

30 & Scow, 2014; Marschner et al., 2003; Zhong et al., 2010). Among these problems are the
31 adverse effects on soil microorganisms and contamination of water reservoirs (Rohila et al.,
32 2017). Soil conservation and enhancement are at the core of sustainable agriculture. At this
33 juncture, the consideration of natural resources and recyclable wastes as alternatives to
34 chemicals gains significance. In this context, introducing the liquid digestate (LD) resulting
35 from biogas energy production to agricultural use carries the potential to reduce environmental
36 pollution while enhancing soil productivity.

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

47 During the biogas production process, energy is transferred to methane molecules from
48 organic waste through anaerobic digestion (Angelidaki and Ellegaard, 2003), while nitrogen
49 (N) and other nutrients are retained in the digestate (Massé et al., 2007). The solid fraction of
50 the waste material released after biogas production represents carbon (C) sequestration, while
51 the liquid fraction signifies richness as a plant nutrient source (Robles-Aguilar et al., 2019;
52 Barduca et al., 2021). Although the solid digestate has the potential to increase the carbon
53 content of the soil (Möller, 2015), it carries a risk of significant $\text{NH}_3\text{-N}$ (ammonia) loss during
54 storage due to its high pH value (Brito et al., 2008). When LD is managed correctly, it can serve
55 as a fertilizer and soil amendment (Chookietwattana et al., 2016). The presence of N,
56 phosphorus (P), and potassium (K) elements in LD and the improvement of soil physical
57 properties due to its organic compounds suggest its agricultural importance (Insam et al., 2009).
58 However, since not all organic material can be utilized by microorganisms within the biogas
59 process, digestate can come with potential risks to the soil and environment (Bationo et al.,
60 2007). Residual organic compounds in the digestate may include phytotoxic substances, heavy
61 metals, or excess nutrients that can accumulate in the soil (Singh et al., 2010). For effective use
62 of digestate, outcomes should be determined based on dosage, cumulative effects, and soil type
63 (Karim et al., 2022), and environmental impacts should be researched (Urrea et al. 2019).

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

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

78

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
82 Meteorology Research Institute's field, located at a latitude of $41^\circ 42' 11''$ N and a longitude of
83 $27^\circ 12' 29''$ E. The region's annual average temperature is 13.3°C , and the total average rainfall
84 is 48.7 mm (TSMS, 2022). There was no rainfall for 9 days following the application of the
85 liquid digestate, but on the 10th day, a precipitation of 5.7 mm was recorded. Rainfall data was
86 monitored with the institute's meteorological station. The soil in the top 0-30 cm layer has a pH
87 of 8.01, a loamy texture, and an organic matter content of 1.39% (Table 1). The soil was
88 analyzed at three depths (0-30 cm, 30-60 cm, and 60-90 cm) to capture the vertical distribution
89 of key soil properties. Soil samples for the results were taken from the 0-30 cm depth. The soil
90 sampling was carried out on 10 May, 20 June, 2 Aug. and 21 Oct. using a soil auger. These
91 dates represent the 1st, 2nd, 3rd, and 4th sampling periods, respectively. These periods were
92 chosen to monitor soil properties at regular intervals from the **time of** LD application to harvest.
93 For each treatment plot, five soil samples were **randomly** collected and combined to form a
94 composite sample for this depth. For the study, the DKC6630 grain corn seed from Monsanto,
95 which is commonly used in the region, was selected as the plant material. This variety belongs
96 to the FAO 600 maturity group and is known for its drought tolerance, and resistance to
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 ± 0.03 pH, 1.10 ± 0.05 dS m⁻¹ EC, 0.14 ± 0.001
 100 NH₄⁺-N, and 0.68 ± 0.02 NO₃⁻-N.

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

| | 0-30 | 30-60 | 60-90 |
|--|---------------------------------|--------------|-------------|
| pH | 8.01 ± 0.1 | 8.04 ± 0.1 | 8.07 ± 0.15 |
| EC (dS/m) | 0.18 ± 0.02 | 0.19 ± 0.02 | 0.19 ± 0.01 |
| CaCO₃ (%) | 11.02 ± 0.3 | 10.78 ± 0.25 | 9.45 ± 0.25 |
| Organic matter (%) | 1.39 ± 0.08 | 1.24 ± 0.07 | 1.07 ± 0.06 |
| NH₄⁺-N (mg kg⁻¹) | 9.22 ± 2 | 9.10 ± 1,8 | 4.17 ± 2,3 |
| NO₃⁻-N (mg kg⁻¹) | 3.41 ± 0,7 | 8.02 ± 1,4 | 14.95 ± 3,6 |
| P₂O₅ (kg da⁻¹) | 9.71 ± 0.3 | 10.14 ± 0.3 | 11.86 ± 0.4 |
| K₂O (kg da⁻¹) | 44.55 ± 2 | 33.12 ± 1.5 | 25.89 ± 1.2 |
| Texture (%) | 47 sand, 30,65 silt, 22,35 clay | | |

103 EC: Electrical Conductivity; NO₃⁻-N: nitrate.
 104 Data are presented as mean ± standard error.

106 2.2. Liquid digestate

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

114 **Table 2.** Chemical characteristic of liquid digestate

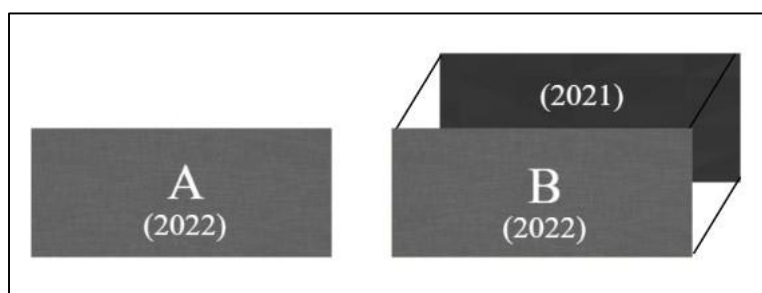
| | | | |
|---|-------------|-------------------------------|---------------|
| pH | 8,90 ± 0.2 | Organic Carbon (%) | 0,67 ± 0.03 |
| Dry matter (%) | 2,41 ± 0.13 | K (mg l⁻¹) | 2.387,00 ± 20 |
| EC (dS m⁻¹) | 23,61 ± 0.4 | P (mg l⁻¹) | 284,20 ± 2.8 |
| Total N (%) | 0,47 ± 0.02 | Cl (mg l⁻¹) | 6.980,00 ± 69 |
| NH₄⁺-N (%) | 0,37 ± 0.01 | Na (mg l⁻¹) | 845,00 ± 8.4 |

115 Data are presented as mean ± standard error.

117 2.3. Experiment set up and practices

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

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



136
 137
 138
 139 **Fig. 1.** Experiment site layout.

