BD + NPK was equivalent to that fertilized with NPK (regardless of compaction condition, n = 10), as well as root K content under L/L (**Table 4**). Here it is important to highlight that although the amount of K added by BD to the substrate was 154% higher than that of NPK fertilizer, BD did not cause an excess of K content in shoot tissue in relation to NPK. Possibly, part of the K applied via BD may have been leached during the period

between the experiment's beginning and soil sampling at maize harvest. In our study, nutrient losses via leaching were not determined. In this sense, we consider leachate evaluation to be addressed in further studies to enhance our knowledge on closing the nutrient cycles in BD-amended soils. Our findings show the importance of supporting and planning the use of BD derived from maize silage+chicken manure in cropping systems. This type of BD, besides sufficiently supplying maize seedlings with N, P, and K, as verified in our study, may contribute to, at least partially, covering maize's increasing K demands in the next development stages, especially if K leaching can be in parallel attenuated.

Interestingly, under C/L condition, root K content was higher in plants fertilized with NPK than with BD and BD + NPK, following an inverse order of amount of K amendment via fertilization (BD > BD + NPK > NPK) (**Table 1**). At 0–25 cm depth, substrate K content was lower under C/L than under L/L condition (regardless of fertilizer type, n = 15). Thus, the top compacted layer in C/L condition may have partially and temporarily reduced the downward movement of K, and subsequently, it may have enabled greater K uptake by plants in the main rooting zone (0–25 cm depth), where its uptake is facilitated due to the greater concentration of roots compared to 25–55 cm depth (**Figure 3**). This is reinforced by the fact that NPK fertilization resulted in lower substrate K content at 0–25 cm depth (regardless of compaction condition, n = 10) and in higher root K content in comparison to BD and BD + NPK. In addition, the effect of compaction on preventing K leaching presumably had particular importance in the substrate with expected lower cation exchange capacity (NPK). In this view, BD fertilization is expected to increase substrate C content cation exchange capacity due to the addition of organic functional groups commonly present in organic residues. Soil cation exchange capacity increase in response to BD application was reported by Glowaca et al. [62]. In our study, increased substrate cation exchange capacity due to BD application may have occurred preferentially at 25–55 cm depth, where substrate C content was higher in BD fertilization compared to NPK (**Table 5**). Yet, the greater substrate C content in BD compared to NPK observed only at 25–55 cm depth may be partially explained by the possible leaching of soluble organic compounds commonly present in digestates [33, 41].

Under L/L condition, a top compacted layer that may partially reduce K leaching is absent, so K leaching is expected to increase (BD < BD + NPK < NPK) as lower is the assumed capacity of the substrate to adsorb it (NPK < BD + NPK < BD). This hypothesis may be confirmed in complementary studies, but this interpretation is corroborated by the observation that root K content under L/L was higher in BD than in NPK, whereas BD + NPK did not differ from the other treatments (**Table 4**). In this view, root K content was greater as higher was the presumed capacity of the substrate to retain cations, and it also seemingly followed the amount of K application via fertilizers (**Table 1**). Also, at 25–55 cm depth, where levels of substrate compaction were similar among C/L and L/L condition (dry bulk density of 0.64 and 0.61 g cm^{-3}, respectively), greater substrate K content was obtained in NPK and BD + NPK treatments compared to BD (**Table 5**). These findings may indicate greater K leaching in treatments receiving NPK (and less in BD). Accordingly, Pfeifer et al. [11] reported greater downward movement of K in loose than in compacted substrate in rhizoboxes receiving similar fertilization and watering. The authors attributed the lower K leaching in the compacted substrate to the physical impedance for downward water flow. The levels of substrate compaction (0.04–1.23 MPa) applied by Pfeifer et al. [11] were comparable to that of our study (0.04–1.00 MPa), giving support to our interpretation. The significant effect of interaction between fertilizer type and compaction

condition on root K content (**Table 2**) is of particular interest once field soils may be subjected to some compaction level during the crop season.

Several studies have reported the benefits of digestates for plant growth and nutrition [36, 37, 39, 63]. However, when digestates do not comply with crop demand for N, P, and K fertilization, then mineral fertilizer supplementation is required. In this case, special attention has to be given to the nutrient balance of fertilizers. Taking into account the N:P:K ratios of BD (1.00:0.26:1.28) and NPK (1.00:0.33:0.53), it seems that the BD + NPK mixture resulted in an intermediate N:P:K ratio (1.00:0.30:0.92) for both P and K and led to plant performance similar to NPK. Regarding P supply, despite the lower P content in the shoots and roots of juvenile maize plants fertilized with BD compared to those fertilized with NPK (regardless of compaction condition, n = 10), it did not affect maize shoot dry weight (**Figure 3**), number of leaves, SLA, and SPAD readings (**Table 3**). However, the lower P content in maize plants fertilized with BD may explain its lower root:shoot ratio in comparison with NPK (**Table 3**). While, BD + NPK promoted similar maize shoot P content and root:shoot ratio to that obtained with NPK, without negative implications to overall shoot parameters. Yet, root system development in terms of visible length of main roots and total lateral roots, as well as root system width and rooting depth of maize plants fertilized with BD + NPK was comparable to that of NPK. These findings reinforce the potential of BD fertilization, possibly in combination with NPK, to reduce environmental liabilities associated with mineral fertilizer utilization and improper BD disposal in the environment, without compromising initial maize development. These findings may be corroborated by studies covering the complete maize growing cycle.

