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Effect of Liquid Digestate on Agricultural Soil – II: Microbial Population Dynamics

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Abstract

The aim of this study was to investigate the effect of liquid digestate (produced from anaerobic digestion of organic municipal solid waste) on the population dynamics of selected soil microbial groups during cultivation of maize plants. Maize seeds where subjected to various concentrations (between 0% to 72%) of the digestate with a total of ten (10) runs. One set of experimental runs were treated with one-time application of the corresponding digestate concentration (OTDA). Another set of experimental runs were treated with two-time application of the corresponding digestate concentration (TTDA). The first application of the digestate (for OTDA and TTDA) was conducted two weeks after sowing while the second application (for TTDA alone) was conducted three weeks after the first application. During the growth of maize plants, the populations of soil aerobic bacteria (AEB), strict anaerobic bacteria (SAB), ammonia oxidizing bacteria (AOB), nitrate reducing bacteria (NRB) and fungi (FUN) where monitored using standard cultural methods. In OTDA and TTDA treatments, the population of soil AEB, SAB, AOB, NRB and FUN ranged from 3.0×10^6 CFU/g to 50.2×10^6 CFU/g and 3.1×10^6 CFU/g to 78.6×10^6 CFU/g; 1.9×10^4 CFU/g to 7.2×10^5 CFU/g and 2.2×10^4 CFU/g to 2.53×10^5 CFU/g; 0MPN/g to 4.3×10^4 MPN/g and 0MPN/g to 3.9×10^5 MPN/g; 0 MPN/g to 4.4×10^5 MPN/g and 0 MPN/g to 1.4×10^5 MPN/g and 1.3×10^3 CFU/g to 2.6×10^4 CFU/g and 1.2×10^3 CFU/g to 9.4×10^3 CFU/g respectively before and 70 days after applying the digestate. Bacteria species isolated appear to belong to genera such as *Arthrobacter, Azotobacter, Flavobacterium, Nocardia, Bacillus, Clostridium, Cellulomonas, Micrococcus* and *Pseudomonas*. Fungi species isolated appear to belong to genera such as *Aspergillus, Cladosporium, Fusarium, Geotrichum, Penicillium, Rhizopus* and *Trichoderma*. Digestate application appears to have increased the population of soil microbes with time during the cultivation of maize plants.

Keywords: Liquid digestate; Soil; Microbes

Introduction

During anaerobic digestion, bacteria consume part of the organic matter to produce biogas and a residue known as digestate [1]. Biogas production from anaerobic digestion of organic matter has increased in recent years and thus, the application of digestates to soil as biofertilizers has become more common. Digestate is a very complex material therefore its application to soil has effect on the wide range of physical, chemical and biological properties of the soil, depending on the soil types. Generally, the application of digestate to soil have been shown to affect soil properties such as pH, macro and micro element contents, organic matter content, microbiological activities and the quality and yield of crops [2-9]. The application of digestate to agricultural soils may strongly influence various microbial groups in the soil. For example, Sapp et al. compared the effect of digestate and chemical fertilizer on soil bacterial community and plant yield, Garcia-Sanchez et al. studied the changes in soil microbial community functionality and structure and plant yield in a metal-polluted site as a result of digestate and fly ash application, while Caracciolo et al. studied the changes in microbial diversity and plant growth in a degraded agricultural soil after the addition of two composts obtained from cattle manure or pig waste slurry anaerobic digestate [4,7,9].

Microbial immobilization of labile nitrogen (N) after the application of organic matter to soil has been shown to reduce nitrification and thus can decrease nitrate leaching [10,11]. Alburquerque et al. reported that highly biodegradable digestates led to the immobilization of nitrogen (N) and retarded nitrification whereas less biodegradable digestates caused rapid nitrification [12]. Therefore, the biodegradability of digestates needs to be evaluated in order to more clearly understand nitrification processes in soils [13]. The oxidation of ammonium to nitrite is the rate limiting step in nitrification and is catalyzed by ammonia monoxygenase (AMO), which is encoded by the amoA genes harboured by both ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) [14]. However, the relative importance of AOA and AOB to the nitrification process is still under debate [15,16]. Using real-time PCR, it is showed that the application of a digestate from wet fermentation only increased AOB and not AOA amoA gene copies however, the relationship between amoA gene copies and nitrification rates currently remains unclear.

In one study, the effect of applying wet digestate (pH 8.2, C/N ratio 4.5), dry digestate (pH 8.8, C/N ratio 23.4) and a chemical fertilizer to Japanese paddy and upland soils on short-term nitrification under laboratory aerobic conditions were compared [17]. All applications led to rapid increases in nitrate nitrogen (NO3-N) contents in both soils and ammonia-oxidizing bacteria but not archaea may have played a critical role in nitrification in the amended soils. The net nitrification rates for both soils were the highest after the application of dry digestates, followed by wet digestates and then the chemical fertilizers in order of decreasing soil pH. Their result suggested that the immediate effects of applying digestates, especially the dry digestate with the highest pH, on nitrate leaching need to be considered when digestates are used as

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alternative fertilizers [13]. In this study, the effect of liquid digestate on the population dynamics of selected soil microbial groups during cultivation of maize (*Zea mays*) was investigated.

Materials and Methods

Collection and characterization of digestate and agricultural soil

The liquid digestate used to cultivate the test plant (*Zea mays*) was obtained from a pilot-scale anaerobic digester (ADH) treating organic fraction of municipal solid waste. The loamy soil used to cultivate the test plant (*Zea mays*) was obtained from an agricultural field at the Nigerian Institute for Oil Palm research (NIFOR) in Edo State, Nigeria. After collection, samples of the digestate and soil were taken to the laboratory to determine parameters such as moisture content (MC), pH, total organic carbon (TOC), biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonia nitrogen (NH4-N), total nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), micro elements (such as Mn, Zn, Fe, Cu, Co and Ni) and heavy metals (such as Pb, Cd, Cr and Hg) using the Standard Methods [18].

Experimental set-up

During cultivate of *Zea mays* (TZESR-W; early maturing streak resistant white grain variety), the plant was subjected to various concentrations (ranging from 0% to 72%) of the digestate using One-Factor Response Design (Design Expert version 9.0) with a total of ten (10) runs in 10L-capacity plastic pots (Table 1). After completely loading the pots with the loamy soil, two seeds of the *Zea mays* were sown into the soil in each of the pots at a depth of 5 cm per hole. After sowing, one set of experimental runs were treated with one-time application of the corresponding digestate concentration. This was tagged “One-time Digestate Application (OTDA)”. However, the other set of experimental runs were treated in such a way that the total amount of the liquid digestate required for each pot was divided into two equal halves and applied with respect to time instead of applying all of it at once. This was tagged “Two-time Digestate Application (TTDA)”. The first application of the liquid digestate (for OTDA and TTDA) was conducted two weeks after sowing while the second application (for TTDA alone) was conducted three weeks after the first application (Table 1). The liquid digestate (as bio-fertilizer) was applied by spraying it into the soil in the pots.

