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Original Article

Influence of anaerobic digestate type on nitrogen dynamics and leaching losses across two soils

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Abstract

To enhance soil fertility and subsequent crop yields, digestate, byproduct of anaerobic digestion, can serve as a supplement or potential alternative to chemical fertilizers when sensibly utilized. This study assessed the impact of two types of digestates on two distinct soils for the perspective of agriculture (affecting pH, EC, organic carbon and mineral nitrogen) and environment (mineral nitrogen leaching under two rainfall patterns). Both soils mainly differed in silt and sand contents: 32% silt and 47% sand for soil-1 and 42% silt and 39% sand for soil-2. Two sets of controlled experiments served the purpose, in which first set involved a soil incubation experiment, applying two digestates to two soil types at 28°C for 60 days. The second set comprised reconstituted soil columns to collect soil solutions at depths of 2.5 cm and 7.5 cm after rainfall application under two patterns for 45 days. Results indicated that application of both digestates in test soils initially increased pH at day 15, followed by a decrease at days 30 and 60. Both digestates significantly elevated soil electrical conductivity compared to control treatments in both soils. Organic carbon content displayed variable impacts, with a slight decrease for solid digestate (12%) and higher decrease for liquid-amended soil (43%) for soil-1. While a significant decrease was observed for soil-2 throughout the incubation period for both amendments (34% and 36% for solid and liquid amended soils respectively). Rapid nitrification occurred with the application of both digestates in both soils, albeit at different rates. Soil-2 exhibited 1.2 to 2 folds higher net nitrification rate (depending upon digestate type and days of incubation) compared to soil-1. Liquid digestate induced more mineral nitrogen compared to solid digestate in both soils. Interestingly, rainfall frequency, digestate type, and soil type influenced the leaching of ammonium and nitrates, with nitrates recording higher levels in both soils, at both depths, and under both rainfall patterns.

Keywords: Slurry, Nitrates, Crop productivity, Nitrification rate, Soil nutrition

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Introduction

World population is escalating at a remarkable pace and expected to reach 10 billion by 2050 (Graham, 2019). It is predicted to stretch out to 11 billion by 2100 (Adam, 2021). This increasing populace will put enormous pressure on global crop production. The 60-70% increase in existing food production should be augmented to meet the food requirements of this increasing population (van Dijk et al., 2021). On the other hand, sustainable development of agriculture sector, being the main food provider, will seriously be affected by climate change, pest attacks, water scarcity and reduction in cultivated land. Keeping in view these challenges and food demands, the use of chemical fertilizers will be on the rise. For example, total nitrogen fertilizer consumption in the world is projected to increase between 125 to 236 million metric tons in 2050 (Good and Beatty, 2011). Similarly in Pakistan, an exponential increase in nitrogen (N) application rates has been observed for major crops in recent past. In 2014, 44-64% higher average input rates of N in Pakistan for major crops were used, than the corresponding global average values (Shahzad et al., 2019). Although augmented application of nitrogenous fertilizers promoted crop production, but nitrogen use efficiency (NUE) is very low in the country than the global average. Due to this low NUE in the country, a handsome amount of surplus N (175 kg N per hectare per year) is being wasted to hydrosphere or atmosphere (Raza et al., 2018). So, the country should focus on sustainable use of nitrogenous fertilization to meet the food requirement and minimize the environmental as well as crop production costs. This increased application of chemical fertilizers will further exacerbate the air, water and soil pollution (Awan et al., 2022). Furthermore, from last few years, the prices of chemical fertilizers are hiking globally as well as in Pakistan with almost 80% of increase in the fertilizer prices has been reported globally in 2021 (Rodríguez-Espinosa et al., 2023).

With increasing population, the global demand for energy is constantly shooting up (Prask et al., 2018). With no exception, huge energy is required by Pakistan to meet its requirement (Irfan et al., 2021). Unfortunately, escalating fertilizer prices and energy shortage are hurting agricultural production and hence Pakistan’s economy. One of the feasible solutions to these problems lies in the valorization of locally available biomass especially through the anaerobic digestion (AD) to generate energy-rich biogas (Zeb et al., 2019). This biogas generation arises out of crop residues, animal waste, kitchen waste or agro-food industry waste (Prask et al., 2018). Huge potential of biogas generation from livestock manure exists for Pakistan (Arshad et al., 2022). There are around 8000 functional biogas units in Pakistan (Khan et al., 2021) with a varying production capacity. Pakistan announced, in October 2020, a new Alternative Renewable Energy Policy 2019 to install more biogas units throughout the country in coming years. According to the analysis, Pakistan will have the potential of producing approximately 24-Terawatt hour of green electricity in 2030 (Arshad et al., 2022). With increasing number of biogas plants, a huge amount of digestate (a byproduct of biogas generation processes) will also be produced. Recently, various strategies (utilization as fertilizer, biochar production, soil conditioner, animal feed and insect transformation) attained the focus in the world for digestate’s sustainable management and resource utilization (Guan et al., 2024).

In this context, this nutrient rich resource (digestate) can be used as an alternative to chemical fertilizer for crop production and subsequently reducing their use in agriculture (Sogn et al., 2018). If this digestate is valorized sensibly (keeping in view the circular economy or zero-waste policy), the use of digestate in agriculture can meet 11% of the total nitrogen and approximately 29% of the total phosphorus requirement for crop production (Abbas et al., 2023). Digestate application results in complex organic carbon (C) composites plus N (NH_4^+), which may play a key role in potentially improving C balance in soil. Mature digestate is already being used, in many developing countries, to improve soil fertility. So, there is a huge potential market for digestate application in Pakistan (Afridi and Qammar, 2020). Originally digestate is composed of solid and liquid fractions which may vary in percentage depending on the inputs. It is well documented that digestates are rich in macro and micronutrients which can be available to the plant in response to their microbial decomposition (Sogn et al., 2018). But the proportion of these nutrients in digestate depends upon the input materials, loading rate of input materials, temperature, pH, retention time, and digestate’s processing technology (separation of liquid and solid fractions) (Nyang’au et al., 2023; Ndubuaku et al., 2013). For this reason, the content of individual components in digestate from different biogas plants may be different (Czekała, 2022).

Despite of the possibility of using digestates as fertilizer, the presence of large quantities of soluble forms of nutrients (Tuszynska et al., 2021) may contaminate surface and groundwaters if used excessively (Nkoa, 2014). It is observed that ammonium (NH_4^+) concentration is higher in digestate from agricultural biogas plants (Tuszynska et al., 2021) compared to conventional organic agricultural fertilizers such as manure. So, when digestates are applied to soils, ammonium may either be adsorbed on negatively charged surfaces of soil particles, or absorbed by plants, or oxidized to nitrates. Furthermore, increased rate of ammonia oxidation and nitrate leaching (Jahangir et al., 2012) can be associated to application of digestate in soil. Some researchers also highlighted the "priming effect" of digestate which can promote the decomposition of already existing soil organic matter (Abubaker et al., 2012; Fontaine et al., 2004) and hence can release mineral nitrogen which is readily available for plants (Mason-Jones et al., 2018) or can be leached down. Therefore, soil solutions should be monitored for the assessment of environmental risk of nitrate leaching to groundwater. The increasing number of biogas plants in Pakistan may lead to growing problem of digestate management. In this context, the current study is focused to valorize the digestate produced at two different biogas plants in two types of soils varying in native properties and management practices. We hypothesized that the type of soil and digestate would drive the nitrogen dynamics within and leaching from varying soil depths. We also investigated the role of rainfall patterns for fluctuations of ammonium and nitrate within the varied depths of test soils. The study further aimed to enquire if such digestates can be applied to soils as a fertilizer and also the extent to which digestate or soil type ultimately affect the studied perspectives.

Material and Methods

Soils and digestates

Two types of soils were used in this experiment, first soil (which was abbreviated as S1) was sampled from experimental area of COMSATS University Islamabad, Abbottabad campus (34°11.56′ N, 73°14.84′ E). Normally, this soil is not cultivated since long. Second soil (which was abbreviated as S2) was sampled from Khanpur (District Haripur) agricultural field (33°54.38′ N, 72°55.68′ E). These soils were selected based on the idea, how two different types of soil (varied cultivation practice and intrinsic soil properties) respond to application of digestates. The soil samples were taken randomly from 0-10 cm depth and ground. The soils were sieved (<4 mm) and stored separately in bags at 4 °C prior to use. All residues were removed manually from soils. The initial characteristics of both soils are summarized in Table 1. The soils majorly varied in silt and sand contents: 32% silt and 47% sand for soil-1 and 42% silt and 39% sand for soil-2 and were loamy in nature (Table 1).