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

| Treatments | Applications | Mineral Fertilization Periods | |
|----------------|--|--------------------------------|--------------------------------|
| | | Sowing Period | Hoeing Period |
| G ₀ | Control | - | - |
| G _M | (456 kg ha ⁻¹ urea) | 228 kg ha ⁻¹ urea | 228 kg ha ⁻¹ urea |
| G ₁ | 10 ton LD + 330,4 kg ha ⁻¹ urea | 102,1 kg ha ⁻¹ urea | 228,2 kg ha ⁻¹ urea |
| G ₃ | 30 ton LD + 78,2 kg ha ⁻¹ urea | - | 78,2 kg ha ⁻¹ urea |
| G ₅ | 50 ton LD | - | - |
| G ₇ | 70 ton LD | - | - |

140
 141 **Table 4.** Agricultural practices.

| Date | Practices | Date | Practices |
|------------|------------------------------|------------|-----------------------|
| 23.04.2022 | Liquid digestate application | 05.06.2022 | Weedicide application |
| 02.05.2022 | Soil tillage | 11.06.2022 | Hoeing |
| 02.05.2022 | Weeding | 07.07.2022 | Second fertilization |
| 11.05.2022 | Fertilization | 14.07.2022 | Weeding |
| 11.05.2022 | Sowing | 19.10.2022 | Harvesting |

142
 143 **2.4. Analysis methods**

144 Soil samples' pH and EC values were determined using a 1:2.5 soil-pure water mixture (Soil
 145 Survey Lab. Staff, 1975). Organic matter content was detected with the modified Walkley-
 146 Black method (Jackson, 1979) while lime (% CaCO₃) contents were measured using the
 147 Scheibler calcimeter method (Loeppert and Suarez, 1996). Micro-elements were determined in
 148 samples digested with a 1:3 HNO₃ solution using Inductively Coupled Plasma Optical Emission
 149 Spectrometry (ICP-OES) (Sutherland, R. A., 2018). Inorganic nitrogen contains values of

150 NH_4^+ -N and NO_3^- -N extracted with KCl solution (Bremner, 1965). Available phosphorus was
151 analyzed in the ICP-OES device after being extracted according to the Olsen method.
152 Exchangeable potassium was determined based on potassium values extracted with 1 N
153 ammonium acetate (Soil Survey Lab., 1975). Soil texture was identified using the Hydrometer
154 method (Gee and Bauder, 1986).

155 In LD, a glass electrode pH meter was used to determine pH values (Soil Survey Lab. Staff,
156 1975), and an electrical conductivity measurement device was used for salinity (EC). Total
157 nitrogen amount was detected using the Kjeldahl method (Bao, 2005). The same method was
158 preferred for inorganic nitrogen values (Apha et al., 2012). Organic carbon was determined
159 after the ashing method. ICP-OES (Kacar and Inal 2010) was chosen for macro-micro elements
160 and heavy metals, and silver nitrate with chromate indicator was selected for Chlorine (Cl)
161 amount (Apha et al., 2012).

162 For maize grain, yield calculation based on moisture was carried out taking weights at
163 15.5% moisture value as the basis. Nitrogen ratio in leaves and grain was analyzed with the
164 Kjeldahl method (Apha et al., 2012). Nitrogen use and uptake efficiencies were calculated using
165 the following formulas (Moll et al., 1982). In these formulas, yield with fertilizer (YF)
166 represents the treatment to which fertilizer was applied, and yield with control (YC) represents
167 the control treatment without fertilizer.

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

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

170

171 **2.5. Statistical analysis**

172 Statistical analysis of the obtained data was conducted in the SPSS software (IBM Corp.,
173 2017). The experimental design used was the Randomized Complete Block Design, and
174 variance analysis was applied to these data. Potential differences between the resulting mean
175 values were evaluated with the help of the Duncan's multiple range test.

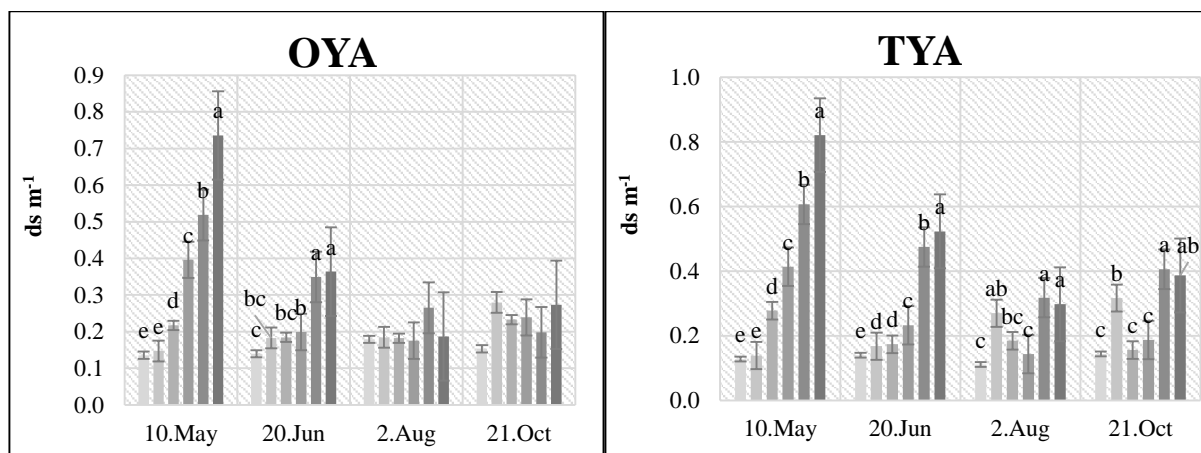
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177 **3. Results**

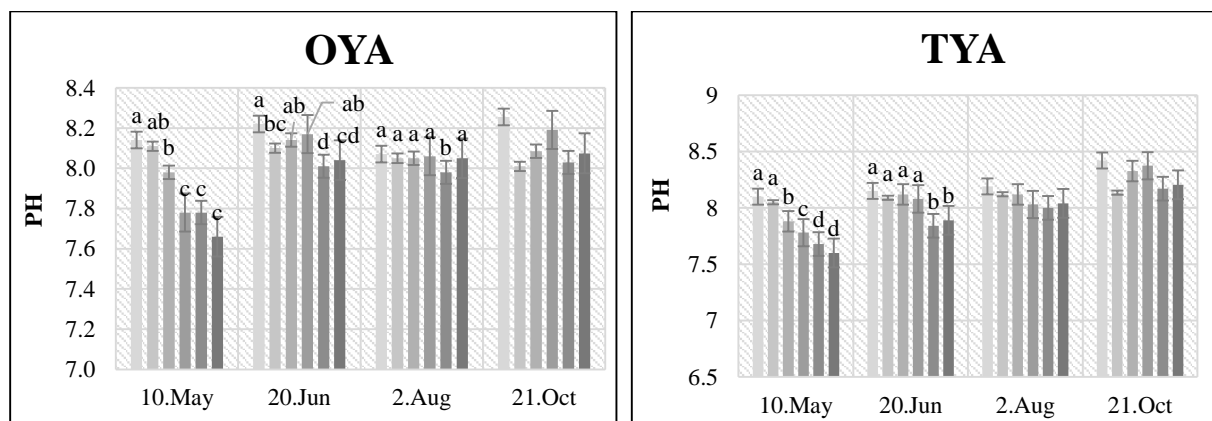
178 **3.1. Soil EC and pH**

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

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



188 **Fig. 2.** Electrical Conductivity results of the soils sampled on different dates in two types of experiments (one year
 189 application, OYA; two consecutive year application, TYA).
 190
 191



192 ■ G0 ■ GM ■ G1 ■ G3 ■ G5 ■ G7

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

194 **Fig. 3.** pH results of the soils sampled on different dates in two types of experiments (one year application, OYA;
 195 two consecutive year application, TYA). Different letters in each histogram indicate significant differences at $p < 0.05$ (Duncan's Multiple Range Test) among means.
 196
 197
 198
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200 Following the LD application, a decrease in soil pH was observed in the 1st period (Fig.
 201 3), and this decrease was more pronounced in TYA. In the 2nd period, the acidifying effect of
 202 the high dose of LD continued in TYA, while the acidic effect of chemical fertilizer application
 203 was determined in OYA. In the subsequent periods, no significant difference in pH was
 204 observed in either method.

