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# The Impact of Single and Cumulative Applications of Biogas Liquid Digestate on Soil and Plant

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# 5 Abstract

The surge in biogas energy production has resulted in an accumulation of liquid digestate, 6 a byproduct with possible agricultural utility. To discern its benefits and shortcomings, a field 7 trial was conducted to evaluate the effects of different doses of liquid digestate on maize yield, 8 soil salinity, leaf and grain nitrogen (N) content. The study included both single-year and 9 consecutive two-year applications of liquid digestate at doses of 10, 30, 50, and 70 t ha<sup>-1</sup>. Based 10 on maize N requirements, any N deficit was supplemented with chemical fertilizers. For the 11 one-year experiment, the highest grain yield was obtained from the chemical fertilization 12 treatment and 70 t ha<sup>-1</sup> dose of liquid digestate. In the two consecutive years, 70 t ha<sup>-1</sup> dose gave 13 the highest grain yield. Liquid digestate provided N to the soil as effectively as chemical 14 fertilization and stabilized the soil pH within approximately 1 month. However high doses of 15 digestate resulted in increased soil salinity and decreased N use efficiency (NUE). Consecutive 16 two-year application increased electrical conductivity (EC) and pH stabilization in the soil to a 17 greater extent than single-year applications. However, there was no difference in the N content 18 19 of the plant between single-year and two consecutive applications. In summary, liquid digestate provides significant agricultural benefits such as pH stabilization and increased inorganic N 20 levels. However, our findings indicate that overuse can lead to soil salinity and N losses, 21 underscoring the importance of balanced application to maximize its benefits while minimizing 22 23 potential drawbacks.

24 Keywords: Liquid digestate, maize, nitrogen, soil, electrical conductivity, pH.

# 2526 1. Introduction

Modern agricultural practices aim to continually increase productivity to meet the food needs of the growing global population. However, the excessive use of chemical fertilizers not only disrupts the natural structure of the soil but also leads to environmental issues (Geisseler

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& Scow, 2014; Marschner et al., 2003; Zhong et al., 2010). Among these problems are the adverse effects on soil microorganisms and contamination of water reservoirs (Rohila et al., 2017). Soil conservation and enhancement are at the core of sustainable agriculture. At this juncture, the consideration of natural resources and recyclable wastes as alternatives to chemicals gains significance. In this context, introducing the liquid digestate (LD) resulting from biogas energy production to agricultural use carries the potential to reduce environmental pollution while enhancing soil productivity.

Biomass-based fuels are increasingly crucial in meeting energy needs (Canisares et al., 37 2017; Rawoof et al. 2021). In particular, biogas stands out as a bioenergy source with a low 38 ecological footprint (Deviren et al., 2017; SAPEA, 2012). The globally rising number of biogas 39 facilities results in an increase in the amount of digestate waste produced (Karimi et al., 2022). 40 Sustainable biogas production necessitates the reuse of by-products to maintain economic 41 balance (Holm-Nielsen et al., 2009). Considering climate change, this can be seen as an 42 43 opportunity to return digestate to the soil, thereby reducing greenhouse gas emissions and enhancing carbon sequestration in the soil (Karimi et al., 2022). When properly utilized, this 44 material can serve as a soil enhancer and fertilizer, strengthening the concept of circular 45 agriculture (Shi et al., 2018; Jurgutis et al., 2021; Sürmen and Kara, 2022). 46

During the biogas production process, energy is transferred to methane molecules from 47 organic waste through anaerobic digestion (Angelidaki and Ellegaard, 2003), while nitrogen 48 49 (N) and other nutrients are retained in the digestate (Massé et al., 2007). The solid fraction of the waste material released after biogas production represents carbon (C) sequestration, while 50 the liquid fraction signifies richness as a plant nutrient source (Robles-Aguilar et al., 2019; 51 Barduca et al., 2021). Although the solid digestate has the potential to increase the carbon 52 53 content of the soil (Möller, 2015), it carries a risk of significant NH<sub>3</sub>-N (ammonia) loss during storage due to its high pH value (Brito et al., 2008). When LD is managed correctly, it can serve 54 as a fertilizer and soil amendment (Chookietwattana et al., 2016). The presence of N, 55 phosphorus (P), and potassium (K) elements in LD and the improvement of soil physical 56 57 properties due to its organic compounds suggest its agricultural importance (Insam et al., 2009). However, since not all organic material can be utilized by microorganisms within the biogas 58 59 process, digestate can come with potential risks to the soil and environment (Bationo et al., 2007). Residual organic compounds in the digestate may include phytotoxic substances, heavy 60 metals, or excess nutrients that can accumulate in the soil (Singh et al., 2010). For effective use 61 of digestate, outcomes should be determined based on dosage, cumulative effects, and soil type 62 (Karim et al., 2022), and environmental impacts should be researched (Urra et al. 2019). 63

Especially concerns arise due to its high ammonium  $(NH_4^+)$  and salinity content, which might 64 lead to adverse effects on soil and plants (Fransman & Nihlgard, 1995). In some regions, these 65 concerns have restricted the agricultural use of LD (Piccoli et al., 2022). Determining the 66 positive and negative effects of this material on soil and plant development is important for 67 68 assessing its agricultural impact (Diacono ve Montemurro, 2010). However, many studies are based solely on one cultivation season or are short-term experiments under controlled 69 conditions (Głowacka et al., 2020). Furthermore, while the high ammonia nitrogen content in 70 LD seems advantageous in terms of making nitrogen available in a form plants can use, it 71 suggests potential issues like ammonia nitrogen evaporating into the atmosphere and losses in 72 the form of nitrates (Fransman & Nihlgard, 1995; Gurbuz & Oz, 2016; Basak et al., 2020). 73

This study aims to examine the effect of LD on some soil properties and the yield of maize
plants under field conditions. The study evaluated the cumulative effects of LD over two years.
Furthermore, effective management and usage of LD in terms of sustainable agriculture were
determined as the primary objectives.