Two types of digestates were used in this experiment: solid (sampled from Pakpattan, 30°13′ N, 72°58.8′ E) and liquid (sampled from Haveliyan, 34°3.07′ N, 73°8.54′ E). At both biogas plants, the input used for anaerobic digestion was livestock manure. Freshly sampled digestates were also stored in refrigerator for further use. Both (solid and liquid digestates) soil nutritional inputs were applied in both soils. The pH values differed between two digestates, with the liquid digestate having a pH of 7.19 and the solid digestate registering a higher pH of 8.86. The electrical conductivity (EC) in liquid digestate was observed to be higher (844 µS/cm) than that in solid digestate (4.4 µS/cm). Liquid digestate contained a higher concentration of ammonium (27 mg/l) as compared to solid digestate (10 mg/l). In contrast, the concentration of nitrates was 22 mg/l and 34.6 mg/l for liquid and solid digestates respectively.

Table-1. Initial properties of soil-1 and soil-2 used in the experiment.

Soil properties	Soil-1	Soil-2
Clay (%)	21	19
Silt (%)	32	42
Sand (%)	47	39
Organic Carbon (%)	1.8±0.1	2.6±0.3
Ammonium (mg/kg)	7.2±0.2	2.9±0.4
Nitrates (mg/kg)	14.3±0.8	17.1±0.1
pH	8.0±0.4	7.8±0.3
Electrical conductivity (EC) (µS/cm)	9.5±0.5	10.1±1.1

Two different sets of experiments were performed in this study. In the first set of experimentation, soil incubation was performed at a controlled temperature to assess the soil nitrogen mineralization, carbon stock, pH and electrical conductivity (EC). While in the second set of experimentation, soil columns were reconstituted based on both soils to assess the leaching of NH_4^+ and NO_3^- following application of both forms of digestates under two patterns of rainfall application.

Soil incubation studies

Both digestates were added to both soils at a rate of 60 g of digestate/kg of soil, mixed into moist soil (30%) and incubated at 28°C for 60 days. The rate of digestate application was deliberately kept low as high doses may affect the biological and chemical parameters of soil negatively (Panuccio et al., 2021). Two factors, i.e. the type of soil and type of digestate were combined into four different treatments (Figure 1). Solid digestate (abbreviated as Sol) and liquid digestate (abbreviated as Li) were applied on soil-1 (SI, sampled from COMSATS) and soil-2 (S2, sampled from Khanpur agriculture farm). The four treatments were further named as Sol-S1, Li-S1, Sol-S2 and Li-S2. Two control (without addition of any input) treatments, 1 for each soil, were also added in the experiment. Control of soil-1 was named as "control-S1" and that of soil-2 was named as "control-S2". Glass jars were used to carry out the incubation. Each glass jar was filled with moist sieved soil. Each jar contained 200g of dry soil for each type of soil keeping the soil density of 1 g/cm^3. All treatments were carried out in triplicates. 30% moisture level was maintained throughout the incubation period by weighing the jars at regular intervals. The top of the bottle was closed with a pierced aluminum foil to avoid the water evaporation and maintain aerobic conditions. Destructive sampling technique was used, and all treatments were taken out from incubator at days 15, 30 and 60 and soil was used for various analyses.

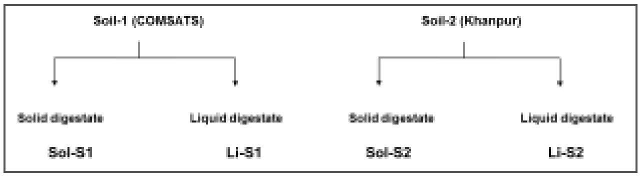

Figure-1. Schematic diagram showing different treatments used in this study.

Leaching experiment

To assess the leaching of NH_4^+ and NO_3^- in soil, both digestates were applied in both soils at the same rate mentioned above. PVC cylinders with the length of 12 inches and diameter of 4.3 inches were used. The columns were filled with soil up to height of 10 cm keeping the soil density of 1 g/cm^3 for both soils in replicates. Inspired by (Iqbal et al., 2015), initially the moist soil (30% moisture level) was filled in PVC columns. Each PVC column had two holes, one at 2.5 cm of soil depth, other at 7.5 cm of soil depth from top of the column. Two rainy seasons (summer and winter) were selected. Summer season was considered from April to September and winter between months of October and March. The amount of rainfall applied at each event and frequency were decided for both seasons based on the rainfall data (not shown) of 3 years (2013-2015) recorded at weather station installed at COMSATS University Islamabad, Abbottabad campus. Generally Abbottabad receives frequent rainfall during the summer season as compared to winter. Keeping this in view, we mimicked the duration between two events of winter and summer seasons. So, total of 7 rainfall events were performed with a 7/8 day interval for summer season and 4 rainfall events with 15 days gap for winter season. First rainfall event was applied at day 0 (just after filling the columns and applying digestates) for both seasons, the rest of 6 events were performed with a 7/8 day interval for summer season and 3 rainfall events with 15 days gap for winter season. 3mm of rainfall was applied to each column at each rainfall event for both seasons, hence varying only frequency but not amount of water applied. Top of the columns was closed with pierced aluminum foil to avoid excessive water evaporation and kept at room temperature. The applications of rain allowed us to obtain soil solution (Golden Vac ^{TM} 8 cm) for the analysis of soluble NH_4^+ and NO_3^- concentrations at two depths of columns (2.5 and 7.5 cm soil depth). The solution was collected after each rainfall event for each treatment, filtered through the Whatman filter paper and refrigerated until further analyses.

Determination of soil texture and moisture content

Hydrometer was used for the determination of soil texture (Moodie et al., 1959; Hafeez et al., 2019) and textural class was determined by the method devised by the United States Department of Agriculture. In brief, 50g of air-dried soil was weighed in a beaker and dissolved in a 50-mL (2%) sodium hexametaphosphate (BDH laboratory supplies, England) solution. One hundred fifty milliliters of distilled water were added to the sample containing beaker. Two successive readings were recorded at

Bouyoucos Hydrometer, after 40 seconds and 2 hours. International soil classification system (ISSS) was used to calculate the percentage of soil particles (sand, silt, and clay). The correction factor for temperature during the reading was also took into account for calculation of percentage of each soil particle size. Soil moisture content was calculated by using the method of (Black, 1965). The soil samples were dried to a constant weight in an oven at 105°C for 24 hours. The calculation for each sample was done by using the formula:

$$\text{Moisture Content} = (\text{Mass of water} / \text{Mass of soil}) \times 100 \quad (1)$$

Measurement of soil pH and EC

To determine pH and electrical conductivity (EC), the following procedure was adopted. In a glass beaker, 4g of soil (or digestate) was finely mixed with 20 ml of distilled water (using 1:5, w/v) and allowed to settle for 20 minutes. After that, the electrode was immersed in soil solution for 30 seconds, and pH and EC measurements were taken (Rayment and Higginson, 1992). pH and EC were measured with pH (Sinotester phs-550) and conductivity (Owell EC meter) meters respectively. The electrode was withdrawn from suspension after each stable reading and washed with distilled water in a separate beaker before taking the next reading.

Assessment of soil carbon and nitrogen

Organic carbon of soils was determined by combustion method as described by (Hafeez et al., 2019). The soil samples were dried at 105°C and weighed in china dish. The dried samples were then combusted at 550°C for 2 hours in a conventional furnace. When the temperature dropped to 50°C, the samples were recovered, and again dried samples were weighed. The volatile solids (VS) were calculated from the change in weight of samples before and after combustion as shown by the formula given below.

$$\text{VS (\%)} = (\text{initial weight} - \text{final weight}) / \text{initial weight}) * 100 \quad (2)$$

The total organic carbon was calculated by following formula:

$$\text{TOC} = \text{VS}/1.8 \quad (3)$$

Ammonium in soil and digestates was determined by the procedure as proposed by (Zaman et al., 2023). The samples were extracted using a K_2SO_4 (BDH laboratory supplies, England) solution (0.5M) with a sample to solution ratio of 1/5 and shaken for 1 hr in orbital shaker at 150 rpm. Extract was filtered by using Whatman filter paper 40. Then the sample was frozen before recording the absorbance from spectrophotometer at 655nm. Nitrates in soil samples and digestates were determined after adjusting moisture contents in soil according to the method of (Downes, 1978). In short, nitrate stock solution was prepared after drying 1 g of potassium nitrate (KNO_3, BDH laboratory supplies, England with minimum 99% purity) in an oven at 105°C for 24 hrs. Then dried 1 g KNO_3 was dissolved in 1000 ml of distilled water. From this stock solution, NO_3^- standards were prepared as 0, 2, 5, 10, 15 and 20 ppm. Against these standards, absorbance of soil and digestate samples was measured at 220 nm using a UV-spectrophotometer (UV-1100) (Hafeez et al., 2019). All the chemicals used for different analyses were of analytical grade. Net nitrification rate (mg N/kg/day), at various incubation dates (15 days, 30 days and 60 days), was calculated by the formula as proposed by (Sawada and Toyota, 2015):

$$\text{Net nitrification rate} = (NO_3\text{-}N_{xd} - NO_3\text{-}N_{0d}) / Xd \quad (4)$$

In this equation (Xd) represents the day of sampling (i.e. 15, 30 or 60) while 0d represents the concentration of nitrates at day 0 (initial concentration).