205 According to the 1st period results of this study, the difference in soil EC results due to the
 206 method is statistically significant. In the 2nd period, both the method difference and the
 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

209 to OYA, an increase in soil EC results was observed with all applications except for the G1
 210 treatment (Fig. 4). The changes in soil pH results are consistent with those observed in EC
 211 results. In the 1st period, only the method difference was statistically significant, whereas in the
 212 2nd period, the interaction between the method and the LD dose also became important. It was
 213 determined that the TYA accelerated the decrease in soil pH values caused by LD (Fig. 5).
 214 However, from the 3rd period onwards, the effect of LD on these parameters has decreased.

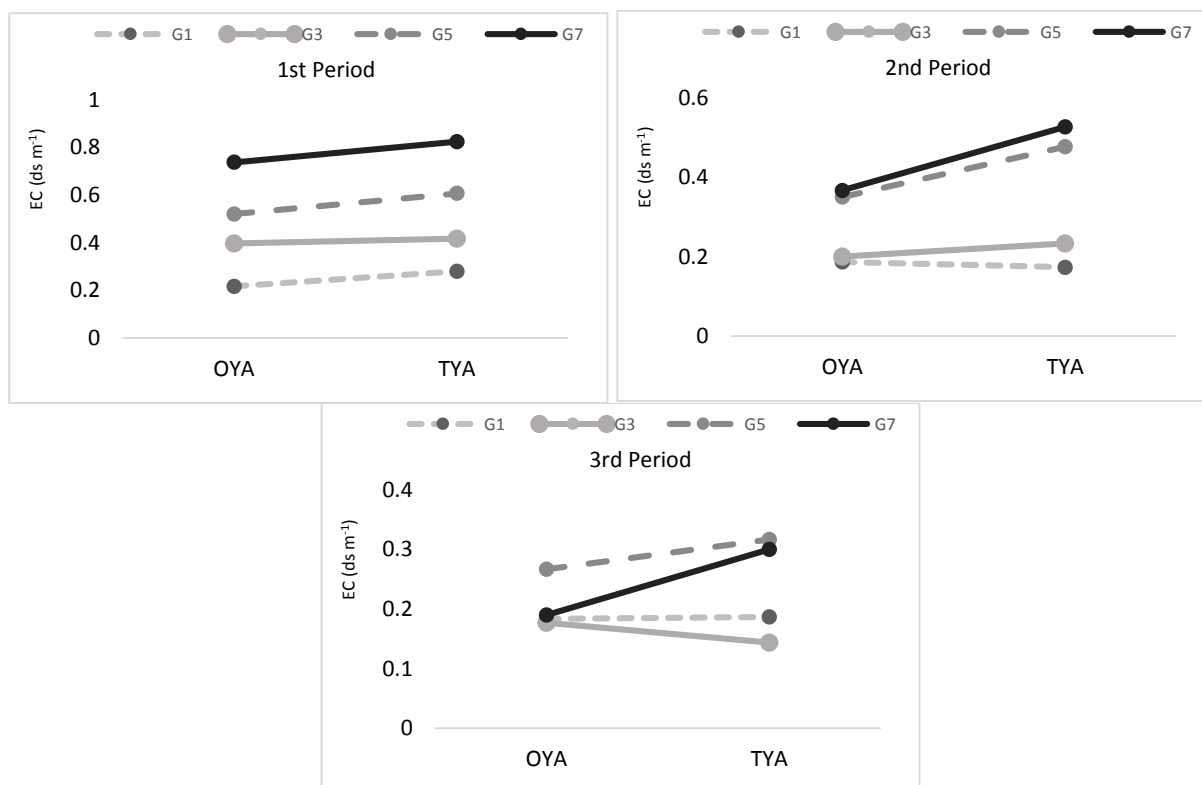
215 **Table 5.** Statistical analysis of EC and pH results based on method.

| | EC (1st Period) | | EC (2nd Period) | | EC (3rd Period) | | PH (1st Period) | | PH (2nd Period) | | PH (3rd Period) | |
|-------------------|-----------------|----|-----------------|----|-----------------|----|-----------------|----|-----------------|----|-----------------|----|
| | F | P. | F | P. | F | P | F | P. | F | P. | F | P |
| 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 |

216 F: Indicate the statistical significance from ANOVA

217 P: ns: not significant, *:<0,05, **:<0,01.

218
 219 In the 2nd period, a significant interaction was observed between the method and the LD
 220 dose. This reveals the impact of both the application method and the LD dose on the soil's pH
 221 and EC values. This pronounced effect was detected in measurements taken two months after
 222 the application of LD.