Collection of soil samples

During the growth of maize plant in all set-ups, composite soil samples were collected with respect to time at depths between 0 cm and 20 cm from each of the set-ups into black polyethylene bags using sterilized cork-borer pushed vertically/horizontally into the soil in the pots. After collection, the polyethylene bags were sealed, labeled and immediately taken to the laboratory in order to estimate the population of soil microbes such as aerobic bacteria (AEB), strict anaerobic bacteria (SAB), ammonia oxidizing bacteria (AOB), nitrate reducing bacteria (NRB) and fungi (FUN) using the cultural techniques.

Determination of soil microbial populations

Aerobic Bacterial (AEB) populations were enumerated and isolated using the spread plate method as described [19]. Strict Anaerobic bacterial (SAB) populations were enumerated and isolated using the agar roll-tube technique described [20,21]. Ammonia Oxidizing Bacterial (AOB) populations were enumerated and isolated as described [22] Nitrate Reducing Bacteria (NRB) populations were enumerated and isolated as described [23]. Fungi (FUN) populations were enumerated and isolated using the soil dilution plate count and soil plate count methods as described [24]. Bacterial isolates were identified according to Bergey’s Manual of Determinative Bacteriology, using morphological and metabolic/biochemical tests [25,26]. Identification of the fungi that were isolated was based on the morphological characteristics of both colonial and microscopic examinations as described by [27-29], with help from additional literatures [24,30-33].

Result and Discussion

Properties of the digestate and the soil

The properties of the liquid digestate produced following anaerobic treatment of organic fraction of municipal solid waste (OFMSW) are presented in Table 2. The Table 2 also shows some properties of the loamy soil used to cultivate the test plant (*Zea mays*). It shows that elements (or compounds) such as carbon (as total organic carbon), total nitrogen, ammonium nitrogen, phosphorus, potassium, calcium, magnesium, manganese, zinc, copper, nickel, cobalt, iron, and molybdenum, which are known to be beneficial for plant growth and to soil microbes were higher in the digestate compared to the soil. Some heavy metals such as copper, zinc, lead, cadmium, mercury and chromium were also present in the digestate. However, their concentrations appear to be low. The Table 2 shows that the digestate contained both aerobic (most likely facultative anaerobic) bacteria and strict anaerobic bacteria [34]. The presence of macro and micro elements in most digestates make them excellent form of fertilizer when applied correctly [2-9,35].

Population dynamics of soil microbes

Aerobic bacteria (AEB): In the control set-up (treated with 0% digestate), the average population of soil aerobic bacteria (AEB) increased from 3.3×10^6 CFU/g to 8.1×10^6 CFU/g of soil after 70 days (Figure 1). In

Std	Run	Conc. of Digestate (%)	Mass of Liquid Digestate (Kg)	Mass of soil per pot (Kg)	One-time application (OTDA)	Two-time application (TTDA)	
						First	Second
5	1	0	0.00	10	0.00	0.00	0.00
1	2	8	0.80	10	0.80	0.40	0.40
6	3	16	1.60	10	1.60	0.80	0.80
8	4	24	2.40	10	2.40	1.20	1.20
4	5	32	3.20	10	3.20	1.60	1.60
9	6	40	4.00	10	4.00	2.00	2.00
2	7	48	4.80	10	4.80	2.40	2.40
10	8	56	5.60	10	5.60	2.80	2.80
3	9	64	6.40	10	6.40	3.20	3.20
7	10	72	7.20	10	7.20	3.60	3.60

Table 1: One Factor Response Design for analysis of fertilizer potential of digestate.

Parameters	Digestate	Loamy soil
Biochemical oxygen demand (mg/L)	54.88	ND
Chemical oxygen demand (mg/L)	843.92	ND
Moisture content (%)	ND	32.40
Total organic carbon (mg/L)	107.20	9.30
Total nitrogen (mg/L)	193.70	6.50
Ammonium nitrogen (mg/L)	135.30	1.10
C/N Ratio	0.55	1.43
Phosphorus (mg/L)	46.40	0.51
Potassium (mg/L)	11.42	0.62
Magnesium (mg/L)	2.40	0.13
Calcium (mg/L)	9.50	0.32
Manganese (mg/L)	1.10	0.61
Zinc (mg/L)	10.4	1.74
Copper (mg/L)	1.20	0.96
Nickel (mg/L)	1.52	0.07
Cobalt (mg/L)	0.42	-
Lead (mg/L)	0.63	0.02
Iron (mg/L)	1.25	0.34
Molybdenum (mg/L)	0.91	0.06
Cadmium (mg/L)	0.05	-
Chromium (mg/L)	0.06	-
Mercury m(g/L)	0.02	-
Aerobic Bacteria (CFU/ml)	3.6 x 10^3	8.6 x 10^6
Strict anaerobic Bacteria (CFU/ml)	5.60 x 10^6	4.1 x 10^3
ND=Not Determined		

Table 2: Characteristics of the liquid digestate and composite soil sample.

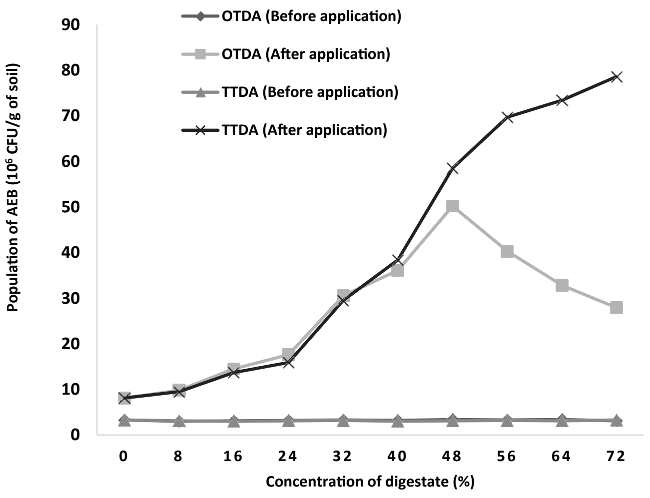

Figure 1: Population dynamics of aerobic bacteria (AEB) with respect to digestate concentration in OTDA and TTDA set-ups before and after 70 days of applying the digestate.