Statistical analyses

Statistical analyses were performed using SigmaPlot (version 12.0). Three way ANOVA was performed to analyze the impact of soil type, digestate type and time by Fisher LSD formula. Pearson correlation was run to investigate the relationship between various parameters. Origin-2018 and Excel were used for graphical representation.

Results and Discussion

Variations in soil pH, EC and Organic Carbon upon application of different digestates

Both soils were alkaline in nature (pH 8 for soil-1 and 7.8 for soil-2) as shown in Table 2. A slight increase in pH was observed for control treatments of both soils over the period of incubation. Increase in pH was more pronounced for control treatment of soil-2 as compared to soil-1 at days 15 and 30. Soil amendment with solid and liquid digestates increased the pH at day 15 (with no significant difference between the treatments) for soil-1. Then a decrease in pH was observed for both treatments (of soil-1) at days 30 and 60. Solid and liquid digestate amendments in soil-2 showed the same trend for pH change as of soil-1 (Table 2). Soils and digestates used in the present study showed alkaline pH. The pH values of both digestates were in line with already reported values (Panuccio et al., 2021; Xia and Murphy, 2016). Application of digestates on both soils immediately increased soil pH (at 15 days) and the same trend is also reported by (García-López et al., 2023). It can be inferred from these results that application of digestates can have positive effect on acidic soils in short term. But pH was decreased afterwards (30 and 60 days) which suggests that long term application of digestates on alkaline soils may favor the drop in soil pH (Kataki et al., 2017). Nitrification process may induce the release of H in soil solution which may ultimately lower the soil pH (Singleton, 2006). The decreasing pH may also be closely related to amino acid formation and decomposition of sugar into acetate and acetic acid during the anaerobic digestion process (Waqas et al., 2018).

The initial (at day 0) electrical conductivity values were almost similar in both soils (9.5 µS/cm for soil-1 and 10.1 µS/cm for soil-2). A gradual and significant increase in EC was observed for control treatments of both soils and this increase was more pronounced in soil-2. Soil amendment with solid and liquid digestates significantly increased EC as compared to control treatments in both soils. In soil-1, soil amendment with solid digestate increased the EC significantly higher than soil amendment with liquid digestate. But in soil-2, inverse trend was observed, soil amendment with liquid digestate showed higher values of EC at days 15 and 30 as compared to soil amendment with solid digestate. At day 60, soil amendment in soil-2 showed a similar trend for EC as of soil-1 (Table 2). Soil EC was significantly and positively correlated with concentration of nitrates and cumulative nitrogen which shows that nitrification process regulated the soil EC (Tables 3 & 4). Furthermore, the difference in EC between two soils upon application of digestates can be attributed to intrinsic properties (pH, organic matter, CEC) of soils as demonstrated by other researchers (Aimrun et al., 2009; Peralta and Costa, 2013). Higher EC may be a matter of concern with high application dosage because increased salinity tends to impart phytotoxic effect particularly during seedling establishment (Panuccio et al., 2021; Kataki et al., 2017).

As shown in Figure 2, there was a significant difference between initial carbon contents of both soils (1.8% for soil-1 and 2.6% for soil-2). Then a gradual and significant decrease was observed for control treatments of both soils over 60 days. The decrease in organic carbon was almost 50% for control-S1 and 62% for control-S2 from day 0 and 60.

Table-2. Changes in pH and EC in soil-1 and soil-2 following application of solid and liquid digestates at various days of incubation. The data are mean values of 3 replicates (n=3)±SD.

Soil type	Parameters	Treatments	Day 0	Day 15	Day 30	Day 60
Soil-1	pH	Control	8.0±0.38	8.3±0.17	8.4±0.69	8.3±0.27
		Solid		8.6±0.14	8.3±0.25	8.1±0.18
		Liquid		8.7±0.11	8.2±0.06	8.2±0.14
	Electrical conductivity (µS/cm)	Control	9.5±0.50	197.0±30.7	173.0±29.7	213.3±8.5
		Solid		379.5±6.4	519.0±69.3	374.3±81.1
		Liquid		237.3±20.4	267.0±15.6	275.0±37.6
Soil-2	pH	Control	7.8±0.30	8.3±0.33	8.7±0.47	7.8±0.08
		Solid		8.7±0.33	7.8±0.37	7.8±0.02
		Liquid		8.4±0.25	7.8±0.24	7.8±0.04
	Electrical conductivity (µS/cm)	Control	10.1±1.1	174.9±12.1	207.2±27.3	274.0±36.6
		Solid		289.5±6.4	311.0±21.5	428.5±2.1
		Liquid		323.5±48.8	315.0±59.2	390.0±36.4

For soil-1, organic carbon gradually decreased for amended soil (either with solid or liquid digestate) over a period of incubation (Figure 2a). Similarly, for soil-2, a slight increase (1%) in carbon contents was observed for soil amended with liquid digestate at day15, then a significant decrease was observed for all treatments throughout the incubation period. The carbon contents in soil-2 remained significantly higher for Sol-S2 and Li-S2 as compared to control treatment (control-S2) at all days of sampling (Figure 2b). Impact of digestate application on soil organic carbon was similar in trend but different in extent in different soils. Organic carbon decreased during 1st month of digestates application with varied degree (less decrease for solid digestate and more for liquid amended soil) for soil-1. A significant decrease in organic carbon was observed for soil-2 after application of both digestates throughout incubation period. It may be inferred that digestate application, in long term, may reduce the soil organic carbon which is in line with the findings of (Johansen et al., 2013). The different results in two soils suggest that soil is the main controlling factor for changes in its fertility as suggested by (Panuccio et al., 2021).

Nitrogen dynamics of soil upon application of different digestates

At day 0, concentration of ammonium in soil-1 was 7.2 mg/kg of soil (Figure 3a). A significant increase in ammonium concentration was observed at day 15 (32.3 mg/kg of soil) and day 30 (36.4 mg/kg of soil). No significant difference was observed in ammonium concentration among solid (11.1 mg/kg) and liquid (12.2 mg/kg) digestate treatments at day 15. At day 60 a significant decrease in ammonium concentration was observed for all three treatments. The final (day 60) concentration of ammonium in soil-1 ranged between 0.5 mg/kg soil (for control) and 3.1 mg/kg of soil (for liquid digestate) (Figure 3a). As demonstrated in Figure 3b, initial (day 0) ammonium concentration in soil-2 was low (2.9 mg/kg of soil) as compared to soil-1. At day 15, all treatments showed a significant increase in concentration of ammonium, while maximum increase was observed for control-S2 (36 mg/kg of soil). Ammonium concentration was 12 mg/kg for solid and 13 mg/kg for liquid digestates at day 15. The concentration of ammonium was decreased significantly for all treatments at day 60 but remained higher for control-S2 (8.8 mg/kg of soil) as compared to solid and liquid treatments (Figure 3b). The final (at day 60) concentration of ammonium in soil-2 ranged between 6.4 mg/kg soil (for liquid digestate) and 8.8 mg/kg of soil (for control).