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#### 79 2. Materials and Methods

## 80 2.1. Trial area and material

81 The trial was conducted in 2022 at the Kırklareli Atatürk Soil, Water, and Agricultural Meteorology Research Institute's field, located at a latitude of 41°42'11" N and a longitude of 82 27°12'29" E. The region's annual average temperature is 13.3 °C, and the total average rainfall 83 is 48.7 mm (TSMS, 2022). There was no rainfall for 9 days following the application of the 84 liquid digestate, but on the 10th day, a precipitation of 5.7 mm was recorded. Rainfall data was 85 monitored with the institute's meteorological station. The soil in the top 0-30 cm layer has a pH 86 of 8.01, a loamy texture, and an organic matter content of 1.39% (Table 1). The soil was 87 analyzed at three depths (0-30 cm, 30-60 cm, and 60-90 cm) to capture the vertical distribution 88 of key soil properties. Soil samples for the results were taken from the 0-30 cm depth. The soil 89 90 sampling was carried out on 10 May, 20 June, 2 Aug. and 21 Oct. using a soil auger. These dates represent the 1st, 2nd, 3rd, and 4th sampling periods, respectively. These periods were 91 chosen to monitor soil properties at regular intervals from the time of LD application to harvest. 92 For each treatment plot, five soil samples were randomly collected and combined to form a 93 composite sample for this depth. For the study, the DKC6630 grain corn seed from Monsanto, 94 which is commonly used in the region, was selected as the plant material. This variety belongs 95 to the FAO 600 maturity group and is known for its drought tolerance, and resistance to 96 97 common diseases. It is cultivated as a main crop in the region due to its adaptability to local

- 98 conditions. Water for irrigation was sourced from a deep well located within the institute's
- 99 premises. The used water has values of  $7.30 \pm 0.03$  pH,  $1.10 \pm 0.05$  dS m<sup>-1</sup> EC,  $0.14 \pm 0.001$

100 NH<sub>4</sub><sup>+</sup>-N, and  $0.68 \pm 0.02 \text{ NO}_3^-$ -N.

1	0	1
1	0	2

**Table 1.** Physical and chemical characteristics of the experiment site soil at different depths (0-30 cm, 30-60 cm, and 60-90 cm).

	0-30	30-60	60-90
рН	$8.01 \pm 0.1$	$8.04 \pm 0.1$	$8.07\pm0.15$
EC (dS/m)	$0.18\pm0.02$	$0.19\pm0.02$	$0.19\pm0.01$
CaCO <sub>3</sub> (%)	$11.02\pm0.3$	$10.78\pm0.25$	$9.45\pm0.25$
Orcanic matter (%)	$1.39\pm0.08$	$1.24\pm0.07$	$1.07\pm0.06$
NH4 <sup>+</sup> -N (mg kg <sup>-1</sup> )	$9.22 \pm 2$	$9.10 \pm 1.8$	$4.17 \pm 2,3$
NO <sub>3</sub> -N (mg kg <sup>-1</sup> )	$3.41 \pm 0.7$	$8.02 \pm 1.4$	$14.95 \pm 3,6$
$P_2O_5$ (kg da <sup>-1</sup> )	$9.71 \pm 0.3$	$10.14\pm0.3$	$11.86\pm0.4$
$K_2O$ (kg da <sup>-1</sup> )	$44.55 \pm 2$	$33.12 \pm 1.5$	$25.89 \pm 1.2$
Texture (%)	47 s	and, 30,65 silt, 22,35	clay
C: Electrical Conductivit	y; NO <sub>3</sub> <sup>-</sup> -N: nitrate.		

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Data are presented as mean  $\pm$  standard error.

## 106 **2.2. Liquid digestate**

LD was sourced from a private biogas plant facility situated in the Babaeski district of 107 Kırklareli. This establishment processes roughly 1,050 tons of animal and agricultural organic 108 waste daily, including cattle manure (60%), plant waste (primarily maize and sunflower 109 residues; 20%), sheep manure (10%), and industrial waste (10%), to generate biogas energy. 110 At the facility, the resultant solid-liquid mixture (slurry) is separated using a centrifuge, and the 111 liquid fraction is hygienized at 70°C for 1 hour to neutralize pathogens. Specific characteristics 112 of the liquid digestate are outlined in Table 2. 113 Table 2. Chemical charactiristic of liquid digestate 114

Organic Carbon (%)  $0,67 \pm 0.03$ pН  $8,90 \pm 0.2$ Drv matter (%)  $2.41 \pm 0.13$  $K (mg l^{-1})$  $2.387.00 \pm 20$ EC (dS m<sup>-1</sup>)  $23.61 \pm 0.4$ **P** (mg l<sup>-1</sup>)  $284.20 \pm 2.8$  $0.47 \pm 0.02$ Cl (mg l<sup>-1</sup>)  $6.980.00 \pm 69$ Total N (%) NH4<sup>+</sup>-N (%)  $0,37 \pm 0.01$ Na (mg l<sup>-1</sup>)  $845.00 \pm 8.4$ 

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Data are presented as mean  $\pm$  standard error.

## 117 **2.3. Experiment set up and practices**

The study was conducted in two separate experiment areas: The first area (A) was chosen 118 for a one year liquid digestate application (OYA), while the second area (B) was selected for 119 consecutive two-year liquid digestate applications (TYA) (Fig. 1). Liquid digestate was applied 120 to plots at different doses (10, 30, 50, and 70 tons ha<sup>-1</sup>) 20 days before planting, and in cases of 121 nitrogen deficiency, the specified doses were supplemented with chemical fertilizer. Each 122 treatment had three replicates. Based on the study by Yakan and Saglam (1997), which 123 recommended 210 kg N ha<sup>-1</sup> to obtain the highest N rate per grain in maize under regional 124 conditions, this amount was adopted for our experiment. The remaining nitrogen requirement 125 after LD treatments (e.g., 10 tons of liquid digestate provides 47 kg N<sup>-1</sup>) was supplemented with 126

127 urea fertilizer as shown in Table 3. Since the soil already contained adequate levels of phosphorus (P), potassium (K), and trace elements necessary for maize plants, no additional 128 fertilization containing these elements was carried out. Drip irrigation was applied based on the 129 plant's water needs. During the growth period of the plant, soil samples were taken five times 130 131 in total, and inorganic nitrogen, organic matter, pH, and salinity analyses were conducted. Harvesting was done upon determining the R6 maturity phase, grains were separated from their 132 cobs, moisture percentages were calculated, and samples were taken for protein analyses. The 133 raw yields of all plots were recorded by weighing the grains. Agricultural practices carried out 134 during the study are presented in Table 4. 135



Fig. 1. Experiment site layout.