the set-ups treated with 8% digestate, the average population of soil AEB in OTDA and TTDA increased from 3.0 × 10^6 CFU/g and 3.1× 10^6 CFU/g to 9.8 × 10^6 CFU/g and 9.5 × 10^6 CFU/g respectively after 70 days (Figure 1). In the set-ups treated with 16% digestate, the average population of soil AEB in OTDA and TTDA increased from 3.1 × 10^6 CFU/g and 3.0 × 10^6 CFU/g to 14.5 × 10^6 CFU/g and 13.7 × 10^6 CFU/g respectively after 70 days (Figure 1). In the set-ups treated with 24% digestate, the average population of soil AEB in OTDA and TTDA increased from 3.2 × 10^6 CFU/g and 3.1 × 10^6 CFU/g to 17.6 × 10^6 CFU/g and 15.9 × 10^6 CFU/g respectively after 70 days (Figure 1). In the set-ups treated with 32% digestate, the average population of soil AEB in OTDA and TTDA increased from 3.3 × 10^6 CFU/g and 3.2 × 10^6 CFU/g to 30.5 × 10^6 CFU/g and 29.5 × 10^6 CFU/g respectively after 70 days (Figure 1). In the set-ups treated with 40% digestate, the average population of soil AEB in OTDA and TTDA increased from 3.2 × 10^6 CFU/g and 3.0 × 10^6 CFU/g to 36.1 × 10^6 CFU/g and 38.4 × 10^6 CFU/g respectively after 70 days (Figure 1). In the set-ups treated with 48% digestate, the average population of soil AEB in OTDA and TTDA increased from 3.4 × 10^6 CFU/g and 3.1 × 10^6 CFU/g to 50.2 × 10^6 CFU/g and 58.5 × 10^6 CFU/g respectively after 70 days (Figure 1). In the set-ups treated with 56% digestate, the average population of soil AEB in OTDA and TTDA increased from 3.3 × 10^6 CFU/g and 3.2 × 10^6 CFU/g to 40.3 × 10^6 CFU/g and 69.1 × 10^6 CFU/g respectively after 70 days (Figure 1). In the set-ups treated with 64% digestate, the average population of soil AEB in OTDA and TTDA increased from 3.4 × 10^6 CFU/g and 3.1 × 10^6 CFU/g to 32.8 × 10^6 CFU/g and 73.4 × 10^6 CFU/g respectively after 70 days (Figure 1). In the set-ups treated with 72% digestate, the average population of soil AEB in OTDA and TTDA increased from 3.1 × 10^6 CFU/g and 3.3×10^6 CFU/g to 27.9 × 10^6 CFU/g and 78.6 × 10^6 CFU/g respectively after 70 days (Figure 1).

Strict anaerobic bacteria (SAB): In the control set-up (treated with 0% digestate), the average population of soil SAB increased from 2.4 × 10^4 CFU/g to 4.5 × 10^4 CFU/g of soil after 70 days (Figure 2). In the set-ups treated with 8% digestate, the average population of soil SAB in OTDA and TTDA increased from 2.1 × 10^4 CFU/g and 2.2 × 10^4 CFU/g to 8.1 × 10^4 CFU/g and 5.3 × 10^4 CFU/g respectively after 70 days (Figure 2). In the set-ups treated with 16% digestate, the average population of soil SAB in OTDA and TTDA increased from 2.2 × 10^4 CFU/g and 2.4 × 10^4 CFU/g to 9.5 × 10^4 CFU/g and 6.5 × 10^4 CFU/g respectively after 70 days (Figure 2). In the set-ups treated with 24% digestate, the average population of soil SAB in OTDA and TTDA increased from 2.0 × 10^4 CFU/g and 2.2 × 10^4 CFU/g to 9.9 × 10^4 CFU/g and 8.2 × 10^4 CFU/g respectively after 70 days (Figure 2). In the set-ups treated with 32% digestate, the average population of soil SAB in OTDA and TTDA increased from 2.3 × 10^4 CFU/g and 2.1 × 10^4 CFU/g to 1.1 × 10^5 CFU/g and 9.1 × 10^4 CFU/g respectively after 70 days (Figure 2). In the set-ups treated with 40% digestate, the average population of soil SAB in OTDA and TTDA increased from 2.1 × 10^4 CFU/g and 2.3 × 10^4 CFU/g to 1.26 × 10^5 CFU/g and 9.5 × 10^4 CFU/g respectively after 70 days (Figure 2). In the set-ups treated with 48% digestate, the average population of soil SAB in OTDA and TTDA increased from 2.3 × 10^4 CFU/g and 2.3 × 10^4 CFU/g to 1.62 × 10^5 CFU/g and 1.15 ×

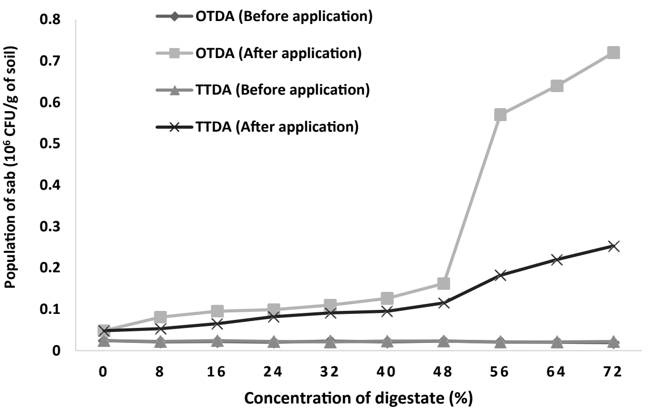

Figure 2: Population dynamics of strict anaerobic bacteria (SAB) with respect to digestate concentration in OTDA and TTDA set-ups before and after 70 days of applying the digestate.

10^5 CFU/g respectively after 70 days (Figure 2). In the set-ups treated with 56% digestate, the average population of soil SAB in OTDA and TTDA increased from 2.1×10^4 CFU/g and 2.0×10^4 CFU/g to 5.70×10^5 CFU/g and 1.82×10^5 CFU/g respectively after 70 days (Figure 2). In the set-ups treated with 64% digestate, the average population of soil SAB in OTDA and TTDA increased from 2.0×10^4 CFU/g and 2.1×10^4 CFU/g to 6.40×10^5 CFU/g and 2.20×10^5 CFU/g respectively after 70 days (Figure 2). In the set-ups treated with 72% digestate, the average population of soil SAB in OTDA and TTDA increased from 1.9×10^4 CFU/g and 2.2×10^4 CFU/g to 7.2×10^5 CFU/g and 2.53×10^5 CFU/g respectively after 70 days (Figure 2).