224 **Fig. 4.** Effect of the method on EC results.
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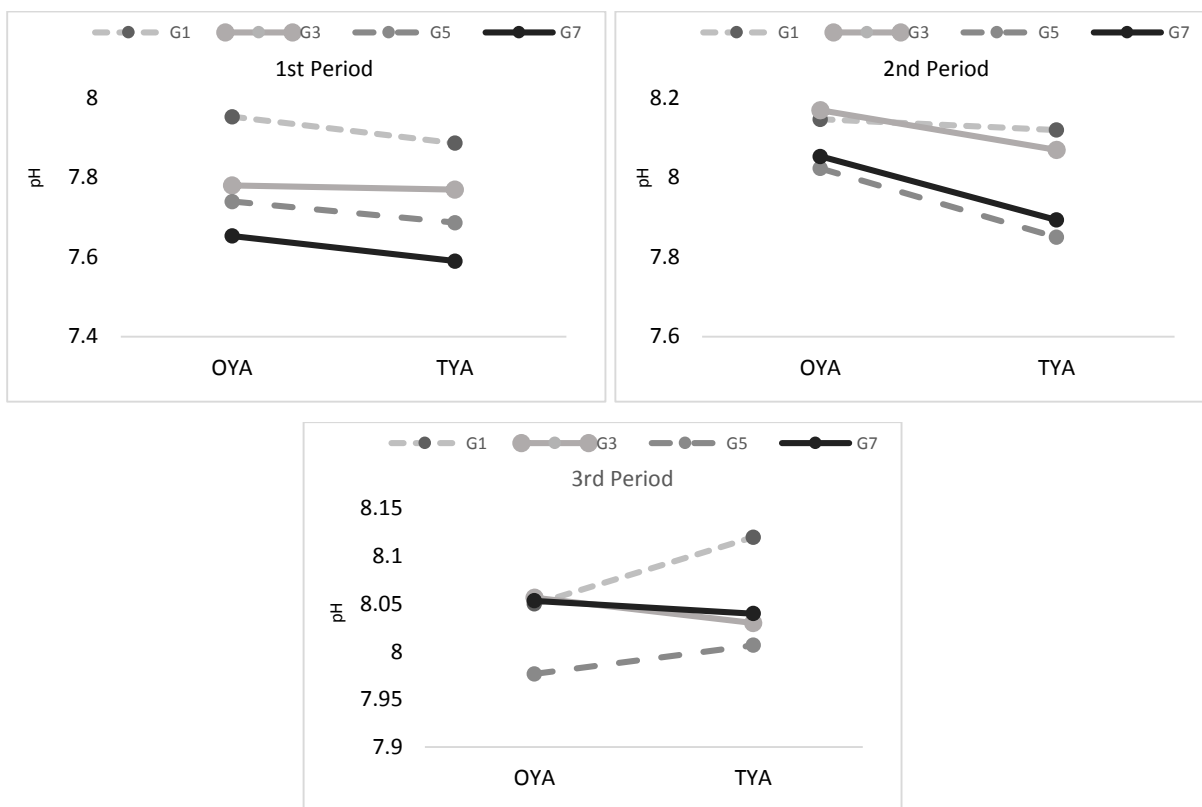
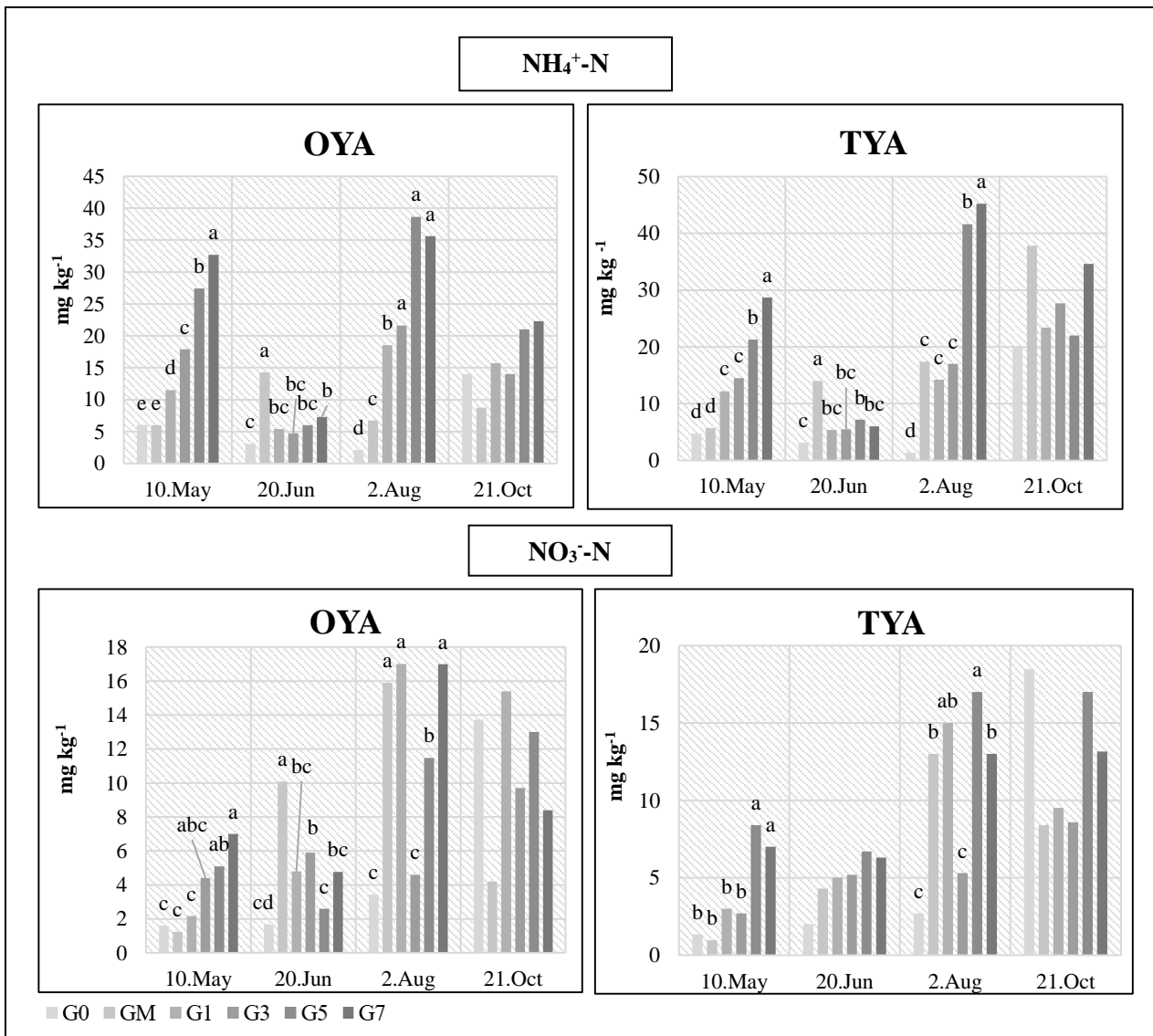


Fig. 5. Effect of the method on pH results.

3.2. Soil inorganic nitrogen content

LD application on April 23 resulted in monitoring soil inorganic nitrogen ($\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$) levels at specific dates (May 10, June 20, August 2, and October 21). When investigating the effect of LD on soil ammonium nitrogen ($\text{NH}_4^+\text{-N}$) levels, it was identified that as the dosage of LD increased, so did this value (Fig. 9). In the plots where LD was applied, the amount of inorganic nitrogen provided by the mineral fertilizer was observed to be added to the soil (Fig. 6). This finding suggests that LD applications are effective in adding the necessary inorganic nitrogen to the soil for maize plants. When the inorganic nitrogen levels of the soils were analyzed periodically, no significant difference was detected between the application methods. Both after mineral fertilizer application and LD applications, the added soil inorganic nitrogen levels showed similar values.



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

243
 244 **Fig. 6.** Values of inorganic nitrogen in the soil in two types of experiments (one year application, OYA; two
 245 consecutive year application, TYA). Different letters in each histogram indicate significant differences at $p < 0.05$
 246 (Duncan's Multiple Range Test) among means.