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**Table 3.** Experiment topics, application amounts, and periods.

Trootmonto	Applications	<b>Mineral Fertilization Periods</b>			
Treatments	Applications	Sowing Period	Hoeing Period		
G <sub>0</sub>	Control	-			
GM	$(456 \text{ kg ha}^{-1} \text{ urea})$	228 kg ha <sup>-1</sup> urea	228 kg ha <sup>-1</sup> urea		
G1	$10 \text{ ton } LD + 330,4 \text{ kg ha}^{-1} \text{ urea}$	102,1 kg ha <sup>-1</sup> urea	228,2 kg ha <sup>-1</sup> urea		
G3	$30 \text{ ton } \text{LD} + 78,2 \text{ kg ha}^{-1} \text{ urea}$	-	78,2 kg ha <sup>-1</sup> urea		
G5	50 ton LD	-	-		
$G_7$	70 ton LD	-	-		
<b>Fable 4.</b> Agrid <b>Date</b>	cultural practices. Practices	Date	Practices		
Table 4. Agrid           Date           23.04.2022	cultural practices. Practices Liquid digestate application	<b>Date</b> 05.06.2022	Practices Weedicide application		
Table 4. Agrid           Date           23.04.2022           02.05.2022	cultural practices. Practices Liquid digestate application Soil tillage	<b>Date</b> 05.06.2022 11.06.2022	<b>Practices</b> Weedicide application Hoeing		
Table 4. Agrid           Date           23.04.2022           02.05.2022           02.05.2022	cultural practices. Practices Liquid digestate application Soil tillage Weeding	Date 05.06.2022 11.06.2022 07.07.2022	Practices Weedicide application Hoeing Second fertilization		
Table 4. Agrid           Date           23.04.2022           02.05.2022           02.05.2022           11.05.2022	cultural practices. Practices Liquid digestate application Soil tillage Weeding Fertilization	Date 05.06.2022 11.06.2022 07.07.2022 14.07.2022	Practices Weedicide application Hoeing Second fertilization Weeding		

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## 2.4. Analysis methods

Soil samples' pH and EC values were determined using a 1:2.5 soil-pure water mixture (Soil
Survey Lab. Staff, 1975). Organic matter content was detected with the modified WalkleyBlack method (Jackson, 1979) while lime (% CaCO<sub>3</sub>) contents were measured using the
Scheibler calcimeter method (Loeppert and Suarez, 1996). Micro-elements were determined in
samples digested with a 1:3 HNO<sub>3</sub> solution using Inductively Coupled Plasma Optical Emission
Spectrometry (ICP-OES) (Sutherland, R. A., 2018). Inorganic nitrogen contains values of

NH4<sup>+</sup>-N and NO3<sup>-</sup>-N extracted with KCl solution (Bremner, 1965). Available phosphorus was
analyzed in the ICP-OES device after being extracted according to the Olsen method.
Exchangeable potassium was determined based on potassium values extracted with 1 N
ammonium acetate (Soil Survey Lab., 1975). Soil texture was identified using the Hydrometer
method (Gee and Bauder, 1986).

In LD, a glass electrode pH meter was used to determine pH values (Soil Survey Lab. Staff, 1975), and an electrical conductivity measurement device was used for salinity (EC). Total nitrogen amount was detected using the Kjeldahl method (Bao, 2005). The same method was preferred for inorganic nitrogen values (Apha et al., 2012). Organic carbon was determined after the ashing method. ICP-OES (Kacar and Inal 2010) was chosen for macro-micro elements and heavy metals, and silver nitrate with chromate indicator was selected for Chlorine (Cl) amount (Apha et al., 2012).

For maize grain, yield calculation based on moisture was carried out taking weights at 15.5% moisture value as the basis. Nitrogen ratio in leaves and grain was analyzed with the Kjeldahl method (Apha et al., 2012). Nitrogen use and uptake efficiencies were calculated using the following formulas (Moll et al., 1982). In these formulas, yield with fertilizer (YF) represents the treatment to which fertilizer was applied, and yield with control (YC) represents the control treatment without fertilizer.

168 Nitrogen uptake amount in grain: % N in grain \* YF (t  $ha^{-1}$ )

169 Nitrogen use efficiency:  $[(YF-YC) / N \text{ application amount } (t ha^{-1})] * 100$ 

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## 171 **2.5. Statistical analysis**

Statistical analysis of the obtained data was conducted in the SPSS software (IBM Corp., 2017). The experimental design used was the Randomized Complete Block Design, and variance analysis was applied to these data. Potential differences between the resulting mean values were evaluated with the help of the Duncan's multiple range test.

177 **3. Results** 

## 178 3.1. Soil EC and pH

The LD, due to its high salinity content (25.86 dS m<sup>-1</sup>), has increased soil salinity levels (Fig 2). In OYA, no significant difference in salinity was observed after the 3rd period, while in the TYA, differences were determined in every period. However, starting from the 3rd period, an increase in salinity was also observed in the GM (mineral fertilizer application), so the effect of LD could not be clearly determined for the periods after this. It is assumed that the rainfall over time has reduced the salt content in the soil.

After high-dose LD applications (G5 and G7), an increase in salinity was observed in the 185 3rd and 4th periods (Fig. 9). However, this increase is not sufficient to change the classification 186 187 of the soil EC values.





application, OYA; two consecutive year application, TYA).

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Agricultural Applications: 23 Apr: LD application.; 11 May: Fertilization, Sowing; 7 Jul: Fertilization; 13 Oct: Harvest

196 Fig. 3. pH results of the soils sampled on different dates in two types of experiments (one year application, OYA; two consecutive year application, TYA). Different letters in each histogram indicate significant differences at p < 1198 0.05 (Duncan's Multiple Range Test) among means. 199

Following the LD application, a decrease in soil pH was observed in the 1st period (Fig. 3), and this decrease was more pronounced in TYA. In the 2nd period, the acidifying effect of the high dose of LD continued in TYA, while the acidic effect of chemical fertilizer application was determined in OYA. In the subsequent periods, no significant difference in pH was observed in either method.