Ammonia oxidizing bacteria (AOB): In the control set-up (treated with 0% digestate), the average population of soil AOB increased to 9.3×10^3 MPN/g of soil after 70 days (Figure 3). In the set-ups treated with 8% digestate, the average population of soil AOB in OTDA and TTDA increased to 1.1×10^4 MPN/g and 9.4×10^3 MPN/g respectively after 70 days (Figure 3). In the set-ups treated with 16% digestate, the average population of soil AOB in OTDA and TTDA increased to 1.5×10^4 MPN/g and 1.3×10^4 MPN/g respectively after 70 days (Figure 3). In the set-ups treated with 24% digestate, the average population of soil AOB in OTDA and TTDA increased to 1.6×10^4 MPN/g and 1.4×10^4 MPN/g respectively after 70 days (Figure 3). In the set-ups treated with 32% digestate, the average population of soil AOB in OTDA and TTDA increased to 1.9×10^4 MPN/g and 1.9×10^4 MPN/g respectively after 70 days (Figure 3). In the set-ups treated with 40% digestate, the average population of soil AOB in OTDA and TTDA increased to 3.5×10^4 MPN/g and 3.6×10^4 MPN/g respectively after 70 days (Figure 3). In the set-ups treated with 48% digestate, the average population of soil AOB in OTDA and TTDA increased to 4.3×10^4 MPN/g and 9.3×10^4 MPN/g respectively after 70 days (Figure 3). In the set-ups treated with 56% digestate, the average population of soil AOB in OTDA and TTDA increased to 1.9×10^4 MPN/g and 1.2×10^5 MPN/g respectively after 70 days (Figure 3). In the set-ups treated with 64% digestate, the average population of soil AOB in OTDA and TTDA increased to 1.3×10^4 MPN/g and 3.4×10^5 MPN/g respectively after 70 days (Figure 3). In the set-ups treated with 72% digestate, the average population of soil AOB in OTDA and TTDA increased to 9.1×10^3 MPN/g and 3.9×10^5 MPN/g respectively after 70 days (Figure 3).

Nitrate reducing bacteria (NRB): In the control set-up (treated with 0% digestate), the average population of soil NRB increased to 4.3×10^3 MPN/g of soil after 70 days (Figure 4). In the set-ups treated with 8% digestate, the average population of soil NRB in OTDA and TTDA increased to 6.1×10^3 MPN/g and 4.6×10^3 MPN/g respectively after 70 days (Figure 4). In the set-ups treated with 16% digestate, the average population of soil NRB in OTDA and TTDA increased to 6.1×10^4 MPN/g and 9.1×10^3 MPN/g respectively after 70 days (Figure 4). In the set-ups treated with 24% digestate, the average population of soil NRB in OTDA and TTDA increased to 7.5×10^4 MPN/g and 9.3×10^3 MPN/g respectively after 70 days (Figure 4). In the set-ups treated with 32% digestate, the average population of soil NRB in OTDA and TTDA increased to 9.2×10^4 MPN/g and 1.4×10^4 MPN/g respectively after 70 days (Figure 4). In the set-ups treated with 40% digestate, the average population of soil NRB in OTDA and TTDA increased to 1.3×10^5 MPN/g and 1.9×10^4 MPN/g respectively after 70 days (Figure 4). In the set-ups treated with 48% digestate, the average population of soil NRB in OTDA and TTDA increased to 1.9×10^5 MPN/g and 3.9×10^4 MPN/g respectively after 70 days (Figure 4). In the set-ups treated with 56% digestate, the average population of soil NRB in OTDA and TTDA increased to 2.4×10^5 MPN/g and 4.6×10^4 MPN/g respectively after 70 days (Figure 4). In the set-ups treated with 64% digestate, the average population of soil NRB in OTDA and TTDA increased to 2.9×10^5 MPN/g and 7.5×10^4 MPN/g respectively after 70 days (Figure 4). In the set-ups treated with 72% digestate, the average population of soil NRB in OTDA and TTDA increased to 4.4×10^5 MPN/g and 1.4 cc 10^5 MPN/g respectively after 70 days (Figure 4).

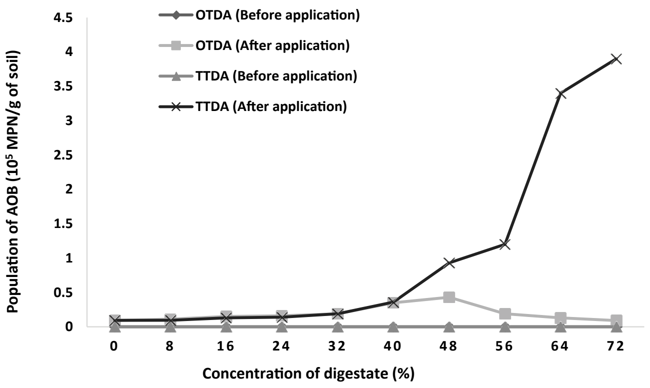

Figure 3: Population dynamics of ammonia oxidizing bacteria (AOB) with respect to digestate concentration in OTDA and TTDA set-ups before and after 70 days of applying the digestate.

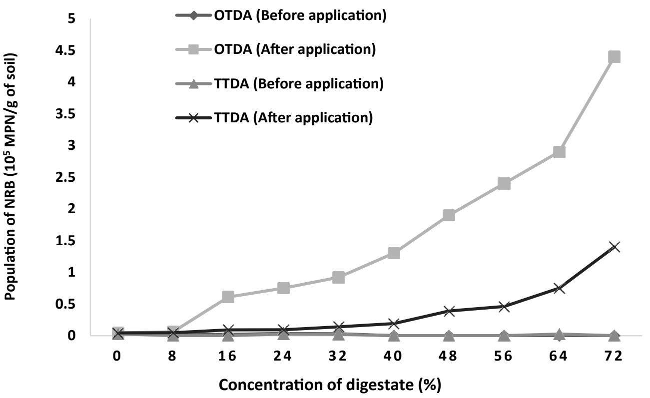

Figure 4: Population dynamics of nitrate reducing bacteria (NRB) with respect to digestate concentration in OTDA and TTDA set-ups before and after 70 days of applying the digestate.

