

## THE IMPACT OF DCD AND DMPP ON NITRIFICATION IN ALKALINE AND SILTY LOAM TEXTURED SOIL

**Murat GENCER, Mustafa GÖK**

Çukurova University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition,  
Balcalı, Sarıçam, Adana, Türkiye

Corresponding author email: mgencer@cu.edu.tr

### **Abstract**

*Nitrogen, which serves an important role in the vital activities of living organisms, is difficult to manage because it is highly mobile in soil. Nitrate, which is a form of mineral nitrogen, can cause economic and environmental effects through losses such as leaching and denitrification. Nitrification inhibitors slow down the biological process of nitrification in soil, preventing nitrate losses. In this study, the effects of DMPP and DCD on the nitrification process in soil with silty loam and alkaline textures were examined in an incubation conditions. The findings showed that the effects of DCD were still present on the 51st sampling day and both inhibitors were successfully inhibiting nitrification for more than 21 days. Soil mineral nitrogen content increased by 9% and 7% in the DCD and DMPP compared to the Control, respectively. DCD was found to be more effective at inhibiting nitrification in alkaline and silty loam soils. The impact of nitrification inhibitors on plant productivity and their function in mineral nitrogen losses require more research.*

**Key words:** DCD, DMPP, nitrogen, nitrification inhibitors, alkaline soil.

### **INTRODUCTION**

Nitrogen (N), one of the macronutrient elements for plants and microbes, is transported to animals and humans via the food chain (Blume et al., 2016). Plants and microorganisms get most of their nitrogen from the soil, but managing it is challenging because nitrogen is such a dynamic and mobile element (Zaman et al., 2009). For instance, organic N forms can be mineralized into ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) can be formed because of nitrification, and appeared nitrate can be either taken up by plants, leached from the soil, or removed from the soil with gaseous nitrous oxide ( $\text{N}_2\text{O}$ ) by the process called denitrification (Gök et al., 1999). Due to the highly mobile nitrogenous compounds in soil, significant losses occur relatively quickly (Zaman et al., 2009). Losses in applied nitrogen fertilizers might result in greater agricultural inputs, groundwater pollution, or environmental issues such as global warming (Blume et al., 2016). There are different strategies for reducing these losses. Nitrification inhibitors (NI) are one of the simple, practical, and successful techniques among the available approaches.

In well aerated soils, nitrifiers rapidly convert ammonium to nitrite and subsequently nitrate (Gök et al., 2004). Therefore, the predominant form of mineral nitrogen ( $\text{N}_{\text{min}}$ ) in agricultural soils is composed of nitrate (Liu et al., 2013). Nitrate is susceptible to leaching and loss in gaseous form. Nitrification inhibitors (NI) delay bacterial oxidation of ammonium ions by inhibiting the activity of *Nitrosomonas* spp. bacteria in soil for a particular time as 4 to 10 weeks (Kim et al., 2012; Liu et al., 2013; Sahrawat, 2008). Thus, ammonium in the soil may be utilized by plants while also protecting them against nitrate losses, either directly or indirectly.

The use of NI increases crop yield as well as nitrogen usage efficiency by reducing nitrogen losses in the soil (Abalos et al., 2014; Linnquist et al., 2013; Liu et al., 2013) while also contributing to the minimized environmental pollution (Cui et al., 2021). In addition, using NI provides farmers with more flexibility by reducing the number of fertilizer treatments and allowing for a wider range of application timings (Grant, 2005; Trenkel, 2010). Inhibiting nitrate formation indirectly reduces nitrogen loss through denitrification (Chaves et al., 2006; Yang et al., 2016). In contrast, the

form of  $\text{NH}_4^+$  releases ammonia gas ( $\text{NH}_3$ ), particularly under alkaline soil conditions, and serves as an indirect source of  $\text{N}_2\text{O}$  (Kim et al., 2012).