247
 248 **3.3. Grain yield and nitrogen parameters in the plant**

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

257

258 **Table 6.** Maize grain yield and percentage of total nitrogen in leaves and grains for different treatments in two
 259 types of experiments (one year application, OYA; two consecutive year application, TYA).

| Method | Treatments | Grain Yield (t ha ⁻¹) | N in Leaf (%) | N in Grain (%) |
|----------|------------|-----------------------------------|-----------------|----------------|
| OYA | G0 | 13,0 ± 0.5 d | 1,86 ± 0.03 d | 1,03 ± 0.02 c |
| | GM | 18,2 ± 0.6 a | 2,67 ± 0.06 ab | 1,17 ± 0.03 a |
| | G1 | 16,0 ± 0.4 bc | 2,50 ± 0.05 c | 1,09 ± 0.02 b |
| | G3 | 15,7 ± 0.5 c | 2,54 ± 0.04 bc | 1,07 ± 0.02 b |
| | G5 | 17,4 ± 0.5 ab | 2,58 ± 0.05 abc | 1,09 ± 0.02 b |
| | G7 | 19,1 ± 0.7 a | 2,70 ± 0.06 a | 1,18 ± 0.03 a |
| <i>F</i> | | 18,09** | 51,89** | 18,34** |
| TYA | G0 | 8,9 ± 0.2 c | 1,59 ± 0.04 d | 0,99 ± 0.02 c |
| | GM | 17,0 ± 0.5 b | 2,79 ± 0.06 a | 1,18 ± 0.03 a |
| | G1 | 16,0 ± 0.4 b | 2,51 ± 0.05 c | 1,09 ± 0.02 b |
| | G3 | 16,4 ± 0.4 b | 2,54 ± 0.05 c | 1,09 ± 0.02 b |
| | G5 | 17,9 ± 0.6 ab | 2,67 ± 0.07 b | 1,14 ± 0.03 a |
| | G7 | 19,4 ± 0.8 a | 2,73 ± 0.06 ab | 1,17 ± 0.03 a |
| <i>F</i> | | 38,64** | 182,19** | 26,01** |

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

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

263
 264 Between the methods, although the effect of LD on yield and nitrogen ratios in the plant
 265 has not shown a statistically significant change (Table 7), higher yields have been achieved
 266 with the TYA in G3, G5, and G7 treatments (Fig. 7). Additionally, in the TYA, a dose of 50
 267 tons ha⁻¹ of LD has provided a higher yield compared to mineral fertilization, which is different
 268 from the OYA. Within the method framework, there is no linear increase in the amount of
 269 nitrogen in the plant with the LD dose.

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

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

271 F: Indicate the statistical significance from ANOVA.

272 P: ns: not significant.

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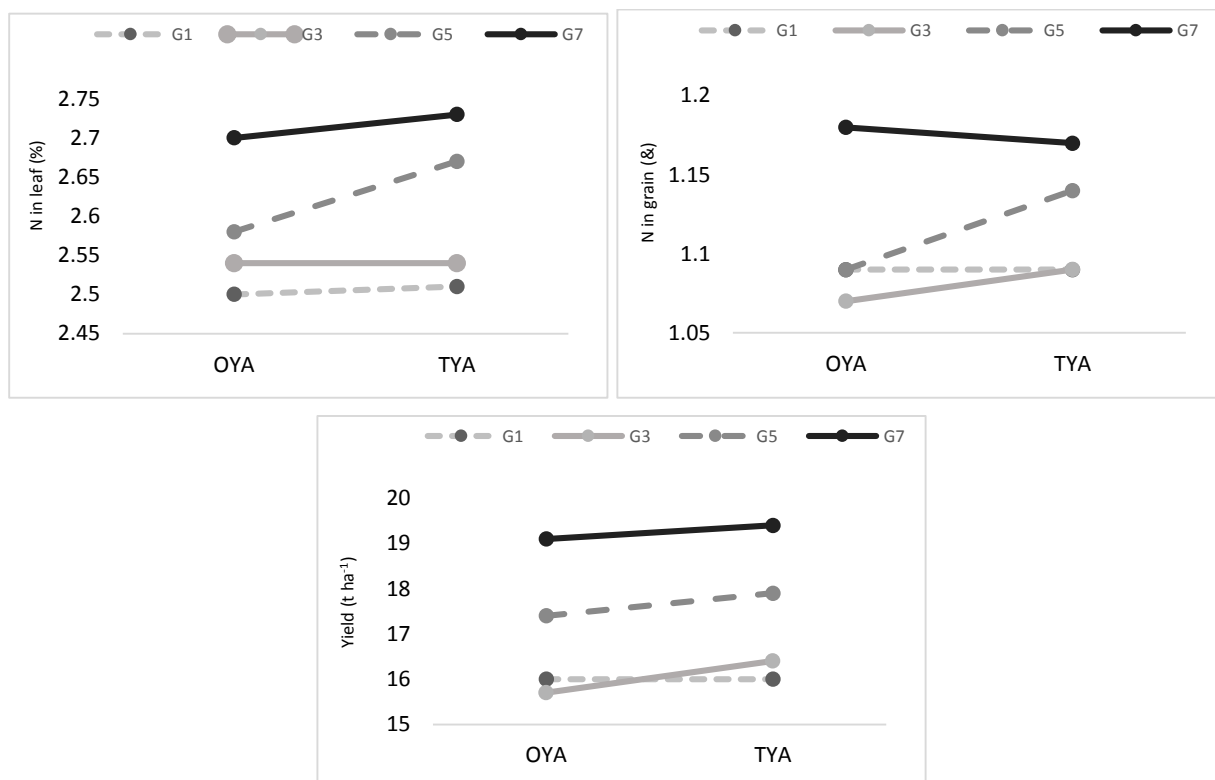
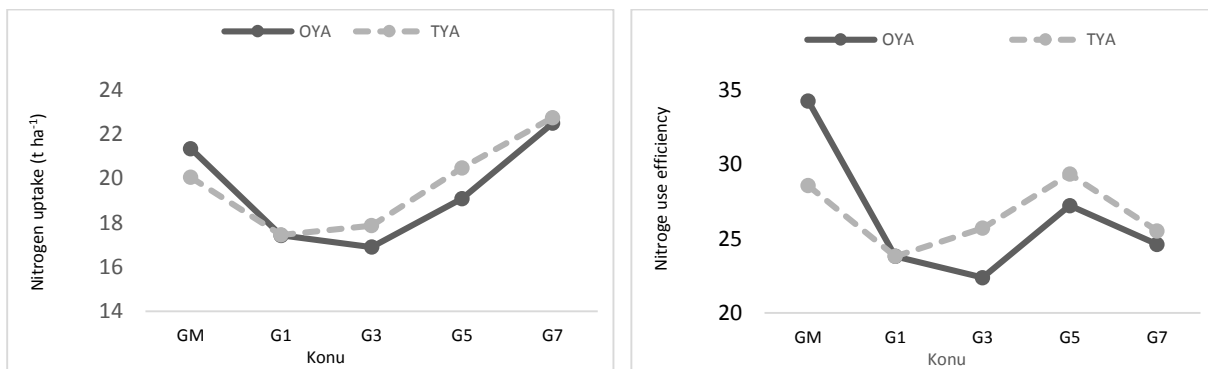


Fig. 7. The effect of the method on grain yield and nitrogen values.