Fungi (FUN): In the control set-up (treated with 0% digestate), the average population of soil FUN increased from 1.6×10^3 CFU/g to 5.9×10^3 CFU/g of soil after 70 days (Figure 5). In the set-ups treated with 8% digestate, the average population of soil FUN in OTDA and TTDA increased from 1.6×10^3 CFU/g and 1.4×10^3 CFU/g to 6.1×10^3 CFU/g and 6.4×10^3 CFU/g respectively after 70 days (Figure 5). In the set-ups treated with 16% digestate, the average population of soil FUN in OTDA and TTDA increased from 1.4×10^3 CFU/g and 1.5×10^6 CFU/g to 7.0×10^3 CFU/g and 7.3×10^3 CFU/g respectively after 70 days (Figure 5). In the set-ups treated with 24% digestate, the average population of soil FUN in OTDA and TTDA increased from 1.5×10^3 CFU/g and 1.3×10^3 CFU/g to 7.2×10^3 CFU/g and 7.8×10^3 CFU/g respectively after 70 days (Figure 5). In the set-ups treated with 32% digestate, the average population of soil FUN in OTDA and TTDA increased from 1.3×10^3 CFU/g and 1.4×10^3 CFU/g to 8.7×10^3 CFU/g and 8.3×10^3 CFU/g respectively after 70 days (Figure 5). In the set-ups treated with 40% digestate, the average population of soil FUN in OTDA and TTDA increased from 1.4×10^3 CFU/g and

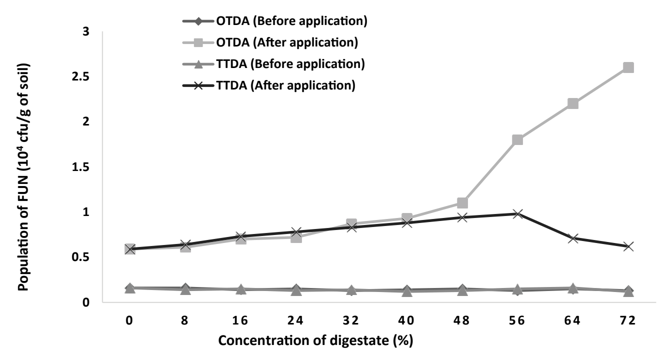

Figure 5: Population dynamics of fungi (FUN) with respect to digestate concentration in OTDA and TTDA set-ups before and after 70 days of applying the digestate.

1.3×10^3 CFU/g to 9.3×10^3 CFU/g and 8.8×10^3 CFU/g respectively after 70 days (Figure 5). In the set-ups treated with 48% digestate, the average population of soil FUN in OTDA and TTDA increased from 1.5×10^3 CFU/g and 1.3×10^3 CFU/g to 1.1×10^4 CFU/g and 9.4×10^3 CFU/g respectively after 70 days (Figure 5). In the set-ups treated with 56% digestate, the average population of soil FUN in OTDA and TTDA increased from 1.3×10^3 CFU/g and 1.5×10^3 CFU/g to 1.8×10^4 CFU/g and 9.8×10^3 CFU/g respectively after 70 days (Figure 5). In the set-ups treated with 64% digestate, the average population of soil FUN in OTDA and TTDA increased from 1.5×10^3 CFU/g and 1.6×10^3 CFU/g to 2.2×10^4 CFU/g and 7.1×10^3 CFU/g respectively after 70 days (Figure 5). In the set-ups treated with 72% digestate, the average population of soil FUN in OTDA and TTDA increased from 1.3×10^3 CFU/g and 1.2×10^3 CFU/g to 2.6×10^4 CFU/g and 6.2×10^3 CFU/g respectively after 70 days (Figure 5).

Figures 1-5 show that the application of liquid digestate (as bio-fertilizer) to the soil in the different set-ups during cultivation of maize plant actually affected the population of soil microbes [6-9]. The population of all the microbial groups considered in this study increased with time after the application of the liquid digestate. This could partly be attributed to the nutrient content of the liquid digestate shown in Table 1. Most of the nutrients are just as beneficial to soil microbes as they are to plants [2-4,8,35]. The result also shows that the one-time digestate application (OTDA) treatment seemed to be less favourable to the growth of aerobic bacteria (AEB) and ammonia oxidizing bacteria (AOB) populations compared to the two-time digestate application (TTDA) treatment which appears to have favoured the populations of AEB and AOB respectively with time. Because these groups of bacteria (especially AOB) are the drivers of nitrification in the soil, it therefore indicates that applying the liquid digestate periodically at smaller quantities (instead of applying it all at once) may have favoured nitrification which is actually beneficial to the plants to some degree [10-14]. The one-time mode of digestate application (OTDA) seemed to have favoured the populations of strict anaerobic bacteria (SAB) and nitrate reducing bacteria (NRB) with time especially at higher concentrations when compared to the two-time mode of digestate application (TTDA).

This is because, application of higher concentrations of the liquid digestate to the soil inside the pots at once (instead of periodically) may have displaced some or most of the oxygen dissolved in the soil solution and this could have promoted some levels of oxygen-limiting condition (or anaerobiosm) which usually favours the populations of SAB and NRB [36,37]. An increase in the population of SAB and especially NRB may indicate an increase in the formation of organic acids, methane (if methanogens are present) and oxides of nitrogen as a result of denitrification which usually leads to the loss of nitrogen from the soil [5,6,12,36-38]. The loss of nitrogen from the soil in the form of oxides (such as NO_2, NO or N_2O) contributes to global warming because these oxides of nitrogen are known to be potent greenhouse gases [36,37]. This condition may be quite unfavourable to plants, soil microbes and the environment at large [36,37]. Therefore, it will be necessary to consider the concentration and mode of application of liquid digestate to agricultural fields in order to prevent the emission of more potent greenhouse gases such as oxides of nitrogen and methane [13].

The result further shows that both (OTDA and TTDA) methods of digestate application increased the growth of fungi (FUN) population to some degree with time. However, the one-time digestate application (OTDA) treatment appeared to have favoured the population of fungi (FUN) more than the two-time digestate application (TTDA) treatment with time especially at higher concentrations (%). This may be possible because the OTDA treatment method appeared to have slightly acidified the soil at higher concentrations of the digestate than the TTDA treatment method. Because fungi appear to thrive better under acidic (or lower) pH, it may be reasonable to assume that this would have been a possible reason why their population appeared to have been slightly higher in the OTDA treatment than in the TTDA treatment with time [17].