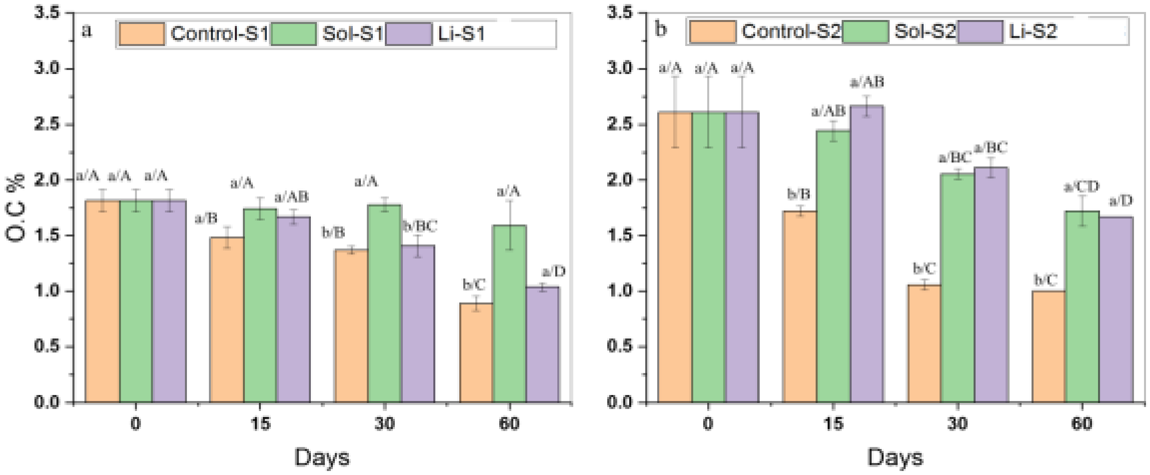

Figure-2. Variations in soil organic carbon upon application of various digestates in soil-1 (a) and soil-2 (b). Control, Sol and Li represent the control, solid digestate and liquid digestate treatments respectively. Small letters (a, b, and c) represent the differences between the treatments at a given incubation time, while the capital letters (A, B, C and D) represent the differences between the incubation times for a given treatment with p = <0.05.

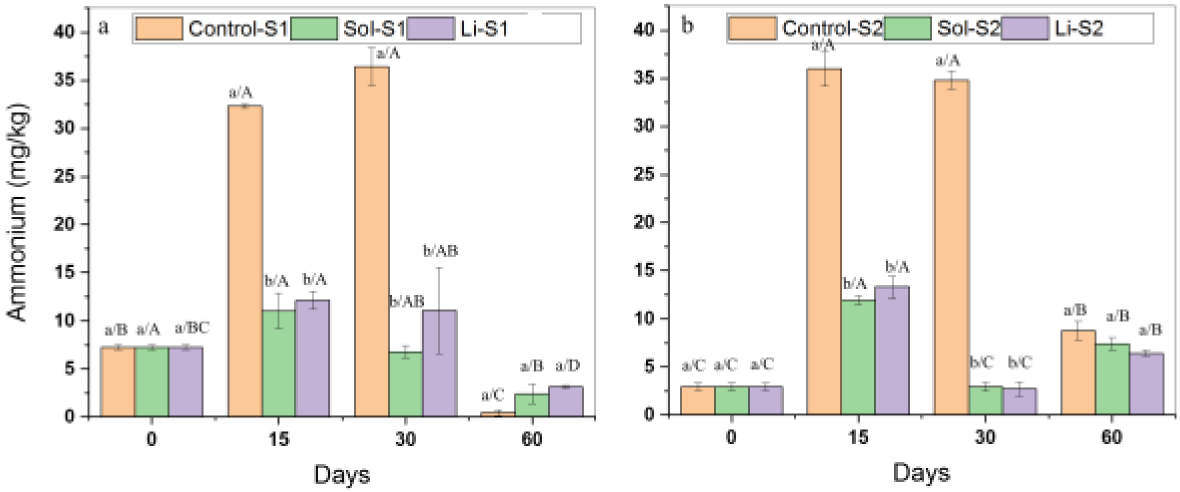

Figure-3. Variations in soil ammonium concentration upon application of various digestates in soil-1 (a) and soil-2 (b). Control, Sol and Li represent the control, solid digestate and liquid digestate treatments respectively. Small letters (a, b, and c) represent the differences between the treatments at a given incubation time, while the capital letters (A, B, C and D) represent the differences between the incubation times for a given treatment with p = <0.05.

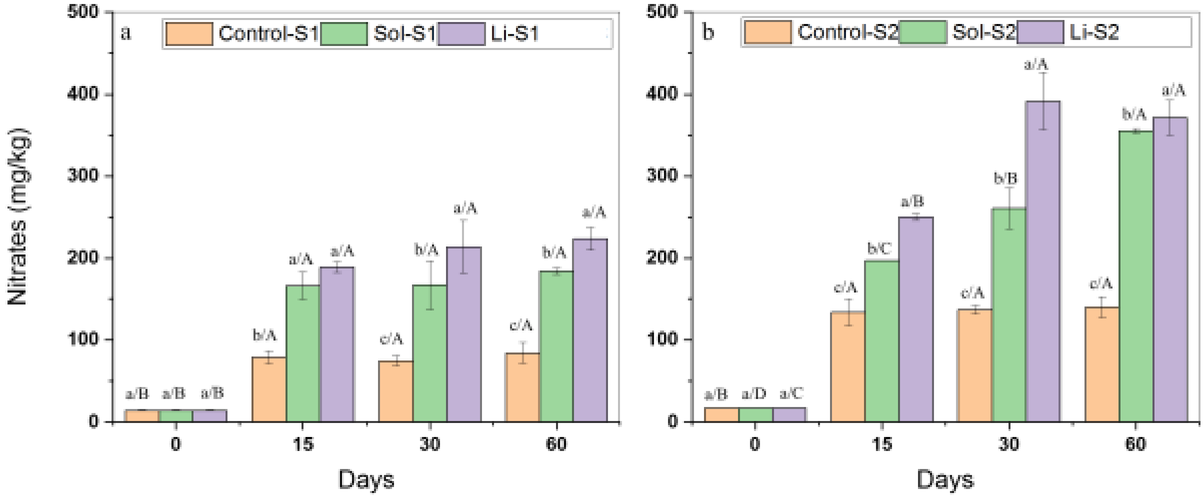

Figure-4. Variations in soil nitrates concentration upon application of various digestates in soil-1 (a) and soil-2 (b). Control, Sol and Li represent the control, solid digestate and liquid digestate treatments respectively. Small letters (a, b, and c) represent the differences between the treatments at a given incubation time, while the capital letters (A, B, C and D) represent the differences between the incubation times for a given treatment with p = <0.05.

The concentration of nitrates in soil-1 was 14.3 mg/kg of soil at 0 day (Figure 4a). A gradual increase in nitrate concentration was observed for all treatments, except for control-S1 with some fluctuations, over the entire period of incubation. At days 15, 30 and 60, the concentration of nitrates remained between 78 – 83 mg/kg soil for control-S1 treatment. No significant difference was observed in nitrates concentration among solid (166 mg/kg) and liquid (189 mg/kg) digestate treatments at day 15.

The final (at day 60) concentration of nitrates in soil-1 ranged between 83 mg/kg of soil (for control-S1) and 223 mg/kg of soil (for liquid digestate) (Figure 4a). Overall, the liquid digestate showed more concentration of nitrates as compared to solid digestate in soil-1 over a period of incubation. The initial (at day 0) concentration of nitrates in soil-2 was 17 mg/kg of soil (Figure 4b). Like in soil-1, a gradual increase in nitrate concentration was observed for all treatments from day 15 to day 60. Both digestates showed significantly higher concentration of nitrates as compared to control-S2. Soil-2 showed the same trend as soil-1 but the increase in nitrate concentration was highly pronounced for soil-2 as compared to soil-1 over the period of incubation. The final concentration of nitrates in soil-2 ranged between 140 mg/kg of soil (for control) and 372 mg/kg of soil (for liquid digestate). Similar to soil-1, soil-2 amended with liquid digestate showed higher concentration of nitrates as compared to soil-2 amended with solid digestate (Figure 4b).

The net nitrification rate was observed highest at day 15 for all treatments and both soils and decreased linearly till days 30 and 60 (Figure 5a, 5b). At day 15 for soil-1, the maximum net nitrification rate (11.6) was observed in soil amended with liquid digestate and the minimum (4.3) was observed for control treatment. The same trend was observed for soil-2 at day 15, but the values for net nitrification rate were higher (15.5 for liquid digestate and 7.8 for control treatment) as compared to soil-1. The lowest net nitrification rates were observed at day 60 for all treatments and both soils. Nevertheless, soil-2 showed higher net nitrification rates at all days of sampling as compared to soil-1.

As illustrated in Figure 6a, the concentration of cumulative mineral nitrogen (NH_4^+-N + NO_3^-N) in soil-1 was 21 mg/kg of soil at day 0. A significant increase in mineral nitrogen was observed for all treatments at day 15, and increase was highest for soil amended with liquid digestate (201 mg/kg of soil). The soil amendment (either with solid or liquied digestates) had a significant impact on soil mineral nitrogen as compared to control throughout the incubation period. The concentration of mineral nitrogen remained constant at day 30 for control-S1 and then decreased at day 60. The soil amended with liquid digestate showed higher mineral nitrogen as compared to soil amended with solid digestate at all days of sampling (Figure 6a). The final (at day 60) concentration of cumulative mineral nitrogen in soil-1 ranged between 80 mg/kg soil (for control) and 227 mg/kg of soil (Li-S1).