205 According to the 1st period results of this study, the difference in soil EC results due to the method is statistically significant. In the 2nd period, both the method difference and the 206 207 interaction between the method and the LD dose are statistically significant (Table 5). TYA led 208 to an increase in soil EC results in both the 1st and 2nd periods. In the same periods, compared

to OYA, an increase in soil EC results was observed with all applications except for the G1
treatment (Fig. 4). The changes in soil pH results are consistent with those observed in EC
results. In the 1st period, only the method difference was statistically significant, whereas in the
2nd period, the interaction between the method and the LD dose also became important. It was
determined that the TYA accelerated the decrease in soil pH values caused by LD (Fig. 5).
However, from the 3rd period onwards, the effect of LD on these parameters has decreased.

215 Table 5. Statistical analysis of EC and pH results based on method.

	EC (1st Per	iod)	EC (2nd	ł	EC (3rd	l	PH (1st Per	iod)	PH (2nd	ł	PH (31	H rd	
			Perio	d)	Perio	d)			Perio	d)	Peri	od)	
	F	Р.	F	Р.	F	Р	F	Р.	F	Р.	F	Р	
Method	15,718	**	58,152	**	2,933	*	6,469	*	91,558	**	0,511	ns	
Method*Treatments	1,012	ns	15,026	**	2,494	ns	0,474	ns	7,763	**	1,096	ns	

F: Indicate the statistical significance from ANOVA

217 <u>P:</u> ns: not significant, \*:<0,05, \*\*:<0,01.

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In the 2nd period, a significant interaction was observed between the method and the LD dose. This reveals the impact of both the application method and the LD dose on the soil's pH and EC values. This pronounced effect was detected in measurements taken two months after the application of LD.







Fig. 5. Effect of the method on pH results.

## 231 **3.2. Soil inorganic nitrogen content**

LD application on April 23 resulted in monitoring soil inorganic nitrogen (NH4<sup>+</sup>-N, NO3<sup>-</sup>-232 N) levels at specific dates (May 10, June 20, August 2, and October 21). When investigating 233 the effect of LD on soil ammonium nitrogen (NH<sub>4</sub><sup>+</sup>-N) levels, it was identified that as the dosage 234 of LD increased, so did this value (Fig. 9). In the plots where LD was applied, the amount of 235 inorganic nitrogen provided by the mineral fertilizer was observed to be added to the soil (Fig. 236 6). This finding suggests that LD applications are effective in adding the necessary inorganic 237 nitrogen to the soil for maize plants. When the inorganic nitrogen levels of the soils were 238 analyzed periodically, no significant difference was detected between the application methods. 239 Both after mineral fertilizer application and LD applications, the added soil inorganic nitrogen 240 levels showed similar values. 241



Agricultural Applications: 23 Apr: LD application.; 11 May: Fertilization, Sowing; 7 Jul: Fertilization; 13 Oct: Harvest.

**Fig. 6.** Values of inorganic nitrogen in the soil in two types of experiments (one year application, OYA; two consecutive year application, TYA). Different letters in each histogram indicate significant differences at p < 0.05 (Duncan's Multiple Range Test) among means.

#### **3.3.** Grain yield and nitrogen parameters in the plant

When comparing the OYA (one-year application) and TYA (two-year application) methods, it was observed that the highest yield in OYA was obtained from the G7 and GM applications, while in TYA, the highest yield was obtained only from the G7 application. During the tassel emergence stage, the highest nitrogen (N) value in the leaves was identified in the G7 application with the OYA. However, in TYA, this value was recorded highest for GM. On the other hand, the lowest nitrogen values were determined for G0 (control) in both methods. Nitrogen ratios of harvested grains were found to be highest in GM and G7 for OYA, and in GM, G5, and G7 for TYA (Table 6). 

Table 6. Maize grain yield and percentage of total nitrogen in leaves and grains for different treatments in two 

types of exp	periments ( <mark>one ye</mark>	ear application, OYA; two co	onsecutive year application,	TYA).
Method	Treatments	Grain Yield (t ha <sup>-1</sup> )	N in Leaf (%)	N in Grain (%)
	G0	$13,0 \pm 0.5 \text{ d}$	$1,86 \pm 0.03 \text{ d}$	$1,03 \pm 0.02 \text{ c}$
	GM	$18,2 \pm 0.6$ a	$2,67 \pm 0.06$ ab	$1,17 \pm 0.03$ a
OVA	G1	$16,0 \pm 0.4 \text{ bc}$	$2,50 \pm 0.05$ c	$1,09 \pm 0.02$ b
UIA	G3	$15,7 \pm 0.5 \text{ c}$	$2,54 \pm 0.04$ bc	$1,07 \pm 0.02 \text{ b}$
	G5	$17,4 \pm 0.5 \text{ ab}$	$2,58 \pm 0.05$ abc	$1,09 \pm 0.02$ b
	G7	$19,1 \pm 0.7$ a	$2,70 \pm 0.06$ a	$1,18 \pm 0.03$ a
F		18,09**	51,89**	18,34**
	G0	$8,9\pm0.2$ c	$1,59 \pm 0.04 \text{ d}$	$0,99 \pm 0.02 \text{ c}$
	GM	$17,0 \pm 0.5 \text{ b}$	$2,79 \pm 0.06$ a	$1,18 \pm 0.03$ a
TVA	G1	$16,0 \pm 0.4 \text{ b}$	$2,51 \pm 0.05 \text{ c}$	$1,09 \pm 0.02 \text{ b}$
IIA	G3	$16,4 \pm 0.4 \text{ b}$	$2,54 \pm 0.05 \text{ c}$	$1,09 \pm 0.02 \text{ b}$
	G5	$17,9 \pm 0.6 \text{ ab}$	$2,67 \pm 0.07$ b	$1,14 \pm 0.03$ a
	G7	$19,4 \pm 0.8 \text{ a}$	$2,73 \pm 0.06 \text{ ab}$	$1,17 \pm 0.03$ a
F		38 64**	182 19**	26.01**

Different letters in each histogram indicate significant differences at p < 0.05 (Duncan's Multiple Range Test) among means.