Distribution and characteristics of soil microbes isolated

Generally, a total of 21 bacteria species and 13 fungi species were isolated. The bacteria include species which belong to genera such as *Arthrobacter*, *Azotobacter*, *Flavobacterium*, *Nocardia*, *Bacillus*, *Clostridium*, *Cellulomonas*, *Micrococcus* and *Pseudomonas* respectively. In the control set-up, a total of 2 *Arthrobacter* sp., 1 *Azotobacter* sp., 1 *Flavobacterium* sp., 2 *Bacillus* sp., 1 *Cellulomonas* sp., and 1 *Pseudomonas* sp. were isolated (Figure 6). In the OTDA set-up, a total of 1 *Arthrobacter* sp., 1 *Azotobacter* sp., 2 *Flavobacterium* sp., 2 *Bacillus* sp., 3 *Clostridium* sp., 2 *Cellulomonas* sp., 2 *Micrococcus* sp. and 1 *Pseudomonas* sp. were isolated (Figure 6). In the TTDA set-up, a total of 2 *Arthrobacter* sp., 1 *Azotobacter* sp., 2 *Flavobacterium* sp., 2 *Nocardia* sp., 4 *Bacillus* sp., 1 *Clostridium* sp., 1 *Cellulomonas* sp., 2 *Micrococcus* sp. and 2 *Pseudomonas* sp. were isolated (Figure 6).

The fungi include species which belong to genera such as *Aspergillus*, *Cladosporium*, *Fusarium*, *Geotrichum*, *Penicillium*, *Rhizopus* and

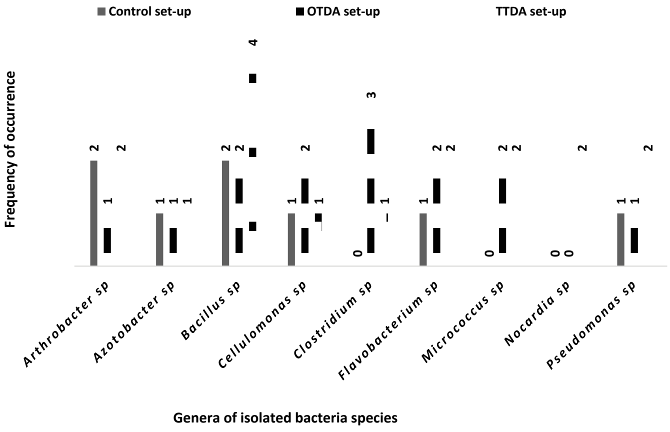

Figure 6: Bacteria species isolated from digestate-treated and untreated soil samples.

Trichoderma respectively (Figure 7). In the control set-up, a total of 2 *Aspergillus* sp., 1 *Fusarium* sp., 1 *Geotrichum* sp., 1 *Penicillium* sp., 1 *Rhizopus* sp. and 1 *Trichoderma* sp. were isolated (Figure 7). In the OTDA set-up, a total of 3 *Aspergillus* sp., 1 *Cladosporium* sp., 2 *Fusarium* sp., 1 *Geotrichum* sp., 2 *Penicillium* sp., 1 *Rhizopus* sp. and 1 *Trichoderma* sp. were isolated (Figure 7). In the TTDA set-up, a total of 2 *Aspergillus* sp., 2 *Fusarium* sp., 1 *Geotrichum* sp., 2 *Penicillium* sp., 1 *Rhizopus* sp. and 1 *Trichoderma* sp. were isolated (Figure 7).

The characteristics of bacteria and fungi isolated from composite soil sample in the control set-up (with 0% digestate application), OTDA set-up and TTDA set-up are presented in Table 3-6 respectively.

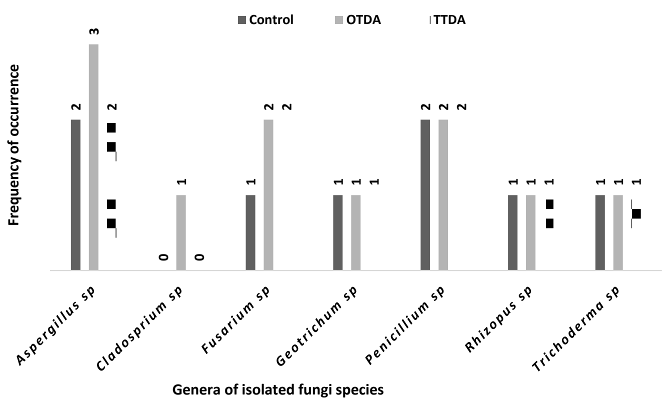

Figure 7: Fungi species isolated from digestate-treated and untreated soils samples.

Conclusion

In conclusion, the two-time mode of digestate application (TTDA) appeared to have favoured the population of aerobic bacteria (AEB) and ammonia-oxidizing bacteria (AOB) better than the one-time

Biochemical Tests	B1	B2	B3	B4	B5	B6	B7
Gram stain	-	-	-	-	-	+	+
Shape	Rod	Club-rod	Rod	Rod	Rod	Filamentous	Filamentous
Arrangement	Single	Single	Paired	Single	Paired	Branched	Branched
Spore	-	-	-	-	-	-	-
Acid fast	-	-	-	-	-	+	-
Motility	-	-	+	-	+	-	-
O_2 requirement	OA	OA	OA	FA	FA	FA	FA
Oxidase	+	+	+	+	+	-	-
Coagulase	-	-	-	-	-	-	-
Citrate	+	+	-	-	+	-	-
Catalase	+	+	+	+	+	-	-
Indole	-	-	-	-	-	-	-
Urease	+	-	-	-	-	-	-
H_2S Production	+	-	-	-	-	-	-
Nitrate red.	-	+	-	+	+	+	-
Methyl red	-	-	-	-	-	-	-
Voges Proskauer	-	-	-	-	-	-	-
Ornithine decarboxylase	-	-	-	-	-	-	-
D-glucose	+/-	+/-	+/-	+/-	+/+	-	+/+
D-mannitol	-	-	+/-	+/-	+/+	-	-
D-sucrose	-	+/-	+/-	+/+	+/-	-	-
Lactose	-	-	-	+/-	+/-	-	-
D-maltose	-	+/-	+/-	+/-	+/-	-	-
D-xylose	-	+/-	+/-	+/-	+/-	-	-
L-arabinose	-	-	-	-	+/-	-	-
Salicin	-	-	-	-	-	-	-
Cellulose	-	-	-	-	-	-	-
Starch	-	+/-	-	+/-	-	+/-	+/-
Gelatin	+/-	+/-	-	+/-	+/-	+/-	+/-
Esculin	-	-	-	-	-	+/-	-
Glycerol	-	-	-	-	+	+/-	-
D-cellobiose	-	-	-	-	-	-	-
D-mannose	-	+/-	-	+	-	-	-
D-melezitose	-	-	-	-	-	-	-
D-raffinose	-	-	-	-	-	-	-
D-sorbitol	-	-	-	-	-	-	-
L-rhamnose	-	-	-	+	-	-	-
D-trehalose	-	-	-	+	-	-	-
Probably identify	*Arthrobacter sp*	*Arthrobacter sp*	*Azotobacter sp*	*Flavobacterium sp*	*Flavobacterium sp*	*Nocardia sp*	*Nocardia sp*
OA: Obligate Aerobe; OAN: Obligate Anaerobe; FA: Facultative Anaerobe: +/+:Acid and gas production; +/-: Acid production without gas production; - = No fermentation							

Table 3: Characteristics of bacteria isolated from digestate-treated soil and the control.