Although there are several effective NIs, such as 2-chloro-6-(trichloromethyl) pyridine (nitrapyrin) and 3-methylpyrazole (3-MP), the most commonly used are dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP) (Lan et al., 2018; Liu et al., 2013; Zaman et al., 2009). Both inhibitors inhibit the enzyme responsible for the oxidation of  $\text{NH}_4^+$  to  $\text{NH}_2\text{OH}$ , delaying the production of  $\text{NO}_3^-$  (Guardia et al., 2018). DCD's low volatility, economics, and water solubility have made it widespread (Zaman et al., 2009). On the other hand, DMPP provides benefits such being heterocyclic, which means it is highly effective even at very low doses (Chaves et al., 2006; Weiske et al., 2001), having less negative effects related to ecotoxicity, and being successful in lowering  $\text{N}_2\text{O}$  emission loss and  $\text{NH}_3$  volatilization (Liu et al., 2013).

The effectiveness of NI's is influenced by various environmental and soil factors (Akiyama et al., 2010). Although several studies have shown that soil pH has a significant impact on NI activity (Abalos et al., 2014), there have been a few investigations in alkaline soils, and the findings are conflicting. For instance, studies are showing that DCD (Cui et al., 2022; Guardia et al., 2018) or DMPP (Gök & Pamiralan, 2017; Zhou et al., 2020) are more effective in suppressing nitrification in alkaline soils ( $\text{pH} \geq 7.5$ ). Nitrification occurs between pH 5.5-10.0, with 8.5 being the optimal pH. Nitrification decreases significantly when soil  $\text{pH} < 5$ , but increases rapidly when  $\text{pH} > 6$  (Sahrawat, 2008). This existing information indicates that nitrification is a very important biochemical process, especially for alkaline soils. Therefore, determining NI efficiency in alkaline soil conditions will increase N use efficiency by reducing N losses.

This study was carried out to examine the effects of DCD and DMPP inhibitors on ammonium and nitrate fluctuation in alkaline and silty loam soil under incubation conditions. Our hypotheses for this research are that in alkaline and silty loam-textured soil, (I) NI's will slow down  $\text{NH}_4^+$  oxidation, causing the less  $\text{NO}_3^-$  formation in the soil and (II) DCD

will be more effective than DMPP in inhibiting nitrification.

## MATERIALS AND METHODS

The experiment's soil was collected from a 0-10 cm depth in an area that is representative of the Çukurova Region in Adana, Turkey. Soil was air-dried at room temperature and then sieved through a 2 mm diameter sieve. Basic soil analyses were performed to determine the typical characteristics of the soil to be used for the experiment, and the findings are presented in Table 1.

Table 1. The characteristics of the soil used in the study

| Properties                       | Unit                | Value      |
|----------------------------------|---------------------|------------|
| Texture                          |                     | Silty loam |
| Sand                             | %                   | 20.7       |
| Silt                             |                     | 53.1       |
| Clay                             |                     | 26.2       |
| Organic matter                   |                     | 2.01       |
| $\text{CaCO}_3$                  |                     | 31.0       |
| pH (1:2.5 $\text{H}_2\text{O}$ ) |                     | 7.84       |
| EC                               | $\text{dS m}^{-1}$  | 0.12       |
| $\text{NH}_4^+\text{-N}$         | $\text{mg kg}^{-1}$ | 6.4        |
| $\text{NO}_3^-\text{-N}$         |                     | 10.4       |
| P                                |                     | 8.72       |
| K                                |                     | 468        |
| Fe                               |                     | 4.57       |
| Zn                               |                     | 0.37       |
| Mn                               |                     | 15.9       |
| Cu                               |                     | 1.75       |

A 300 g of soil were weighed into containers of 5 cm height and 10 cm in diameter. As an ammonium source,  $(\text{NH}_4)_2\text{SO}_4$  (Sigma 1.01217) was used. Dicyandiamide source was DCD (%99 DCD; Sigma D76609), and DMPP source was ENTEC Solub 21 (%0.168 DMPP + %21  $\text{NH}_4^+\text{-N}$ ) treatment. For the N+DMPP treatment, since ENTEC Solub 21 contains 21%  $\text{NH}_4^+\text{-N}$ , the remaining amount of N was provided with  $(\text{NH}_4)_2\text{SO}_4$  to achieve a total of 300  $\text{mg N kg}^{-1}$ . Treatments were Control (300  $\text{mg N kg}^{-1}$ ), N+DCD (300  $\text{mg N kg}^{-1}$  + 30  $\text{mg DCD kg}^{-1}$ ) and N+DMPP (300  $\text{mg N kg}^{-1}$  + 3  $\text{mg DMPP kg}^{-1}$ ). Because DMPP is recommended to be at concentrations ten times lower than DCD (Weiske et al., 2001), similar doses were employed in this incubation trial. During the experiment, the soil's moisture content was increased to 70% of the water holding capacity, and the decreased moisture was given back with pure water at regular

intervals. The 12 pots prepared following the above-described procedure were placed in an incubator set at 30°C.