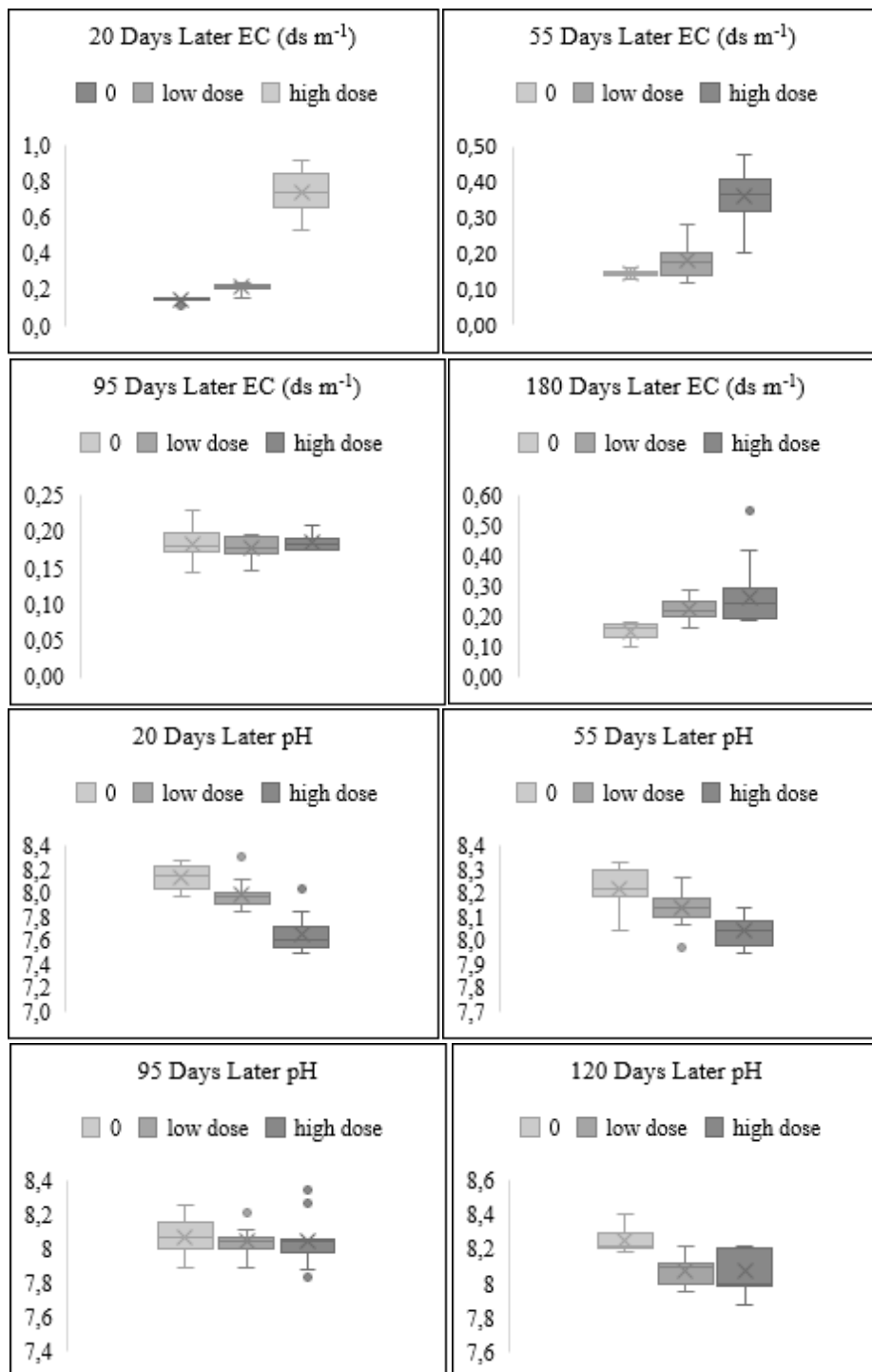
Nitrogen uptake and nitrogen use efficiencies have followed similar trends for both methods. An increase in LD dosage has increased yield and thus grain nitrogen uptake, and a higher value has been determined at a dosage of 70 tons ha^{-1} compared to mineral fertilization.

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 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 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^{-1} LD did not create a significant change in NUE. In G1 and G3 applications, which are combinations of LD with chemical fertilizer, NUE has shown a similar trend. However, in the TYA, G3 has had higher nitrogen uptake and NUE values compared to G1.



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Fig. 8. Grain nitrogen uptake and nitrogen use efficiency.



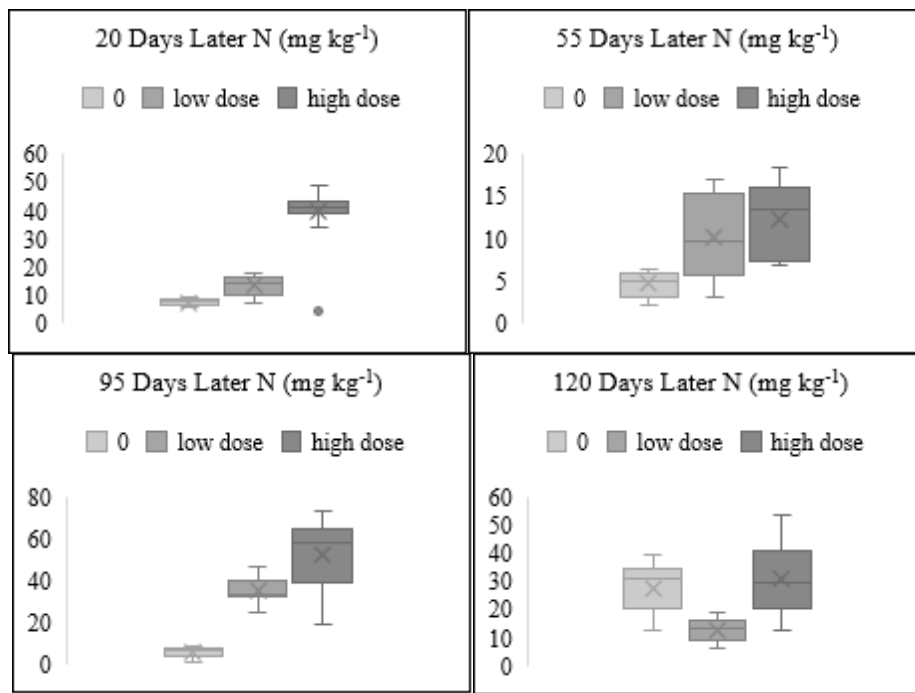
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Low dose: 10 t ha⁻¹ LD, High dose: 70 t ha⁻¹ LD

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

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4. Discussion

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4.1. EC and pH

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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 were observed in the first period with doses of 3.5 and 7 tons/da, whereas in the second period, it was determined that chemical fertilizer applications also caused a slight acidifying effect in the soil. The acidification became more pronounced with the impact of LD but diminished in subsequent periods. While Jia et al. (2013) stated that the LD raised soil pH values, Ren et al. (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 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⁺-N concentrations in the soil could lead to acidification by releasing H⁺ ions through nitrification.

333 4.2. Yield and nitrogen results

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

343 According to the study, the effect of LD on the soil's inorganic nitrogen amount is similar
344 to that of chemical fertilizer application. Due to the high $\text{NH}_4^+\text{-N}$ content of the LD, it is
345 anticipated to have a positive impact on grain yield compared to organic fertilizers (Möller &
346 Müller, 2012; Nkoa, 2014; Du vd., 2019). This positive effect has been identified by Al-Juhaimi
347 et al. (2014) on alfalfa and by Ernst et al. (2008) as well as Chantigny et al. (2008) on other
348 plants. Barlóğ et al. (2020) have suggested that the digestate can replace urea fertilizer as a
349 nitrogen source in the soil.