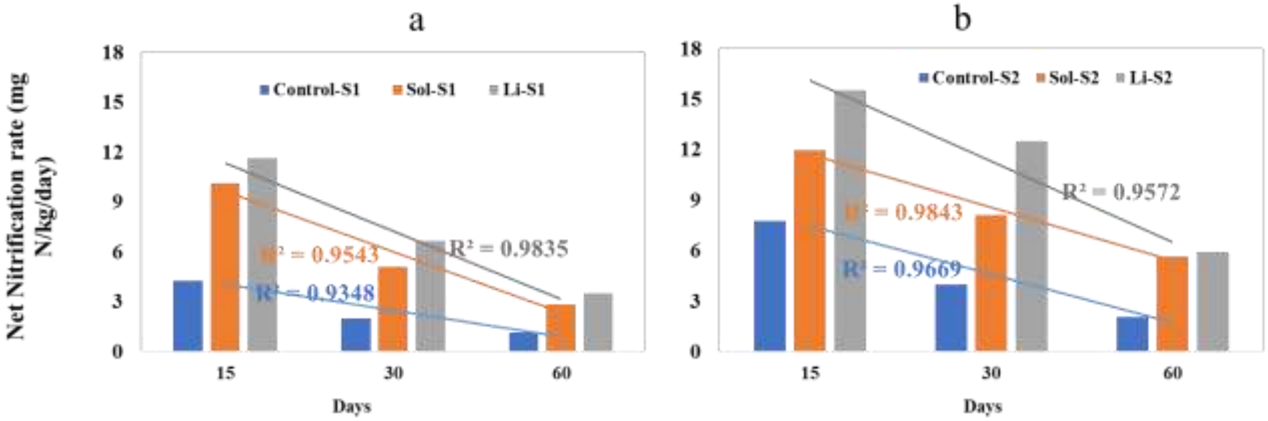

Figure-5. Soil net nitrification rate upon application of various digestates in soil-1 (a) and soil-2 (b). Control, Sol and Li represent the control, solid digestate and liquid digestate treatments respectively.

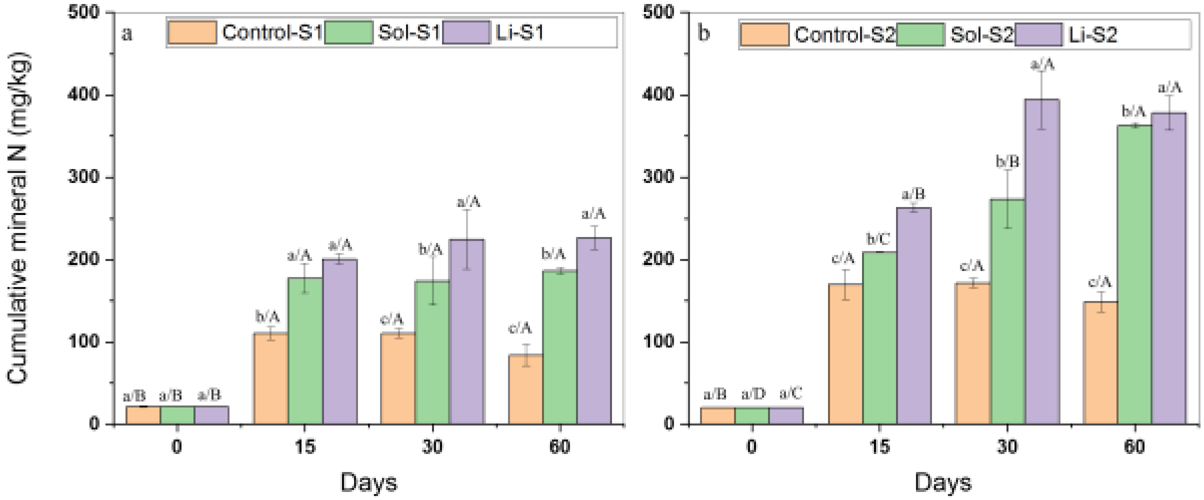

Figure-6. Variations in concentration of soil total nitrogen upon application of various digestates in soil-1 (a) and soil-2 (b). Control, Sol and Li represent the control, solid digestate and liquid digestate treatments respectively. Small letters (a, b, and c) represent the differences between the treatments at a given incubation time, while the capital letters (A, B, C and D) represent the differences between the incubation times for a given treatment with $p = <0.05$.

Soil-2 showed the same trend as soil-1 for mineral nitrogen (Figure 6b). But the concentration of mineral nitrogen remained significantly high in soil-2 as compared to soil-1 for all treatments despite having same initial concentrations (21 and 20 mg/kg of soil respectively). Similarly, as in soil-1, a significant increase in mineral nitrogen for soil-2 was observed for all treatments at day 15, and increase was highest for soil amended with liquid digestate (263 mg/kg of soil). The soil amendment (either with solid or liquid digestates) had a significant impact on soil mineral nitrogen as compared to control throughout the incubation period. The soil amended with liquid digestate showed higher mineral nitrogen as compared to soil amended with solid digestate over the entire period of incubation. The increase in concentration of mineral nitrogen for Sol-S2 treatment was significantly pronounced between days 30 and 60 as compared to days 15 and 30 (Figure 6b). These results suggest that swift nitrification occurred upon application of solid and liquid digestates to both soils, but at different extent, which is evident from the accumulation of soil nitrates after amendment with solid and liquid digestates. The increase in concentration of soil nitrates after digestate application is also established in previous studies (Johansen et al., 2013; Barłóg et al., 2020). Ammonium present in digestate is rapidly nitrified into nitrates and, furthermore, addition of digestate in soil may induce priming effect and as a result ammonium is nitrified into nitrates (Barłóg et al., 2020). Soil amended with liquid digestate promoted the accumulation of nitrates in both soils as compared to soil amended with solid digestate but the difference between two amendments was more pronounced in soil-2 in comparison to soil-1. Net nitrification rate for soil amended with liquid digestate also confirmed these findings. Higher nitrification in soil amended with liquid digestate can be attributed to initial high ammonium contents in liquid digestate as compared to solid digestate. Doyeni et al. (2021) also proposed that the high proportion of NH_4^+-N present in the digestate has the potential to increase NH_4^+-N content of the amended soil. Petraityte et al. (2022) also revealed that the highest nitrates concentration was observed following the application of liquid organic fertilizer during winter wheat vegetation. The net nitrification rate at 15 days for both soils suggests that no nitrogen immobilization was recorded and application of both digestates promoted nitrification without lag phase as suggested by (Sawada and Toyota, 2015). As revealed by another study, cumulative nitrogen content in soil were increased in all digestates amended soils in comparison to control (Yang et al., 2015). We also obtained similar results, where the highest soil cumulative nitrogen was in the digestate treatments. In current study, we observed differences between two soils with respect to the trends in soil cumulative nitrogen among the various treatments, consistent with the findings of another study (Cristina et al., 2020). These differences are likely related to differences in the intrinsic soil properties (Li et al., 2023).

Correlation between various parameters

The Pearson correlation between various parameters was summarized in Table 3 and Table 4. In soil-1, nitrates showed a negative correlation with organic carbon in solid and liquid digestate amended soils. While in soil-2, ammonium showed a strong positive correlation with pH for all treatments. Nitrates and cumulative nitrogen showed a positive correlation with EC in both soils and both digestates. Both these parameters showed a negative correlation with organic carbon in soil-2. Overall, nitrates, ammonium and net nitrification rate showed a positive correlation with EC and pH and a negative correlation with organic carbon. Such relationships among the soil edaphic factors have also been reported across a range of soil ecosystems (Zaman et al., 2023). A positive correlation between nitrification and pH was also established by (Sawada and Toyota, 2015) after application of digestates.

Table-3. Pearson correlation coefficients of various studied parameters of soil-1.