4.2 Rhizosphere and bulk substrate pH over days after germination

The acidic pH of the substrate after the application of the fertilizers (BD, BD + NPK and NPK) (**Table 6**) in relation to the pH of the field soil (6.3), peat (6.0) and BD (8.2), is probably associated with a combination of factors, likely: (i) fast ammonium nitrification ($NH_4^+ + 2O_2\ NO_3^- + H_2O + 2H^+$), since about 60, 61 and 63% of the total N of BD, BD + NPK and NPK was in the form of NH_4^+, respectively, and drainage holes at the bottom of the rhizoboxes allowed oxygenation of substrate [64, 65]; (ii) reaction between Mg^{2+}, NH_4^+ and PO_4^{3-} ions resulting in a release of H^+ ions to the solution [65]; (iii) partial and temporal occupation of substrate cation exchange sites by NH_4^+ instead of by basic cations as Ca^{2+}, Mg^{2+} and K^+; and, (iv) leaching of basic cations as Ca^{2+}, Mg^{2+}, Na^+ and K^+, especially due to the low cation exchange capacity of the substrates (mainly the one without BD application) and its replacement with H^+. The H^+ in this case is also expectedly derived from the fast mineralization of the organic materials in the substrate, e.g., soil organic matter from the field soil used and BD, due to their low C:N ratio, 11 and 6, respectively.

Organic matter mineralization is intensified under these circumstances due to the fast and high input of available N in the substrates (derived from BD and NPK) and substrate wetting, stimulating microbial activity, and because BD usually contains easily biodegradable organic compounds [66]. Here, it is important to mention that about 4 to 5 days elapsed between the substrate's preparation with fertilizers and the first bulk substrate pH measurements with planar optodes (2 DAG). During this time, the installation of the planar pH optode sensors into rhizoboxes was performed, the 30 rhizoboxes were prepared with the substrates and levels of compaction, and maize was sown. This has been shown to be sufficient time to trigger a significant part of the substrate acidification mechanisms aforementioned [40, 49, 65–68], also

due to substrate watering performed to stimulate seed germination. Rhizosphere pH measurements started at 4 DAG, when the first maize roots reached the planar optode sensors positioned at 15 cm depth.

The substrate acidification and NH_4^+ nitrification seem to have strongly driven the rhizosphere pH changes induced by maize roots observed in our study. Both factors settled the acidic bulk substrate pH in which maize roots were growing (**Table 6**), and due to nitrification, NO_3^- possibly became the main N source to plants over DAG instead of NH_4^+. Additionally, NO_3^- was also directly added to the substrates via both NPK and BD fertilizers (see Section 2.1). This may explain the overall trend of rhizosphere pH alkalinization induced by maize roots at both 15 and 55 cm depth during the experimental period. Slight and exceptional rhizosphere acidification was observed in a few measurements (**Figure 6**). According to Custos et al. [24], in aerobic soils, NO_3^- was the most absorbed nutrient (compared to K, NH_4^+, Ca, Mg, SO_4^{2-}, and P ions) by maize plants within the 1 and 4 weeks of growth, resulting in a strong depletion of anions (especially NO_3^-) and ultimately in a positive charge balance and alkalinization of rhizosphere pH. The authors estimated that absorption of NO_3^- was 837 and 830% greater than that of NH_4^+ within the 1 and 4 weeks of maize growth, respectively. The authors also stated that rhizosphere alkalinization by maize roots was associated with NO_3^- depletion in the rhizosphere and that NO_3^- uptake was equilibrated by H^+ absorption by maize roots with equivalent excretion of OH^-. Conversely, in the case of NH_4^+ uptake, plants tend to maintain the electro-neutrality across the root membrane by releasing H^+, which would consequently lead to rhizosphere acidification [22, 69]. In this way, plant N uptake as NH_4^+ or as NO_3^- usually results in rhizosphere acidification or alkalinization, respectively [29, 70–74], and such plant N uptake mechanisms have been reported for maize and other species such as ryegrass, rice, alpine pennycress, and wheat [24, 31, 52, 75, 76].

Faget et al. [77] found similar temporal and magnitude of rhizosphere alkalinization induced by maize roots (measured with planar pH optodes) compared to our study. The authors observed maximum alkalinization of up to 0.62 pH units in relation to the substrate at 8 days after maize transplantation to rhizoboxes. The authors attributed their results to the fact that NO_3^- was the only source of N for maize plants. Similarly to our study, the authors found that alkalinization persisted when coming closer to harvest (14 days after transplanting), but in a lower magnitude compared to our data.