350 The nitrogen levels detected in leaves and grains have been found to be consistent with the
351 effect of LD on the inorganic nitrogen values in the soil. Variability in the concentration of
352 $\text{NH}_4^+\text{-N}$ or $\text{NO}_3^-\text{-N}$ in the soil can be a determinant on the nitrogen uptake of plants (Pan et al.,
353 1995; Barber et al., 1992). In maize, in particular, the amount of inorganic N in the soil, as well
354 as the form in which the plant takes up nitrogen, can influence the nitrogen rate in the leaves at
355 different growth stages. Significant findings on the dynamics of $\text{NH}_4^+\text{-N}$ in the soil have been
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 $\text{NH}_4^+\text{-N}$ in the form of $\text{NH}_3\text{-N}$ may be present depending
358 on the dose of LD.

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

363 Compared to chemical fertilization, the NUE has decreased with LD applications, but there
364 has been no significant difference in terms of NUE between the 10 and 30 t ha⁻¹ applications.
365 This suggests that LD application could lead to nitrogen losses in the soil. Materials with a low
366 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**

372 This study comprehensively examines the effects of the liquid digestate (LD) obtained
373 following biogas energy production on soil and maize plants. The impacts of various LD doses
374 (10, 30, 50, and 70 tons ha⁻¹) have been evaluated in one-year and consecutive two-year
375 applications.

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

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

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

398

399 **Acknowledgements**

400 The authors would like to express their gratitude to TAGEM (General Directorate of
401 Agricultural Research and Policies) for providing the necessary facilities for conducting this

402 research. This study was supported under the TAGEM project with the reference number
403 TAGEM/TSKAD/2021/B/A9/P1/5391.

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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 *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.
414 Miller, R. Ellis Jr., & J. D. Rhoades (Eds.), *Methods of soil analysis, Part 2- Chemical and*
415 *Microbiological Properties* (pp. 831-871). ASA-SSSA.

416 Anderson, J. P. E., & Domsch, K. H. (1978). A physiological method for the quantitative
417 measurement of microbial biomass in soils. *Soil Biology and Biochemistry*, 10(3), 215-221.

418 Anderson, T. H., & Domsch, K. H. (1986). Carbon assimilation and microbial activity in soil.
419 *Zeitschrift für Pflanzenernährung und Bodenkunde*, 149(4), 457-468.

420 Angelidaki, I., & Ellegaard, L. (2003). Codigestion of manure and organic wastes in centralized
421 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.

424 Aydın, B., Öztürk, O., Çobanoğlu, F., Çebi, U., Özkan, E., & Özer, S. (2020). Effects of drip
425 irrigation subsidies on silage maize production: A case study from Edirne. *International*
426 *Journal of Agriculture and Wildlife Science*, 6(3), 496-505.

427 Bationo, A., Kihara, J., Vanlauwe, B., Waswa, B., & Kimetu, J. (2007). Soil organic carbon
428 dynamics, functions and management in West African agro-ecosystems. *Agricultural*
429 *systems*, 94(1), 13-25.

430 Bao, S. D. (2005). *Soil and Agricultural Chemistry Analysis*. Beijing: Agricultural Press.

431 Barduca, L., Wentzel, S., Schmidt, R., Malagoli, M., & Joergensen, R. G. (2021).
432 Mineralisation of distinct biogas digestate qualities directly after application to soil. *Biology*
433 *and Fertility of Soils*, 57(2), 235-243.

- 434 Barlóg, P., Hlisnikovský, L., & Kunzová, E. (2020). Effect of digestate on soil organic carbon
435 and plant-available nutrient content compared to cattle slurry and mineral fertilization.
436 *Agronomy*, 10(3), 379.
- 437 Barber, K. L., Maddux, L. D., Kissel, D. E., Pierzynski, G. M., & Bock, B. R. (1992). Maize
438 responses to ammonium-and nitrate-nitrogen fertilization. *Soil Science Society of America
439 Journal*, 56(4), 1166-1171.
- 440 Brady, N. C., & Weil, R. R. (2008). *The soils around us. The nature and properties of soils*
441 (14th ed.). Pearson Prentice Hall, New Jersey and Ohio, 1-31.
- 442 Bremner, J. T. (1965). Inorganic forms of nitrogen. In *Methods of Soil Analysis: Part 2*
443 *Chemical and Microbiological Properties* (Vol. 9, pp. 1179-1237).
- 444 Brito, L. M., Coutinho, J., & Smith, S. R. (2008). Methods to improve the composting process
445 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
447 byproduct with low concentration of nutrients increased greenhouse gas emissions from soil.
448 *Geoderma*, 307, 81-90.
- 449 Chantigny, M. H., Angers, D. A., Bélanger, G., Rochette, P., Eriksen-Hamel, N., Bittman, S.,
450 ... & Gasser, M. O. (2008). Yield and nutrient export of grain maize fertilized with raw and
451 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.
- 458 Deviren, H., İlkılıç, C., & Aydın, S. (2017). Biyogaz üretiminde kullanılabilen materyaller ve
459 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.
- 462 Du, H., Gao, W., Li, J., Shen, S., Wang, F., Fu, L., & Zhang, K. (2019). Effects of digested
463 biogas slurry application mixed with irrigation water on nitrate leaching during wheat-maize
464 rotation in the North China Plain. *Agricultural Water Management*, 213, 882-893.
- 465 Du, Z., Xiao, Y., Qi, X., Liu, Y., Fan, X., & Li, Z. (2018). Peanut-shell biochar and biogas
466 slurry improve soil properties in the North China Plain: a four-year field study. *Scientific
467 Reports*, 8(1), 1-9.

- 468 El-Khatib, A. A., Al-Muhtaseb, A. H., Al-Makhadmeh, L. A., & Abu-Nameh, E. S. (2018).
469 Impact of liquid fermented organic waste on soil characteristics, plant growth, and yield.
470 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.
- 475 Fransman, B., & Nihlgård, B. (1995). Water chemistry in forested catchments after topsoil
476 treatment with liming agents in South Sweden. Water, Air, and Soil Pollution, 85(2), 895-
477 900.
- 478 Gee, G. W., & Bauder, J. (1986). Particle size analysis. In Methods of Soil Analysis, Part 1,
479 383-411.
- 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.
- 482 Głowacka, A., Szostak, B., & Klebaniuk, R. (2020). Effect of biogas digestate and mineral
483 fertilisation on the soil properties and yield and nutritional value of switchgrass forage.
484 Agronomy, 10(4), 490.
- 485 Gürbüz, M. A., & Öz, T. A. (2016). Ayçiçeğinin Azotlu Gübreleme Önerilerinde İndeks Olarak
486 Kullanılabilecek Parametrelerin Araştırılması [Investigation of Parameters that can be Used
487 as an Index in Nitrogen Fertilization Recommendations for Sunflower]. Çukurova Tarım ve
488 Gıda Bilimleri Dergisi, 31(3), 147-152.
- 489 Holm-Nielsen, J. B., Al Seadi, T., & Oleskiewicz-Popiel, P. (2009). The future of anaerobic
490 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.
- 492 Insam, H., Franke-Whittle, I. H., Knapp, B. A., & Plank, R. (2009). Use of wood ash and
493 anaerobic sludge for grassland fertilization: Effects on plants and microbes. Die
494 Bodenkultur, 60(2), 39-50.
- 495 Jackson, M. L. (1979). Soil Chemical Analysis: Advanced Course. Dept. Soil Science,
496 University of Wisconsin, Madison, WI, USA.
- 497 Jia, Y., Sun, G. X., Huang, H., & Zhu, Y. G. (2013). Biogas slurry application elevated arsenic
498 accumulation in rice plant through increased arsenic release and methylation in paddy soil.
499 Plant and Soil, 365(1), 387-396.
- 500 Jurgutis, L., Šlepetienė, A., Amalevičiūtė-Volungė, K., Volungevičius, J., & Šlepetys, J.
501 (2021). The effect of digestate fertilisation on grass biogas yield and soil properties in field-