		NO_3^-	$NH_4^+ + NO_3^-$	Net nitrification rate	Organic carbon	pH	EC
Control-S1	NH_4^+	0.34	0.69	**0.72**	0.25	-0.15	0.30
	NO_3^-		**0.92**	0.65	**-0.80**	0.67	**0.99****
	$NH_4^+ + NO_3^-$			**0.81**	-0.51	0.45	**0.89**
	Net nitrification rate				0.11	0.56	0.65
	Organic carbon					-0.62	**-0.81**
	pH						**0.72**
Solid-S1	NH_4^+	-0.16	-0.12	0.64	0.66	**0.75**	-0.05
	NO_3^-		**0.99****	0.64	-0.65	0.53	**0.93**
	$NH_4^+ + NO_3^-$			0.68	-0.62	0.57	**0.93**
	Net nitrification rate				-0.05	**0.98***	0.63
	Organic carbon					0.13	-0.37
	pH						0.57
Liquid-S1	NH_4^+	0.06	0.11	0.71	0.58	0.56	0.09
	NO_3^-		**0.99****	0.63	**-0.75**	0.47	**1.0****
	$NH_4^+ + NO_3^-$			0.65	**-0.73**	0.49	**1.0****
	Net nitrification rate				0.02	**0.94**	0.64
	Organic carbon					-0.09	-0.74
	pH						0.49

The numbers in bold indicate a correlation, with $p = <0.05(*), <0.01(**)$.

Table-4. Pearson correlation coefficients of various studied parameters of soil-2.

		NO_3^-	NH_4^+ + NO_3^-	Net nitrification rate	Organic carbon	pH	EC
Control-S2	NH_4^+	0.66	**0.78**	**0.89**	-0.43	**0.90**	0.38
	NO_3^-		**0.98***	0.66	**-0.91**	0.53	**0.94***
	NH_4^+ + NO_3^-			**0.76**	**-0.86**	0.65	**0.87**
	Net nitrification rate				-0.32	0.60	0.40
	Organic carbon					-0.46	**-0.96***
	pH						0.30
Solid-S2	NH_4^+	0.31	0.31	**0.71**	-0.01	**0.89**	0.45
	NO_3^-		**1.0****	0.53	**-0.93**	-0.01	**0.99***
	NH_4^+ + NO_3^-			0.55	**-0.92**	-0.00	**0.99***
	Net nitrification rate				-0.19	**0.75**	0.65
	Organic carbon					0.36	**-0.86**
	pH						0.14
Liquid-S2	NH_4^+	0.11	0.14	0.61	0.30	**0.93**	0.44
	NO_3^-		**1.0****	0.62	**-0.72**	-0.13	**0.93**
	NH_4^+ + NO_3^-			0.64	**-0.71**	0.01	**0.94**
	Net nitrification rate				0.08	**0.70**	**0.70**
	Organic carbon					0.57	-0.61
	pH						0.26

The numbers in bold indicate a correlation, with $p = <0.05$(*), <0.01(**).

Impact of two rainfall patterns on leaching of NH_4^+ and NO_3^-

Rainfall frequency affected the leaching of both ammonium and nitrates in soil-1 (Table 5). Highest ammonium concentration in soil solution was observed for soil amended with liquid digestate (4.3 mg/l) during summer season at 2.5 cm soil depth and the lowest was for solid digestate amended soil (0.3 mg/l) during winter season. For summer season and soil amended with solid digestate, NH_4^+ concentration in soil solution was higher in the upper soil layer (2.5 cm from top) as compared to lower soil layer (7.5 cm) with one exception at day 22. For the same season, soil amended with liquid digestate showed a mix pattern of ammonium concentration in different soil depths at different days. Ammonium concentration in soil solution for solid digestate amended soil in winter season largely remained lower as compared to summer season. The same trend was observed in liquid amended soil except at day 45. By and large, ammonium concentration was higher in upper soil layer as compared to lower soil depth in soil amended with solid digestate. While soil amended with liquid digestate showed contradictory behavior. Overall, the concentration of nitrates was higher at both depths in both rainfall patterns as compared to ammonium. The concentration of nitrates in soil solution ranged between 1.1 mg/l and 14.2 mg/l. For the most part, nitrate concentration was higher in lower soil depth (7.5 cm) as compared to upper soil layer regardless of treatment and rainfall pattern. Contrary to ammonium concentration, overall, less concentration of nitrates was observed in soil solution for summer season as compared to winter season at later stages of incubation.

As far as soil-2 is concerned, highest ammonium concentration in soil solution was 4.7 mg/l and lowest was 0.2 mg/l (Table 6). Ammonium concentration in soil solution was higher in solid digestate amended soil as compared to soil amended with liquid digestate irrespective of soil depth and rainfall pattern. Same as in soil-1, concentration of nitrates in soil solution remained lower during summer season as compared to winter for both treatments (except for liquid amended soil at day 15). The concentrations of both ammonium and nitrate in soil solutions showed irregular variations over the period of experiment for both depths, treatments and rainfall patterns. Overall, the ammonium concentration in soil solution remained higher in soil amended with solid digestate as compared to liquid digestate for both seasons at both depths. The concentration of nitrates in soil solution ranged between 0.4 mg/l and 14.2 mg/l. Concentration of nitrates observed higher as compared to ammonium at both soil depths, both seasons and both treatments. In general, nitrates concentration was observed higher at lower soil depth (7.5 cm) as compared to upper soil layer for both treatments and both seasons (Table 6).

Table-5. Concentration of ammonium and nitrates in soil solutions from soil-1 at two soil depths (2.5 cm, 7.5 cm) following two rainfall patterns for two treatments.

			Depth	Day-0	Day-7	Day-15	Day-22	Day-30	Day-37	Day-45
NH_4^+ concentration in Soil-1 (mg/l)	Summer	Solid	2.5 cm	3.1±0.0	3.1±0.3	0.9±0.0	0.9±0.0	3.4±0.0	2.8±0.0	2.3±0.1
			7.5 cm	2.8±0.1	0.9±0.1	0.5±0.0	1.4±0.0	0.4±0.0	1.0±0.0	0.9±0.1
		Liquid	2.5 cm	1.0±0.1	1.3±0.0	2.7±0.0	0.7±0.0	0.5±0.0	4.3±0.1	1.7±0.0
			7.5 cm	4.2±0.1	0.6±0.0	2.2±0.1	1.3±0.0	0.7±0.1	0.6±0.0	2.8±0.3
	Winter	Solid	2.5 cm	2.7±0.0	-	1.9±0.0	-	0.7±0.1	-	0.3±0.0
			7.5 cm	2.5±0.1		0.8±0.0		0.8±0.0		0.9±0.0
		Liquid	2.5 cm	1.2±0.0		1.8±0.0		0.5±0.0		3.3±0.0
			7.5 cm	3.6±0.1		1.9±0.0		1.0±0.2		5.2±0.2
NO_3^- concentration in Soil-1 (mg/l)	Summer	Solid	2.5 cm	14.2±0.1	13.0±0.4	3.5±0.0	1.0±0.1	1.0±0.1	1.8±0.1	1.3±0.1
			7.5 cm	14.2±0.0	14.2±0.0	2.0±0.0	7.0±0.2	1.7±0.0	1.9±0.1	1.1±0.4
		Liquid	2.5 cm	12.3±0.1	14.2±0.1	3.8±0.4	4.3±0.4	1.1±0.2	2.4±0.1	2.8±0.0
			7.5 cm	13.2±0.1	6.4±0.0	2.8±0.1	5.0±0.1	2.1±0.1	8.0±0.1	3.4±0.5
	Winter	Solid	2.5 cm	12.9±0.0	-	13.9±0.4	-	1.1±0.1	-	9.6±0.0
			7.5 cm	12.7±0.1		13.7±0.1		12.9±0.4		4.5±0.1
		Liquid	2.5 cm	13.1±0.2		11.1±0.4		10.2±0.1		2.5±0.2
			7.5 cm	14.2±0.1		5.5±0.0		5.8±0.5		7.9±0.0

Table-6. Concentration of ammonium and nitrates in soil solutions from soil-2 at two soil depths (2.5 cm, 7.5 cm) following two rainfall patterns for two treatments.