Our data showed that when maize plants were supplied with less P (without P deprivation) and more N, as in the case of BD compared to BD + NPK and NPK fertilizers, roots still tended to alkalinize the rhizosphere (**Figure 6**). Li et al. [78] observed that under comparable sufficient N and deficient P supply, maize roots alkalinized the rhizosphere, while faba bean acidified it. According to the authors, faba bean roots mobilized P by releasing organic acids and protons to the rhizosphere, which also favored P uptake by maize when both species were intercropped. These findings reinforce that mobilization of P by maize roots is unlikely to occur. Therefore, the type of BD used in this experiment should be ideally complemented with high-soluble P sources, such as other organic materials or mineral P, in order to avoid possible P deficiency in maize plants. Also, maize could be intercropped with legume plants able to mobilize P. In this view, rhizosphere alkalinization promoted by maize roots under monoculture and intercropping was reported by Zhou et al. [79]. Furthermore, these authors observed that alkalinization by maize roots was higher under low P supply, very similarly to what was observed at 15 cm depth for plants of BD treatment in our study (**Figure 6**). At 35 cm depth, such observation was not as clear as it was for

the upper depth, but still the highest values of rhizosphere alkalinization at 35 cm depth were verified in a BD fertilization treatment (BD + NPK) and not in NPK alone (**Figure 6**), which supplied the highest P dose to maize (**Table 1**).

In this context, the general pattern of rhizosphere alkalinization observed for most treatments over the experimental period, as well as the different magnitude of such alkalinization between treatments, may be closely related to the dynamics of NH_4^+ nitrification, corroborating previous findings of rhizosphere pH change induced by maize roots [78, 79]. Overall, our rhizosphere pH investigation indicates that although the main form of N in the BD was NH_4^+, the N uptake by maize in BD treatments may have occurred predominantly as NO_3^-. The short time between BD amendment, nitrification, and NO_3^- uptake by maize should be taken into account when BD fertilization and maize sowing are planned in order to further close N cycle in BD-amended soils. Therefore, specific BD mineralization and N speciation studies should be performed, and microbiological parameters should be addressed to improve our understanding of the dynamics of N uptake by early growth of crops fertilized with BD.

In our study, the acid-growth mechanism, characterized by a local restricted and preferential cation uptake (mainly K^+ and NH_4^+) in maize root sections [31], was not evidenced by our data, although this was not the focus of our investigation.

5. Conclusion

Efficient maize fertilization with BD from the co-digestion of maize silage and chicken manure offers a great opportunity to improve nutrient cycling in bioenergy-crops production. Negative effects of BD on maize germination were not observed in this study. Our findings indicated that both NPK and BD fertilizers, alone or combined, supplied maize sufficiently with N, P, and K, and plants had similar specific leaf area and chlorophyll content. Nevertheless, plants fertilized solely with BD had lower shoot and root P content compared to NPK, irrespective of compaction condition. Yet, compaction led to lower shoot and root P contents compared to loose substrate. This may be associated with the fact that P in BD may not be readily available to plants as in NPK. In response to lower shoot and root P content, plants fertilized with BD had reduced root biomass (0–55 cm) (C/L) and main root length (L/L) in relation to NPK, and apparently plants fertilized with BD compensated for it by growing deeper under loose substrate in relation to NPK. The lower root:shoot ratio of plants receiving BD fertilization in relation to NPK was attributed to lower maize P uptake in BD.

Overall, maize roots induced rhizosphere alkalinization, regardless of treatment and substrate depth. This was attributed to preferential NO_3^- uptake, even though most of N in the fertilizers was initially in the form of NH_4^+. This was assigned to the possible fast nitrification of NH_4^+. Magnitude of rhizosphere alkalinization between treatments varied, and it was attributed to possible specific dynamics of nitrification in each treatment. To address such hypotheses, studies dedicated to investigating mineralization of BD and nitrification depending on fertilizer type are needed to help find time-wise BD amendment and crop sowing. Maize plants supplied with less P (not P deprivation) did not induce rhizosphere acidification to mobilize P, as reported in the literature for some legume species.

In general, we confirmed our hypothesis that maize shoot and root seedling parameters under mineral NPK fertilization alone or mixed with BD would be equivalent. Exceptions occurred only for root P content and visible rooting depth in

loose substrate, which were slightly higher in NPK fertilization used alone. Our findings suggest that maize fertilization with the type of BD tested in our study (maize silage+chicken manure) may be combined with fast-soluble P sources, or maize should be intercropped with P-mobilizing plant species to possibly deliver maize performance equivalent to regular NPK fertilization. These hypotheses may be tested in future studies, also possibly covering more maize development stages, aiming for further insights for optimization of BD as fertilizer.

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Conflict of interest

The authors declare no conflict of interest.

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