F: Indicate the statistical significance from ANOVA.; \*: < 0.05, \*\*: < 0.01 

Between the methods, although the effect of LD on yield and nitrogen ratios in the plant has not shown a statistically significant change (Table 7), higher yields have been achieved with the TYA in G3, G5, and G7 treatments (Fig. 7). Additionally, in the TYA, a dose of 50 tons ha<sup>-1</sup> of LD has provided a higher yield compared to mineral fertilization, which is different from the OYA. Within the method framework, there is no linear increase in the amount of nitrogen in the plant with the LD dose. 

	Yield		N in Leaf		N in Grain	
-	F	Р.	F	Р	F	P.
Method	1,023	ns	1,284	ns	2,532	ns
Method*Treatments	0,135	ns	0,463	ns	1,604	ns

 Table 7. Statistical analysis of yield and nitrogen results based on the method.

F: Indicate the statistical significance from ANOVA.

<u>P:</u> ns: not significant.







Fig. 7. The effect of the method on grain yield and nitrogen values.

Nitrogen uptake and nitrogen use efficiencies have followed similar trends for both methods.
An increase in LD dosage has increased yield and thus grain nitrogen uptake, and a higher value
has been determined at a dosage of 70 tons ha<sup>-1</sup> compared to mineral fertilization.

292 G5 and G7 applications have had the lowest nitrogen use efficiency (NUE) compared to other treatments (Fig. 8). This is because the amount of N applied in these treatments was higher 293 294 than in other treatments. In terms of NUE results, GM, G1, and G3 treatments can be compared as they were given the same amount of nitrogen to the soil. GM had the highest nitrogen use 295 296 efficiency. However, despite the difference in LD dosages between G1 and G3 applications, obtaining similar NUE values indicates that an increase in dosage after 1 t ha<sup>-1</sup> LD did not create 297 a significant change in NUE. In G1 and G3 applications, which are combinations of LD with 298 299 chemical fertilizer, NUE has shown a similar trend. However, in the TYA, G3 has had higher nitrogen uptake and NUE values compared to G1. 300







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**Fig. 9.** The effect of low and high doses of LD on inorganic nitrogen (N), pH, and EC parameters in the OYA.

## 313 4. Discussion

## **4.1. EC and pH**

Throughout the first two periods, an increase in the application of the LD was observed to proportionally elevate the salinity (EC) values in the soil. However, after the 3rd period, this rise was noted to decrease. This suggests that the rainfall during the trial period might have reduced the salt content in the soil. Additionally, the high sand content of the research soil has facilitated the leaching of EC. While Panuccio et al. (2021) emphasized that the solid fraction of biogas increased the EC value in the soil, Aimrun et al. (2009) have pointed out that soil salinity can vary with many factors and determining these dynamics is complex.

A notable decrease in soil pH values was recorded after LD application. Similar reductions 322 were observed in the first period with doses of 3.5 and 7 tons/da, whereas in the second period, 323 it was determined that chemical fertilizer applications also caused a slight acidifying effect in 324 the soil. The acidification became more pronounced with the impact of LD but diminished in 325 subsequent periods. While Jia et al. (2013) stated that the LD raised soil pH values, Ren et al. 326 327 (2020) observed a slight decrease. Panuccio et al. (2021) also pointed out that LD application neutralized the pH value in high pH soil. El-Khatib et al. (2018) noted that biogas fermentation 328 329 residues lowered the soil pH and that the main reason for this decrease was organic acids and ammonium ions. Brady and Weil (2016) also mentioned that high NH4<sup>+</sup>-N concentrations in 330 331 the soil could lead to acidification by releasing H<sup>+</sup> ions through nitrification.

#### 333 4.2. Yield and nitrogen results

The application of LD positively affects grain yield and increases the yield as the dosage 334 335 amount increases. The results of various studies have demonstrated the positive effects of LD on different plants. Specifically, Zhao et al. (2022) and Yaraşır (2018) noted a significant 336 337 increase in plant height, branch count, pod count, and yield in the rapeseed plant due to LD. These findings are corroborated by another study conducted by Du et al. (2019) in maize. 338 Furthermore, Głowacka et al. (2020) reported the potential to obtain a higher biomass by 339 reducing the use of mineral fertilizers with digestate. In their study, where they applied doses 340 of biogas digestate compared with irrigation water in a maize experiment, it was determined 341 that an increase in the applied dose amount also increased the yield. 342

According to the study, the effect of LD on the soil's inorganic nitrogen amount is similar to that of chemical fertilizer application. Due to the high NH<sub>4</sub><sup>+</sup>-N content of the LD, it is anticipated to have a positive impact on grain yield compared to organic fertilizers (Möller & Müller, 2012; Nkoa, 2014; Du vd., 2019). This positive effect has been identified by Al-Juhaimi et al. (2014) on alfalfa and by Ernst et al. (2008) as well as Chantigny et al. (2008) on other plants. Barłóg et al. (2020) have suggested that the digestate can replace urea fertilizer as a nitrogen source in the soil.

The nitrogen levels detected in leaves and grains have been found to be consistent with the 350 effect of LD on the inorganic nitrogen values in the soil. Variability in the concentration of 351 NH4<sup>+</sup>-N or NO<sub>3</sub><sup>-</sup>-N in the soil can be a determinant on the nitrogen uptake of plants (Pan et al., 352 353 1995; Barber et al., 1992). In maize, in particular, the amount of inorganic N in the soil, as well as the form in which the plant takes up nitrogen, can influence the nitrogen rate in the leaves at 354 different growth stages. Significant findings on the dynamics of NH<sub>4</sub><sup>+</sup>-N in the soil have been 355 356 provided by Köster et al. (2011) and Nyberg et al. (2004). Notably, it has been indicated that 357 certain substances that limit losses of NH<sub>4</sub><sup>+</sup>-N in the form of NH<sub>3</sub><sup>-</sup>-N may be present depending 358 on the dose of LD.

In the study, no difference was determined between the one-year application (OYA) and the consecutive two-year application (TYA) methods in terms of inorganic nitrogen amounts in the soil. However, De França et al. (2021) in their study pointed out that the effect of the digestate on the amount of nitrogen in the soil is more effective with the OYA than the TYA.