			Depth	Day-0	Day-7	Day-15	Day-22	Day-30	Day-37	Day-45
NH_4^+ concentration in Soil-2 (mg/l)	Summer	Solid	2.5 cm	4.7±0.1	2.1±0.1	1.0±0.0	2.3±0.2	0.8±0.1	0.9±0.1	0.9±0.0
			7.5 cm	2.0±0.1	0.6±0.1	1.1±0.1	1.0±0.1	2.4±0.1	1.1±0.1	1.0±0.1
		Liquid	2.5 cm	0.8±0.1	1.0±0.3	0.7±0.1	0.6±0.1	0.9±0.1	1.0±0.0	0.2±0.1
			7.5 cm	0.6±0.2	1.0±0.0	0.9±0.3	0.7±0.0	0.5±0.0	0.7±0.1	1.4±0.0
	Winter	Solid	2.5 cm	4.1±0.0	-	1.3±0.0	-	0.4±0.0	-	2.1±0.1
			7.5 cm	1.8±0.0		1.4±0.0		0.6±0.0		0.5±0.0
		Liquid	2.5 cm	0.2±0.1		0.9±0.0		0.9±0.0		0.2±0.0
			7.5 cm	0.7±0.0		2.1±0.0		0.9±0.0		0.2±0.0
NO_3^- concentration in Soil-2 (mg/l)	Summer	Solid	2.5 cm	14.2±0.1	2.3±0.0	0.4±0.1	6.4±0.0	3.1±0.2	0.8±0.0	1.9±0.0
			7.5 cm	14.1±0.2	5.2±0.0	4.2±0.1	3.7±0.0	3.2±0.0	5.3±0.0	1.6±0.1
		Liquid	2.5 cm	8.6±0.1	2.0±0.1	8.3±0.0	3.2±0.0	1.5±0.0	2.6±0.1	0.5±0.0
			7.5 cm	14.2±0.0	4.4±0.1	6.5±0.0	4.3±0.0	1.5±0.0	2.4±0.1	3.1±0.1
	Winter	Solid	2.5 cm	14.2±0.1	-	1.2±0.0	-	4.2±0.0	-	4.8±0.1
			7.5 cm	10.3±0.2		5.2±0.1		4.3±0.0		4.1±0.0
		Liquid	2.5 cm	6.6±0.0		1.7±0.0		2.2±0.1		5.4±0.1
			7.5 cm	12.4±0.1		3.9±0.0		8.6±0.1		2.6±0.3

Generally, ammonium concentration was higher in upper soil layer as compared to lower soil depth in soil amended with solid digestate regardless of season and soil type. These findings are in line with (Lili et al., 2016), who showed that maximum ammonium concentration was found at soil surface (0-2 cm soil depth). While soil amended with liquid digestate showed contradictory behavior to above statement and could be explained by the fact that liquid digestate traveled to bottom after rainfall and induced accumulation of ammonium in depth. Ammonium concentration in soil solution for solid digestate amended soil in winter season largely remained lower as compared to summer season. The same trend was observed in liquid amended soil except at day 45. Overall, the concentration of nitrates was higher in both soils, at both depths and both rainfall patterns as compared to ammonium. These results indicate that the nitrates could infiltrate more easily than ammonium, which is in line with the findings of (Lili et al., 2016; Svoboda et al., 2013). It can be deduced from these findings that with higher dose and deep application of digestates, groundwater could get contaminated with nitrates. In contradiction to ammonium, overall, less concentration of nitrates was observed in soil solution for summer season as compared to winter season at later stages of incubation irrespective of soil type and digestate type. Kabala et al. (2017) also linked the higher concentration of nitrates in soil solution with preceding droughts. Current study also demonstrated more nitrates in soil solution during winter season, which received rainfall after 15 days in comparison to 7 days gap during summer season.

Conclusion

Two types of digestates (solid and liquid) were applied at two soils, which differed in cultivation practices and innate properties, and incubated at a controlled temperature for 60 days. Destructive soil sampling was done at days 15, 30 and 60 for measurement of pH, EC, O.C, ammonium and nitrate. The acquired results from this experiment showed that fluctuations of above cited parameters were controlled by both digestate and soil type. pH of soil-1 increased from 8.0 (day 0) to 8.6 (at days 15) and then decreased to 8.1 (at day 60) following the application of both digestates. Soil amendment with solid and liquid digestates increased EC up to 1.7 and 1.5 times (for soil-1 and soil-2 respectively) as compared to respective control treatments in both soils at the end of incubation. Impact of digestate application on soil organic carbon was similar in trend but different in extent within two soils. Generally at 60 days, the trend for organic carbon contents was Sol-S1> Sol-S2>Li-S2>Li-S1> Control-S1>Control-S2. Overall, concentration of ammonium remained higher in controls of both soils compared to amended soils (irrespective of digestate type). On the contrary, concentration of nitrate was observed higher for amended soils as compared to controls of both soils and the increase was more pronounced for liquid treatment. Furthermore, concentration of nitrates was higher in soil-2 as compared to soil-1. The applied digestates produced different cumulative nitrogen content in the studied soils with similar dose of application. Overall, the concentration of nitrates was higher in both soils, at both depths and at both rainfall patterns as compared to ammonium, which indicate that the nitrates could infiltrate more easily than ammonium. It can be deduced from these results that leaching of nitrates may affect the crop productivity and may also exacerbate the groundwater pollution. Therefore, special attention is needed regarding the amounts and timing of the application of digestates in various soils. Finally it can be inferred from this study that the advantages and disadvantages associated with digestates' application in soil are dependent on soil characteristics, digestate type and water availability (rainfall patterns in this case).

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Contribution of Authors

Bano S: Conducted experiments, collected data and wrote the first draft

Rashid MI: Article review, editing and validation

Akhtar A: Conducted experiments and collected data

Hafeez F & Nazir R: Helped in data interpretation, edited manuscript and acquired funds

Ullah F: Co-supervised the student, provided the resources and helped in data interpretation

Irshad M & Ondrasek G: Data interpretation, proofreading of the manuscript and editing

Iqbal A: Conceived idea, designed research, supervised the study, data interpretation, statistical analyses, helped in write up, final approval of the manuscript and acquired funding.

References

Abbas Y, Yun S, Mehmood A, Shah FA, Wang K, Eldin ET, Al-Qahtani WH, Ali S and Bocchetta P, 2023. Co-digestion of cow manure and food waste for biogas enhancement and nutrients revival in bio-circular economy. Chemosphere. 311, 137018.

Abubaker J, Risberg K and Pell M, 2012. Biogas residues as fertilizer effects on wheat growth and soil microbial activities. Appl. Energy. 99: 26–134.

Adam D, 2021. How far will global population rise? Researchers can't agree. 597: 462–465.

Afridi ZUR and Qammar NW, 2020. Technical challenges and optimization of biogas plants. Chem Bio Eng Reviews. 7: 119–129. https://doi.org/10.1002/cben.202000005

Aimrun W, Amin MSM, Rusnam M, Ahmad DESA, Hanaf MM and Anuar AR, 2009. Bulk soil electrical conductivity as an estimator of nutrients in the maize cultivated land. Eur. J. Sci. Res. 31: 37–51.

Arshad M, Ansari AR, Qadir R, Tahir MH, Nadeem A, Mehmood T, Alhumade H and Khan N, 2022. Green electricity generation from biogas of cattle manure: An assessment of potential and feasibility in Pakistan. Front. Energy Res, 10, 911485.

Awan MI, Raza S, Farooq A, Nawaz A and Aziz T, 2022. Chapter 4 - Drivers of increased nitrogen use in Pakistan. Nitrogen Assessment, Pakistan as a Case-Study. 53-71.

Barłóg P, Hlisnikovský L and Kunzová E, 2020. Effect of Digestate on Soil Organic Carbon and Plant-Available Nutrient Content Compared to Cattle Slurry and Mineral Fertilization. Agronomy. 10, 379. https://doi.org/10.3390/agronomy10030379

Black CA, 1965. "Methods of Soil Analysis: Part I Physical and mineralogical properties". American Society of Agronomy. Madison, Wisconsin, USA.

Cristina G, Camelin E, Tommasi T, Fino D and Pugliese M, 2020. Anaerobic digestates from sewage sludge used as fertilizer on a poor alkaline sandy soil and on a peat substrate: Effects on tomato plants growth and on soil properties. J Environ Manage. 269, 110767. https://doi.org/10.1016/j.jenvman.2020.110767

Czekała W, 2022. Digestate as a Source of Nutrients: Nitrogen and Its Fractions. Water. 14, 4067. https://doi.org/10.3390/w14244067

Downes MT, 1978. An improved hydrazine reduction method for the automated determination of low nitrate levels in freshwater. Water Res. 12: 673–675.

Doyeni MO, Stulpinaite U, Baksinskaite A, Suproniene S and Tilvikiene V, 2021. The Effectiveness of Digestate Use for Fertilization in an Agricultural Cropping System. Plants. 10, 1734. https://doi.org/10.3390/plants10081734

Fontaine S, Bardoux G, Abbadie L and Mariotti A, 2004. Carbon input to soil may decrease soil carbon content. Ecol. Lett. 7: 314–320.

García-López M, Delgado A, Anjos O and Horta C, 2023. Digestate Not Only Affects Nutrient Availability but Also Soil Quality Indicators. Agronomy. 13, 1308. https://doi.org/10.3390/agronomy13051308

Good AG and Beatty PH, 2011. Biotechnological Approaches to Improving Nitrogen Use Efficiency in Plants: Alanine Aminotransferase as a Case Study. In: Hawkesford, M.J. and Barraclough, P., Eds., the Molecular and Physiological Basis of Nutrient Use Efficiency in Crops, Wiley-Blackwell, Oxford. 165-192.