Biochemical Tests	B8	B9	B10	B11	B12	B13	B14
Gram stain	+	+	+	+	+	+	+
Shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Arrangement	Chain	Chain	Chain	Chain	Single	Single	Single
Spore	+	+	+	+	+	+	+
Acid fast	-	-	-	-	-	-	-
Motility	+	+	+	+	+	-	-
O_2 requirement	FA	OA	FA	FA	OAN	OAN	OAN
Oxidase	-	-	+	-	-	-	-
Coagulase	-	-	-	-	-	-	-
Citrate	-	+	-	+	+	+	+
Catalase	+	+	+	+	-	-	-
Indole	-	-	+	-	-	+	-
Urease	-	-	-	+	+	-	+
H_2S Production	-	-	-	-	-	-	-
Nitrate red.	+	-	-	+	-	-	+
Methyl red	+	-	-	-	+	+	+
Voges Proskauer	-	+	+	+	-	-	-
Ornithine decarboxylase	-	-	-	-	-	-	-
D-glucose	+/+	+/-	+/+	+/+	+/+	+/+	+/+
D-mannitol	+/-	-	-	+/+	-	+/+	-
D-sucrose	+/+	+/-	+/+	+/-	+/+	+/+	+/+
Lactose	+/-	-	-	-	+/+	-	+/+
D-maltose	+/-	+/-	+/-	+/+	+/+	+/+	+/+
D-xylose	+/+	+/-	-	-	-	+/-	-
L-arabinose	-	-	-	-	-	+/+	-
Salicin	-	+/-	-	-	+/-	+/+	+/+
Cellulose	+/-	-	+/-	-	+/+	+/+	-
Starch	+/-	-	+/-	-	-	-	-
Gelatin	-	+/-	+/-	+/-	-	+/-	+/-
Esculin	+/-	+/-	+/-	+/-	+/-	-	-
Glycerol	-	+	-	+/-	+/+	-	-
D-cellobiose	+/+	+/-	+/+	+/-	+/+	+/+	-
D-mannose	-	+	-	-	+/-	+/+	-
D-melezitose	-	-	-	-	-	-	-
D-raffinose	-	-	-	-	+/+	-	+/+
D-sorbitol	-	-	+/+	+/-	-	+/+	-
L-rhamnose	-	+/-	-	+/+	+/+	-	+/-
D-trehalose	-	+/-	-	-	+/+	-	+/+
Probably identify	*Bacillus lentus*	*Bacillus pumellus*	*Bacillus alvei*	*Bacillus pasteurii*	*Clostridium sp*	*Clostridium sp*	*Clostridium sp*
OA: Obligate Aerobe; OAN: Obligate Anaerobe; FA: Facultative Anaerobe: +/+:Acid and gas production; +/-: Acid production without gas production; - = No fermentation							

Table 4: Characteristics of bacteria isolated soil from digestate-treated and the control.

Biochemical Tests	B15	B16	B17	B18	B19	B20	B21
Gram stain	-	+	-	+	+	+	+
Shape	Rod	Rod	Rod	Cocci	Cocci	Rod	Rod
Arrangement	Single	Single	Single	Cluster	Cluster	Single	Single
Spore	-	-	-	-	-	-	-
Acid fast	-	-	-	-	-	-	-
Motility	+	+	-	-	-	+	+
O_2 requirement	FA	FA	FA	OA	OA	FA	OA
Oxidase	+	+	+	+	+	+	+
Coagulase	-	-	-	-	-	+	-
Citrate	-	-	+	-	+	+	+
Catalase	+	+	+	+	+	+	+
Indole	-	-	-	-	-	-	-
Urease	-	-	-	-	+	+	+
H_2S Production	-	-	-	-	-	-	-

Nitrate red.	+	-	-	+	-	+	-
Methyl red	-	-	-	+	-	-	-
Voges Proskauer	-	-	-	+	+	-	-
Ornithine decarboxylase	-	-	-	-	-	-	-
D-glucose	+/-	+/-	+/+	+/-	+/+	-	+/+
D-mannitol	+/-	-	-	+/-	-	+/-	+/-
D-sucrose	+/-	+/+	+/+	+/-	-	-	-
Lactose	+/-	+/-	+/-	-	-	-	+/+
D-maltose	+/-	+/+	+/-	+/-	-	-	+/+
D-xylose	-	+/-	+/-	-	-	-	-
L-arabinose	-	+/-	-	-	-	-	-
Salicin	-	-	-	-	-	-	-
Cellulose	+/+	+/+	+/-	-	-	-	-
Starch	+/-	+/-	+/-	-	-	-	-
Gelatin	+/-	+/-	-	+/-	+/-	+/-	+/-
Esculin	-	-	+/-	-	-	+/-	-
Glycerol	+/-	+/-	-	-	-	+/-	-
D-cellobiose	+/+	+/+	+/-	-	-	-	-
D-mannose	-	+/-	-	-	-	-	-
D-melezitose	-	-	-	-	-	-	-
D-raffinose	+/+	-	+/-	-	-	-	-
D-sorbitol	-	-	+/+	-	-	-	-
L-rhamnose	+/-	-	-	-	-	-	-
D-trehalose	-	+/+	-	-	-	-	-
Probably identify	*Cellulomonas sp*	*Cellulomonas sp*	*Cellulomonas sp*	*Micrococcus sp*	*Micrococcus sp*	*Pseudomonas sp*	*Pseudomonas sp*
OA: Obligate Aerobe; OAN: Obligate Anaerobe; FA: Facultative Anaerobe: +/+:Acid and gas production; +/-: Acid production without gas production; - = No fermentation							

Table 5: Characteristics of bacteria isolated from digestate-treated soil and the control.