Compared to chemical fertilization, the NUE has decreased with LD applications, but there has been no significant difference in terms of NUE between the 10 and 30 t ha<sup>-1</sup> applications. This suggests that LD application could lead to nitrogen losses in the soil. Materials with a low C/N ratio can promote a rapid mineralization process in the soil, leading to nitrogen losses 367 (Brady & Weil, 2008). The fermentation process reduces the C/N ratio of organic wastes. In
368 this context, the C/N ratio of 1.42 of the LD applied to the soil in our study may have triggered
369 nitrogen losses.

370

#### 371 **5.** Conclusion

This study comprehensively examines the effects of the liquid digestate (LD) obtained following biogas energy production on soil and maize plants. The impacts of various LD doses (10, 30, 50, and 70 tons ha<sup>-1</sup>) have been evaluated in one-year and consecutive two-year applications.

The results show that for maize grain yield and nitrogen parameters, mineral fertilization 376 treatment and 70 tons ha<sup>-1</sup> liquid digestate dose were the most efficient in one-year application, 377 while 70 tons ha<sup>-1</sup> dose application was the most efficient in consecutive two-year application. 378 Especially, the grain yield obtained with a 70 tons ha<sup>-1</sup> dose was found to be considerably higher 379 than the control group and other doses. However, it was determined that with the increase in 380 liquid digestate doses, soil salinity also increased. This became particularly pronounced in two-381 382 year applications. It was observed that within the first two months, the digestate application lowered the soil pH value, but this effect did not show significant change in the subsequent 383 months. The effect of the liquid digestate on the soil's inorganic nitrogen amount is similar to 384 chemical fertilizer applications. However, it was observed that nitrogen use efficiency is lower 385 in 50 and 70 tons ha<sup>-1</sup> dose applications. In the applications of liquid digestate, potential 386 nitrogen losses were determined due to its high ammonium content. 387

Based on the findings of this study, future experiments should consider the application of similar N, P, and K inputs, as well as uniform water application in each period for all treatments, to ensure a comprehensive evaluation of the effects of liquid digestate.

In conclusion, it was noted that the liquid digestate has potential value for agricultural applications. However, it was concluded that with an increase in the dose amount of this product, soil salinity might increase and ammonia and nitrate losses should not be overlooked in high-dose applications. This study contributes to the accurate evaluation of the impacts of the liquid digestate obtained after biogas energy production in agricultural applications. Taking into consideration the sustainability and environmental effects of the applications will ensure the efficient and effective use of liquid digestate.

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## 405 **References**

- 406 Aimrun, W., Amin, M. S., Rusnam, M., Ahmad, D. E. S. A., Hanaf, M. M., & Anuar, A. R.
- 407 (2009). Bulk soil electrical conductivity as an estimator of nutrients in the maize cultivated
  408 land. European Journal of Scientific Research, 31, 37-51.
- 409 Al-Juhaimi, F. Y., Hamad, S. H., Al-Ahaideb, I. S., Al-Otaibi, M. M., & El-Garawany, M. M.
- 410 (2014). Effects of fertilization with liquid extracts of biogas residues on the growth and
  411 forage yield of alfalfa (Medicago sativa L.) under arid zone conditions. Pakistan Journal of
  412 Determined (20), 471, 475
- 412 Botany, 46(2), 471-475.
- 413 Anderson, J. P. E. (1982). Soil respiration. In A. L. Page, D. R. Keeney, D. E. Baker, R. H.
- Miller, R. Ellis Jr., & J. D. Rhoades (Eds.), Methods of soil analysis, Part 2- Chemical and
  Microbiological Properties (pp. 831-871). ASA-SSSA.
- Anderson, J. P. E., & Domsch, K. H. (1978). A physiological method for the quantitative
  measurement of microbial biomass in soils. Soil Biology and Biochemistry, 10(3), 215-221.
- Anderson, T. H., & Domsch, K. H. (1986). Carbon assimilation and microbial activity in soil.
  Zeitschrift für Pflanzenernährung und Bodenkunde, 149(4), 457-468.
- Angelidaki, I., & Ellegaard, L. (2003). Codigestion of manure and organic wastes in centralized
  biogas plants. Applied Biochemistry and Biotechnology, 109(1-3), 95-105.
- 422 Apha, A., & Wpcf. (2012). Standard methods for the examination of water and wastewater.
  423 American Public Health Association, Washington.
- Aydın, B., Öztürk, O., Çobanoğlu, F., Çebi, U., Özkan, E., & Özer, S. (2020). Effects of drip
  irrigation subsidies on silage maize production: A case study from Edirne. International
  Journal of Agriculture and Wildlife Science, 6(3), 496-505.
- Bationo, A., Kihara, J., Vanlauwe, B., Waswa, B., & Kimetu, J. (2007). Soil organic carbon
  dynamics, functions and management in West African agro-ecosystems. Agricultural
  systems, 94(1), 13-25.
- 430 Bao, S. D. (2005). Soil and Agricultural Chemistry Analysis. Beijing: Agricultural Press.
- Barduca, L., Wentzel, S., Schmidt, R., Malagoli, M., & Joergensen, R. G. (2021).
  Mineralisation of distinct biogas digestate qualities directly after application to soil. Biology
  and Fertility of Soils, 57(2), 235-243.