502 biomass-biogas-field renewable energy production approach in Lithuania. *Biomass and*
503 *Bioenergy*, 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).
509 Rapid shift from denitrification to nitrification in soil after biogas residue application as
510 indicated by nitrous oxide isotopomers. *Soil Biology and Biochemistry*, 43(8), 1671-1677.

511 Loeppert, R. H., & Suarez, D. L. (1996). Carbonate and gypsum. *Methods of soil analysis: Part*
512 *3 chemical methods*, 5, 437-474.

513 Marschner, P., Kandeler, E., & Marschner, B. (2003). Structure and function of the soil
514 microbial community in a long-term fertilizer experiment. *Soil Biology and Biochemistry*,
515 35(3), 453-461.

516 Massé, D. I., Croteau, F., & Masse, L. (2007). The fate of crop nutrients during digestion of
517 swine manure in psychrophilic anaerobic sequencing batch reactors. *Bioresource*
518 *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-](https://www.mgm.gov.tr/veridegerlendirme/il-ve-ilceler-istatistik.aspx?k=A&m=KIRKLARELI)
521 [istatistik.aspx?k=A&m=KIRKLARELI](https://www.mgm.gov.tr/veridegerlendirme/il-ve-ilceler-istatistik.aspx?k=A&m=KIRKLARELI)

522 Moll, R. H., Kamprath, E. J., & Jackson, W. A. (1982). Analysis and interpretation of factors
523 which contribute to efficiency of nitrogen utilization. *Agronomy Journal*, 74(3), 562-564.

524 Möller, K. (2015). Effects of anaerobic digestion on soil carbon and nitrogen turnover, N
525 emissions, and soil biological activity. A review. *Agronomy for Sustainable Development*,
526 35(3), 1021-1041.

527 Möller, K., & Müller, T. (2012). Effects of anaerobic digestion on digestate nutrient availability
528 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.

531 Nyberg, K., Sundh, I., Johansson, M., & Schnürer, A. (2004). Presence of potential ammonia
532 oxidation (PAO) inhibiting substances in anaerobic digestion residues. *Applied Soil*
533 *Ecology*, 26(2), 107-112.

- 534 Pan, W. L., Camberato, J. J., Moll, R. H., Kamprath, E. J., & Jackson, W. A. (1995). Altering
535 source–sink relationships in prolific maize hybrids: Consequences for nitrogen uptake and
536 remobilization. *Crop Science*, 35(3), 836-845.
- 537 Panuccio, M. R., Romeo, F., Mallamaci, C., & Muscolo, A. (2021). Digestate application on
538 two different soils: Agricultural benefit and risk. *Waste and Biomass Valorization*, 12(8),
539 4341-4353.
- 540 Piccoli, I., Francioso, O., Camarotto, C., Delle Vedove, G., Lazzaro, B., Giandon, P., & Morari,
541 F. (2022). Assessment of the short-term impact of anaerobic digestate on soil C stock and
542 CO₂ emissions in shallow water table conditions. *Agronomy*, 12(2), 504.
- 543 Rawoof, S. A. A., Kumar, P. S., Vo, D. V. N., & Subramanian, S. (2021). Sequential production
544 of hydrogen and methane by anaerobic digestion of organic wastes: a review. *Environmental*
545 *Chemistry Letters*, 19, 1043-1063.
- 546 Ren, T., Yu, X., Liao, J., Du, Y., Zhu, Y., Jin, L., ... & Ruan, H. (2020). Application of biogas
547 slurry rather than biochar increases soil microbial functional gene signal intensity and
548 diversity in a poplar plantation. *Soil Biology and Biochemistry*, 146, 107825.
- 549 Robles-Aguilar, A. A., Temperton, V. M., & Jablonowski, N. D. (2019). Maize silage digestate
550 application affecting germination and early growth of maize modulated by soil type.
551 *Agronomy*, 9(8), 473.
- 552 Rohila, A. K., Maan, D., Kumar, A., & Kumar, K. (2017). Impact of agricultural practices on
553 environment. *Asian Journal of Microbiology, Environmental Science*, 19(2), 145-148.
- 554 SAPEA. (2012). Facheverhan biogas. Retrieved from
555 http://www.biogas.org/edcom/webfvb.nsf/id/DE_Branchenzahlen
- 556 Shi, L., Simplicio, W. S., Wu, G., Hu, Z., Hu, H., & Zhan, X. (2018). Nutrient recovery from
557 digestate of anaerobic digestion of livestock manure: a review. *Current Pollution Reports*, 4,
558 74-83.
- 559 Singh, A., Agrawal, M., & Marshall, F. M. (2010). The role of organic vs. inorganic fertilizers
560 in reducing phytoavailability of heavy metals in a wastewater-irrigated area. *Ecological*
561 *Engineering*, 36(12), 1733-1740.
- 562 Soil Survey Lab Staff, USDA. (1975). *Soil Taxonomy: A basic system of soil classification for*
563 *making and interpreting soil surveys* (No. 436.4 So3). US Govt. Print. Off.
- 564 Sutherland, R. A. (2018). Guidelines for the use of ICP-OES for the characterisation of urban
565 road-deposited sediment particle size fractions. *Environmental Pollution*, 238, 926–939.
- 566 Sürmen, M., & Kara, E. (2022). High-Quality Fertilizers from Biogas Digestate. *Environment*
567 *and Climate-smart Food Production*, 319-347.

- 568 Urta, 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.
- 570 Yakan, H., & Sağlam, T. (1997). Kırklareli koşullarında yetiştirilen hibrit mısır azotlu ve
571 fosforlu gübrelemenin dane verimi ve bazı kalite özelliklerine etkisi [Doctoral thesis, Trakya
572 University Faculty of Agriculture]. Edirne.
- 573 Yaraşır, N. (2018). The Effect Of Different Doses of Liquid Biogas Wastes on Yield and
574 Quality of Wheat (*Triticum aestivum* L.) (Master's thesis, Adnan Menderes University
575 Graduate School of Natural and Applied Sciences).
- 576 Zhao, Q., Cheng, J., Zhang, T., Cai, Y., Sun, F., Li, X., & Zhang, C. (2022). Biogas slurry
577 increases the reproductive growth of oilseed rape by decreasing root exudation rates at
578 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.