Morphological characteristics of fungi isolated from digestate-treated and untreated soil set-up.			
S/N	**Cultural features**	**Microscopic features**	**Probably identified**
1.	This fungal isolate had a woolly texture with yellowish-green colouration on the surface and brow colouration on the reverse. Growth was rapid (within 3 days) with a colony diameter of 46.3mm after 4 days.	The fungal isolate had septate hyphae with biseriate and globose vessicle. The surface of the conidial walls was finely roughened and the conidiophores were hyaline and slightly roughened at the apex. The metulae covered about ¾ of the entire surface of the vessicle.	*Aspergillus flavus*
2.	This fungal isolate had a powdery texture with greyish-green colouration on the surface, a narrow white border and a pale-yellow colouration on the reverse. Growth was rapid (within 3 days) with a colony diameter of 44.6mm after 4 days.	The fungal isolate had septate hyphae with a uniseriate and a roughened subclavate vessicle. The surface of the conidial walls was smooth and the conidiophores were hyaline and smooth-walled. The metulae covered about 2/3 of the entire surface of the vessicle.	*Aspergillus fumigatus*
3.	This fungal isolate had a woolly texture with black colouration on the surface and greyish on the reverse. Growth was rapid (within 3 days) with a colony diameter of 42.5mm after 4 days.	The fungal isolate had septate hyphae with a biseriate and spherical vessicl at the apex. The surface of the conidial walls was rough and the conidiophores were dark-brown and roughened. The metuale almost covered the entire surface of the vessicle.	*Aspergillus niger*
4.	This fungal isolate had a velutinous texture with cream-buff colouration on the surface and a brown colouration on the reverse. Growth moderate (within 5 days) with colony diameter of 40.7mm after 7 days.	The fungal isolate had septate hyphae with biseriate and globose vessicle. The surface of the conidial walls was very rough and the conidiophores were slightly brown and smooth-walled. The metulae only covered the upper surface of the vessicle.	*Aspergillus nidulans*
5.	This fungal isolate had a wet texture with a yellow colouration surface and a cream colouration on the reverse. Growth was rapid (within 3 days) with a colony diameter of 49.3mm after 4 days.	The fungal isolate had septate hyphae with uniseriate and globose vessicle. The surface of the conidial walls was smooth and conidiophores were hyaline and rough-walled. The metulae covered around ½ of the surface of the vessicle.	*Aspergillus oryzae*
6.	This fungal isolate a powdery texture with dark-green colouration on the surface, black edges and brownish-green colouration on the reverse. Growth was slow (within 6days) with a colony diameter of 13.8mm after 7 days.	The fungal isolate had septate hyphae with ellipsoidal vesicle. The surface of the conidia was smooth-walled and the conidiophores were dark-brown and slightly roughened walls. The metulae covered about ¼ of the surface of the vessicle.	*Cladosporium herbarum*
7.	This fungal isolate had a woolly texture with aerial mycelium that was pale purple in colour on the surface and a dark blue colouration on the reverse. Growth was rapid (within 3 days) with a colony diameter of 45.4mm after 4 days.	The fungal isolate had both septate and aseptate hyphae. The fusiform shaped macro-conidia were septate while the micro-conidia were aseptate. The conidiophores were arranged in branched clusters. Chlamydospores were terminal, hyaline and smooth-walled.	*Fusarium oxysporum*
8.	This fungal isolate had a woolly texture with greyish-white colouration on the surface and a cream colouration on the reverse. Growth was rapid (within 3 days) with a colony diameter of 49.2mm after 4 days.	The fungal isolate had septate hyphae. The macro-conida were curved fusiform in shape while the micro-conidia were oval and unicelled. Chlamydospores which were globose and slightly roughened were present in pairs on the lateral hyphae branches.	*Fusarium solani*
9.	This fungal isolate had a butyrous texture with creamy colouration on the surface but no reverse colouration. The isolate was flat and suede-like in appearance. Growth was fast (within 4 days) with a colony diameter of 43.4mm after 4 days.	The isolate had septate hyphae which were hyaline, branched and broke up into chains. The hyphae were smooth-walled and possessed cylindrical arthroconidia. The arthroconidia varied in size with some having the appearance of a bud.	*Geotrichum sp*

10.	This fungal isolate had a velutinous texture with green colouration on the surface and a yellow colouration on the reserve. Growth was moderate (within 5 days) with a colony diameter of 50.5mm after 6 days.	The fungal isolate had septate hyphae. The penicillia were asymmetrical with divergent branching pattern. The conidiophores were smooth-walled. The phialides were aggregated in clusters and there were about six metulae. The conidia were subglobose, smooth-walled and arranged in slightly long chains.	*Penicillium chrysogenium*
11.	This fungal isolate had a powdery texture with a green colouration on the surface and a pale red on the reverse. The borders on the surface and reverse were yellow. Growth was rapid (within 3 days) with a colony diameter of 55.6mm after 4 days.	The fungal isolate had septate hyphae. The penicillia were monoverticillate. Conidiophores were smoo-walled and swollen at the apex, bearing phialides. The conidia were globose and smooth-walled.	*Penicillium frequentans*
12.	This fungal isolate had a woolly texture with a brownish grey colouration on the surface and pale white on the reverse. The aerial mycelium was very abundant. Growth was rapid (within 3 days) with a colony diameter of 63.7mm after 4 days.	The fungal isolate had aseptate hyphae that were white and bright. Rhizoids and stolons were present. The sporangiophores were smooth-walled and branched. The sporangia were globose with a greyish black colouration. The columella was sub-globose. The sporangiospores were angular and ellipsoidal with fairly rough surfaces.	*Rhizopus oryzae*
13.	This fungal isolate had a moderate and compact woolly texture with a yellowish-green colouration. The reverse colouration was pale yellow. Growth was fast (within 4 days) with a colony diameter of 40.2mm after 5 days.	The fungal isolate had thick septate hyphae. The conidiophores were repeatedly branched, bearing clusters of divergent flask-shape phialides. The conidia were green in colour with slightly roughened walls. The conidia were slightly oval in shape and clustered at the tips of the phialides.	*Trichoderma sp*

Table 6: Characteristics of fungi isolated from digestate-treated set-up and the control.

mode of digestate application (OTDA) with time. This suggests that the TTDA treatment method may have favoured nitrification better than the OTDA treatment method. In addition, the TTDA treatment method appeared to have been less favourable to the population of strict anaerobic bacteria (SAB) and nitrate reducing bacteria (NRB) compared the OTDA treatment method with time. This suggests that the OTDA method may have promoted soil denitrification more than the TTDA treatment method with time. From the current study, it appears that the two-time mode of digestate application (TTDA) to agricultural soils may have been more beneficial to the growth of the test plants (*Zea mays*) as well as minimizing greenhouse gas (GHG) emission compared to one-time digestate application (OTDA) treatment which appeared to have promoted conditions which may have favoured the emission of greenhouse gases such as oxides of nitrogen (and may be methane as well).

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