- 434 Barłóg, P., Hlisnikovský, L., & Kunzová, E. (2020). Effect of digestate on soil organic carbon
- and plant-available nutrient compared to cattle slurry and mineral fertilization.Agronomy, 10(3), 379.
- 437 Barber, K. L., Maddux, L. D., Kissel, D. E., Pierzynski, G. M., & Bock, B. R. (1992). Maize
- responses to ammonium-and nitrate-nitrogen fertilization. Soil Science Society of America
  Journal, 56(4), 1166-1171.
- Brady, N. C., & Weil, R. R. (2008). The soils around us. The nature and properties of soils
  (14th ed.). Pearson Prentice Hall, New Jersey and Ohio, 1-31.
- Bremner, J. T. (1965). Inorganic forms of nitrogen. In Methods of Soil Analysis: Part 2
  Chemical and Microbiological Properties (Vol. 9, pp. 1179-1237).
- Brito, L. M., Coutinho, J., & Smith, S. R. (2008). Methods to improve the composting process
  of the solid fraction of dairy cattle slurry. Bioresource Technology, 99(18), 8955-8960.
- 446 Canisares, L. P., Do Carmo, J. B., Pitombo, L. M., & Pires, E. C. (2017). Digested bioenergy
- byproduct with low concentration of nutrients increased greenhouse gas emissions from soil.Geoderma, 307, 81-90.
- Chantigny, M. H., Angers, D. A., Bélanger, G., Rochette, P., Eriksen-Hamel, N., Bittman, S.,
  ... & Gasser, M. O. (2008). Yield and nutrient export of grain maize fertilized with raw and
  treated liquid swine manure. Agronomy Journal, 100(5), 1303-1309.
- 452 Chookietwattana, K., Chumpol, J., & Sumphanwanich, P. (2016). Liquid Fermented Organic
  453 Products: A Potential Alternative for Chemical Fertilizer and Soil Amendments. In Liquid
  454 Organic Fertilizer (pp. 79-100). Springer.
- 455 De França, A. A., von Tucher, S., & Schmidhalter, U. (2021). Effects of combined application
  456 of acidified biogas slurry and chemical fertilizer on crop production and N soil fertility.
  457 European Journal of Agronomy, 123, 126224.
- Deviren, H., İlkılıç, C., & Aydın, S. (2017). Biyogaz üretiminde kullanılabilen materyaller ve
  biyogazın kullanım alanları. Batman Üniversitesi Yaşam Bilimleri Dergisi, 7(2/2), 79-89.
- 460 Diacono, M., & Montemurro, F. (2011). Long-term effects of organic amendments on soil
  461 fertility. Sustainable Agriculture Volume 2, 761-786.
- Du, H., Gao, W., Li, J., Shen, S., Wang, F., Fu, L., & Zhang, K. (2019). Effects of digested
  biogas slurry application mixed with irrigation water on nitrate leaching during wheat-maize
  rotation in the North China Plain. Agricultural Water Management, 213, 882-893.
- Du, Z., Xiao, Y., Qi, X., Liu, Y., Fan, X., & Li, Z. (2018). Peanut-shell biochar and biogas
  slurry improve soil properties in the North China Plain: a four-year field study. Scientific
  Reports, 8(1), 1-9.

- El-Khatib, A. A., Al-Muhtaseb, A. H., Al-Makhadmeh, L. A., & Abu-Nameh, E. S. (2018).
  Impact of liquid fermented organic waste on soil characteristics, plant growth, and yield.
  Journal of Plant Nutrition, 41(4), 499-508.
- 471 Ernst, G., Müller, A., Göhler, H., & Emmerling, C. (2008). C and N turnover of fermented
  472 residues from biogas plants in soil in the presence of three different earthworm species
  473 (Lumbricus terrestris, Aporrectodea longa, Aporrectodea caliginosa). Soil Biology and
  474 Biochemistry, 40(6), 1413-1420.
- Fransman, B., & Nihlgård, B. (1995). Water chemistry in forested catchments after topsoil
  treatment with liming agents in South Sweden. Water, Air, and Soil Pollution, 85(2), 895900.
- Gee, G. W., & Bauder, J. (1986). Particle size analysis. In Methods of Soil Analysis, Part 1,
  383-411.
- 480 Geisseler, D., & Scow, K. M. (2014). Long-term effects of mineral fertilizers on soil
  481 microorganisms–A review. Soil Biology and Biochemistry, 75, 54-63.
- Głowacka, A., Szostak, B., & Klebaniuk, R. (2020). Effect of biogas digestate and mineral
  fertilisation on the soil properties and yield and nutritional value of switchgrass forage.
  Agronomy, 10(4), 490.
- Gürbüz, M. A., & Öz, T. A. (2016). Ayçiçeğinin Azotlu Gübreleme Önerilerinde İndeks Olarak
  Kullanılabilecek Parametrelerin Araştırılması [Investigation of Parameters that can be Used
  as an Index in Nitrogen Fertilization Recommendations for Sunflower]. Çukurova Tarım ve
  Gıda Bilimleri Dergisi, 31(3), 147-152.
- Holm-Nielsen, J. B., Al Seadi, T., & Oleskowicz-Popiel, P. (2009). The future of anaerobic
  digestion and biogas utilization. Bioresource Technology, 100(22), 5478-5484.
- 491 IBM Corp. (2017). IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.
- Insam, H., Franke-Whittle, I. H., Knapp, B. A., & Plank, R. (2009). Use of wood ash and
  anaerobic sludge for grassland fertilization: Effects on plants and microbes. Die
  Bodenkultur, 60(2), 39-50.
- Jackson, M. L. (1979). Soil Chemical Analysis: Advanced Course. Dept. Soil Science,
  University of Wisconsin, Madison, WI, USA.
- Jia, Y., Sun, G. X., Huang, H., & Zhu, Y. G. (2013). Biogas slurry application elevated arsenic
  accumulation in rice plant through increased arsenic release and methylation in paddy soil.
  Plant and Soil, 365(1), 387-396.
- Jurgutis, L., Šlepetienė, A., Amalevičiūtė-Volungė, K., Volungevičius, J., & Šlepetys, J.
  (2021). The effect of digestate fertilisation on grass biogas yield and soil properties in field-