Graham F, 2019. Daily briefing: world population will push 10 billion by 2050. https://www.nature.com/articles/d41586-019-01959-0

Guan D, Zhao J, Wang Y, Fu Z, Zhang D, Zhang H, Xie J, Sun Y, Zhu J and Wang D, 2024. A critical review on sustainable management and resource utilization of digestate. Process Saf. Environ. Protect. 183, 339–354.

Hafeez F, Zafar N, Nazir R, Javeed HMR, Rizwan M, Faridullah, Asad SA and Iqbal A, 2019. Assessment of Flood Induced Changes in Soil Heavy Metal and Nutrient Status in Rajanpur, Pakistan. Environ. Monit. Assess. 191, 234.

Iqbal A, Aslam S, Alavoine G, Benoit P, Garnier P and Recous S, 2015. Rain regime and soil type affect the C and N dynamics in soil columns that are covered with mixed-species mulches. Plant Soil. 393: 319-334.

Irfan M, Zhao ZY, Rehman A, Ozturk I and Li H, 2021. Consumers' intention−based influence factors of renewable energy adoption in Pakistan: a structural equation modeling approach. Environ Sci Pollut Res, 28. 1: 432–445.

Jahangir MMR, Khalil MI., Johnston P, Cardenas L M, Hatch D J, Butler M, Barrett M, O'flaherty V and Richards KG, 2012. Denitrification potential in subsoils: A mechanism to reduce nitrate leaching to groundwater. Agric. Ecosyst. Environ. 147: 13-23.

Johansen A, Carter MS, Jensen ES, Hauggard-Nielsen H and Ambus P, 2013. Effects of digestate from anaerobically digested cattle slurry and plant materials on soil microbial community and emission of CO2and N2O. Appl. Ecol. 63: 36–44.

Kabala C, Karczewska A, Gałka B, Cuske M and Sowiński J, 2017. Seasonal dynamics of nitrate and ammonium ion concentrations in soil solutions collected using MacroRhizon suction cups. Environ. Monit. Assess. 189, 304.

Kataki S, Hazarika S and Baruah DC, 2017. Assessment of by-products of bioenergy systems (anaerobic digestion and gasification) as potential crop nutrient. Waste Manage. 59: 102–117. http://dx.doi.org/10.1016/j.wasman.2016.10.018.

Khan MU, Ahmad M, Sultan M, Sohoo I, Ghimire PC, Zahid A, Sarwar A, Farooq M, Sajjad U, Abdeshahian P and Yousaf M, 2021. Biogas Production Potential from Livestock Manure in Pakistan. Sustainability, 13, 6751.

Li F, Yuan Y, Shimizu N, Magaña J, Gong P and Na R, 2023. Impact of organic fertilization by the digestate from by‑product on growth, yield and fruit quality of tomato (Solanum lycopersicon) and soil properties under greenhouse and field conditions. Chem. Biol. Technol. Agric. 10, 70. https://doi.org/10.1186/s40538-023-00448-x

Lili W, Wenzhe L, Zhongjiang W, Zhiwu W, Chao S and Yan L, 2016. Effects of digestate application depth on soil nitrogen volatilization and vertical distribution. Int J Agric & Biol Eng. 9. 5: 101−107.

Mason-Jones K, Schmücker N and Kuzyakov Y, 2018. Contrasting effects of organic and mineral nitrogen challenge the N-mining hypothesis for soil organic matter priming. Soil Biol. Biochem. 124: 38–46.

Moodie CD, Smith WH and McCreery RA, 1959. Laboratory manual of soil fertility Department of Agronomy Pullman: State College of Washington. 31–39.

Ndubuaku U, Imegwu C and Ndubuaku N, 2013. Nutrient compositions of liquid and solid fractions of organic waste fermentation and the influence on growth and yield of okra. Int J Curr Tr Res. 21: 415–424.

Nkoa R, 2014. Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review. Agron. Sustain. Dev. 34, 473–492.

Nyang'au JO, Sørensen P and Møller HB, 2023. Nitrogen availability in digestates from full-scale biogas plants following soil application as affected by operation parameters and input feedstocks. Bioresour. Technol. Rep. 24, 101675.

Panuccio R, Romeo F, Mallamaci C and Muscolo A, 2021. Digestate Application on Two Different Soils: Agricultural Benefit and Risk. Waste Biomass Valori. 12: 4341-4353.

Peralta NR and Costa JL, 2013. Delineation of management zones with soil apparent electrical conductivity to improve nutrient management. Comput. Electron. Agric. 99: 218–226.

Petraityte D, Arlauskiene A and Ceseviciene J, 2022. Use of Digestate as an Alternative to Mineral Fertilizer: Effects on Soil Mineral Nitrogen and Winter Wheat Nitrogen Accumulation in Clay Loam. Agronomy. 12, 402. https://doi.org/10.3390/agronomy12020402

Prask H, Szlachta J, Fugol M, Kordas L, Lejman A, Tużnik F and Tużnik FS, 2018. Sustainability Biogas Production from Ensiled Plants Consisting of the Transformation of the Digestate into a Valuable Organic-Mineral Granular Fertilizer. Sustainability. 10 (3), 585.

Rayment GE and Higginson FR, 1992. Australian laboratory handbook of soil and water chemical methods. Melbourne: Inkata Press Pty Ltd.

Raza S, Zhou J, Aziz T, Afzal MR, Ahmed M, Javaid S and Chen Z, 2018. Piling up reactive nitrogen and declining nitrogen use efficiency in Pakistan: a challenge not challenged (1961–2013). Environ. Res. Lett, 13 (3), 034012.

Rodríguez-Espinosa T, Papamichael I, Voukkali I, Gimeno AP, Candel MBA, Navarro-Pedreño J, Zorpas AA and Lucas IG, 2023. Nitrogen management in farming systems under the use of agricultural wastes and circular economy. Sci. Total Environ. 876, 162666.

Sawada K and Toyota K, 2015. Effects of the Application of Digestates from Wet and Dry Anaerobic Fermentation to Japanese Paddy and Upland Soils on Short-Term Nitrification. Microbes and Environment. 1: 37-43.

Shahzad AN, Qureshi MK, Wakeel A and Misselbrook T, 2019. Crop production in Pakistan and low nitrogen use efficiencies. Nat. Sustain. 2: 1106–1114. https://doi.org/10.1038/s41893-019-0429-5

Singleton P, 2006. Nutrient Management Concepts: pH & Nutrient Formulation. University of Hawaii Cooperative Extension Service.

Sogn TA, Dragicevic I, Linjordet R, Krogstad T, Eijsink VGH and EichGreatorex S, 2018. Recycling of biogas digestates in plant production: NPK fertilizer value and risk of leaching. Int. J. Recycl. Org. Waste Agric. 7: 49–58.

Svoboda N, Taube F, Wienforth B, Klu C, Kage H and Herrmann A, 2013. Nitrogen leaching losses after biogas residue application to maize. Soil Tillage Res. 130. 6: 69–80.

Tuszynska A, Wilinska A and Czerwionka K, 2021. Phosphorus and nitrogen forms in liquid fraction of digestates from agricultural biogas plants, Environ. Technol. 42: 3942-3954. https://doi.org/10.1080/09593330.2020.1770339

van Dijk M, Morley T, Rau ML and Saghai Y, 2021. A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. 2: 494–501.

Waqas M, Rehan M, Khan MD and Nizami AS, 2018. Conversion of food waste to fermentation products. In: Ferranti P, Berry EM, Anderson JR (Eds.), Encyclopedia of Food Security and Sustainability. 501–509.

Xia A and Murphy JD, 2016. Microalgal cultivation in treating liquid digestate from biogas systems. Trends Biotechnol. 34: 264–275.

Yang J, Gao W and Ren S, 2015. Long-term effects of combined application of chemical nitrogen with organic materials on crop yields, soil organic carbon and total nitrogen in fluvo-aquic soil. Soil Tillage Res. 151: 67–74.

Zaman T, Asad SA, Irshad M, Faridullah, Shahzad M, Nazir R, Arefeen A, Iqbal A and Hafeez F, 2023. Unraveling the impact of human perturbation on nitrogen cycling in terrestrial ecosystems of lower Himalaya, Pakistan. Environ. Monit. Assess. 95, 1324.

Zeb I, Kafle GK, Xue X and Ndegwa PM, 2019. Enhanced treatment of flush-dairy manure in anaerobic sequencing batch reactors using a cationic polymer. Biosyst. Eng. 186: 195-203.