- biomass-biogas-field renewable energy production approach in Lithuania. Biomass andBioenergy, 153, 106211.
- 504 Kacar, B., & İnal, A. (2010). Bitki analizleri. Nobel Yayın Dağıtım.
- 505 Karimi, B., Sadet-Bourgeteau, S., Cannavacciuolo, M., Chauvin, C., Flamin, C., Haumont, A.,
- 506 ... & Ranjard, L. (2022). Impact of biogas digestates on soil microbiota in agriculture: A
  507 review. Environmental Chemistry Letters, 20(5), 3265-3288.
- 508 Köster, J. R., Cárdenas, L., Senbayram, M., Bol, R., Well, R., Butler, M., ... & Dittert, K. (2011).
- Rapid shift from denitrification to nitrification in soil after biogas residue application as
  indicated by nitrous oxide isotopomers. Soil Biology and Biochemistry, 43(8), 1671-1677.
- Loeppert, R. H., & Suarez, D. L. (1996). Carbonate and gypsum. Methods of soil analysis: Part
  3 chemical methods, 5, 437-474.
- Marschner, P., Kandeler, E., & Marschner, B. (2003). Structure and function of the soil
  microbial community in a long-term fertilizer experiment. Soil Biology and Biochemistry,
  35(3), 453-461.
- Massé, D. I., Croteau, F., & Masse, L. (2007). The fate of crop nutrients during digestion of
  swine manure in psychrophilic anaerobic sequencing batch reactors. Bioresource
  technology, 98(15), 2819-2823.
- 519 Turkish State Meteorological Service [TSMSM]. (2020). Resmi istatistikler. Retrieved from
  520 https://www.mgm.gov.tr/veridegerlendirme/il-ve-ilceler-
- 521 istatistik.aspx?k=A&m=KIRKLARELI
- Moll, R. H., Kamprath, E. J., & Jackson, W. A. (1982). Analysis and interpretation of factors
  which contribute to efficiency of nitrogen utilization. Agronomy Journal, 74(3), 562-564.
- Möller, K. (2015). Effects of anaerobic digestion on soil carbon and nitrogen turnover, N
  emissions, and soil biological activity. A review. Agronomy for Sustainable Development,
  35(3), 1021-1041.
- Möller, K., & Müller, T. (2012). Effects of anaerobic digestion on digestate nutrient availability
  and crop growth: A review. Engineering in Life Sciences, 12(3), 242-257.
- 529 Nkoa, R. (2014). Agricultural benefits and environmental risks of soil fertilization with
  530 anaerobic digestates: a review. Agronomy for Sustainable Development, 34(2), 473-492.
- Nyberg, K., Sundh, I., Johansson, M., & Schnürer, A. (2004). Presence of potential ammonia
  oxidation (PAO) inhibiting substances in anaerobic digestion residues. Applied Soil
  Ecology, 26(2), 107-112.

- Pan, W. L., Camberato, J. J., Moll, R. H., Kamprath, E. J., & Jackson, W. A. (1995). Altering
  source–sink relationships in prolific maize hybrids: Consequences for nitrogen uptake and
  remobilization. Crop Science, 35(3), 836-845.
- Panuccio, M. R., Romeo, F., Mallamaci, C., & Muscolo, A. (2021). Digestate application on
  two different soils: Agricultural benefit and risk. Waste and Biomass Valorization, 12(8),
  4341-4353.
- Piccoli, I., Francioso, O., Camarotto, C., Delle Vedove, G., Lazzaro, B., Giandon, P., & Morari,
  F. (2022). Assessment of the short-term impact of anaerobic digestate on soil C stock and
  CO2 emissions in shallow water table conditions. Agronomy, 12(2), 504.
- Rawoof, S. A. A., Kumar, P. S., Vo, D. V. N., & Subramanian, S. (2021). Sequential production
  of hydrogen and methane by anaerobic digestion of organic wastes: a review. Environmental
  Chemistry Letters, 19, 1043-1063.
- Ren, T., Yu, X., Liao, J., Du, Y., Zhu, Y., Jin, L., ... & Ruan, H. (2020). Application of biogas
  slurry rather than biochar increases soil microbial functional gene signal intensity and
  diversity in a poplar plantation. Soil Biology and Biochemistry, 146, 107825.
- Robles-Aguilar, A. A., Temperton, V. M., & Jablonowski, N. D. (2019). Maize silage digestate
  application affecting germination and early growth of maize modulated by soil type.
  Agronomy, 9(8), 473.
- Rohila, A. K., Maan, D., Kumar, A., & Kumar, K. (2017). Impact of agricultural practices on
  environment. Asian Journal of Microbiology, Environmental Science, 19(2), 145-148.
- 554SAPEA.(2012).Facheverhanbiogas.Retrievedfrom555http://www.biogas.org/edcom/webfvb.nsf/id/DE\_Branchenzahlen
- Shi, L., Simplicio, W. S., Wu, G., Hu, Z., Hu, H., & Zhan, X. (2018). Nutrient recovery from
  digestate of anaerobic digestion of livestock manure: a review. Current Pollution Reports, 4,
  74-83.
- Singh, A., Agrawal, M., & Marshall, F. M. (2010). The role of organic vs. inorganic fertilizers
  in reducing phytoavailability of heavy metals in a wastewater-irrigated area. Ecological
  Engineering, 36(12), 1733-1740.
- Soil Survey Lab Staff, USDA. (1975). Soil Taxonomy: A basic system of soil classification for
  making and interpreting soil surveys (No. 436.4 So3). US Govt. Print. Off.
- Sutherland, R. A. (2018). Guidelines for the use of ICP-OES for the characterisation of urban
   road-deposited sediment particle size fractions. Environmental Pollution, 238, 926–939.
- Sürmen, M., & Kara, E. (2022). High-Quality Fertilizers from Biogas Digestate. Environment
  and Climate-smart Food Production, 319-347.

- 568 Urra, J., Alkorta, I., & Garbisu, C. (2019). Potential benefits and risks for soil health derived
  569 from the use of organic amendments in agriculture. Agronomy, 9(9), 542.
- Yakan, H., & Sağlam, T. (1997). Kırklareli koşullarında yetiştirilen hıbrıt mısır azotlu ve
  fosforlu gübrelemenin dane verimi ve bazı kalite özelliklerine etkisi [Doctoral thesis, Trakya
  University Faculty of Agriculture]. Edirne.
- Yaraşır, N. (2018). The Effect Of Different Doses of Liquid Biogas Wastes on Yield and
  Quality of Wheat (Triticum aestivum L.) (Master's thesis, Adnan Menderes University
  Graduate School of Natural and Applied Sciences).
- Zhao, Q., Cheng, J., Zhang, T., Cai, Y., Sun, F., Li, X., & Zhang, C. (2022). Biogas slurry
  increases the reproductive growth of oilseed rape by decreasing root exudation rates at
  bolting and flowering stages. Plant and Soil, 1–16.
- 579 Zhong, W., Gu, T., Wang, W., Zhang, B., Lin, X., Huang, Q., & Shen, W. (2010). The effects
- 580 of mineral fertilizer and organic manure on soil microbial community and diversity. Plant
- 581 and Soil, 326(1), 511–522.