### Determination of mineral nitrogen in soils

The soil's  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  concentrations were determined on the 1st, 8th, 21st, 51st, and 81st days after the incubation experiment. To determine  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$ , 10 g of fresh soil were extracted with a 1%  $\text{AlK}(\text{SO}_4)_2$  solution. The  $\text{NH}_4^+\text{-N}$  content was determined by spectrophotometric measurement of the green-colored complex formed by nitroprusside salicylate with ammonium at 675 nm (DEZWAS, 1983). The  $\text{NO}_3^-\text{-N}$  content was also determined by spectrophotometric measurement of the yellowish color formed by nitrate with sodium salicylate at a wavelength of 430 nm (Fabig et al., 1978). The concentrations were determined by taking into consideration the moisture content of the soil. The results are expressed in grams per dry soil.

### Statistical analysis

This research was carried out according to completely randomized experimental design. Two-way repeated measures analysis of

variance (ANOVA) was used in the statistical analysis, which was conducted using the OriginPro 2021 program. In addition, the Tukey test was used to compare mean differences. The data was graphed using the "Paired Comparison Plot" add-on in OriginPro 2021.

## RESULTS AND DISCUSSIONS

### Changes in $\text{NH}_4^+\text{-N}$ concentration

The concentration of  $\text{NH}_4^+\text{-N}$  throughout the experiment is presented in Figure 1. The  $\text{NH}_4^+\text{-N}$  values determined in the sampling carried out just after the applications were lower than the initial  $\text{NH}_4^+\text{-N}$  values. This may have been due to the fixation of ammonium by colloids. On the second sampling day, the 8th day, the  $\text{NH}_4^+\text{-N}$  values in the control decreased radically, while the others decreased at levels not statistically different from the initial values. The  $\text{NH}_4^+\text{-N}$  values at the 21st sampling day, the differences between DCD and DMPP became apparent, and DCD was more effective than DMPP. This effect continued on the day 51. In the last sampling day, all values had decreased considerably and the differences between the treatments disappeared.

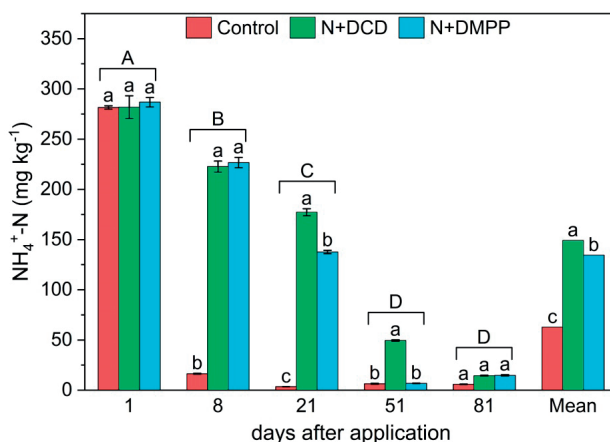


Figure 1. Effect of nitrification inhibitors on  $\text{NH}_4^+\text{-N}$  concentration in soil (According to the Tukey test, lowercase letters in the figure represent differences between treatments, whereas uppercase letters show differences between sampling days at a significance level of  $p < 0.05$ )

According to these findings, NI's prevented nitrification more than 74% of the applied  $\text{NH}_4^+\text{-N}$  within the first 8 days and 46% on the 21st day. The Control treatment had 58% and 53% lower  $\text{NH}_4^+\text{-N}$  concentrations than the

N+DCD and N+DMPP treatments, respectively ( $p < 0.05$ ). The obtained data are following the findings of Cui et al. (2021) that DCD and DMPP treatments had significantly ( $p < 0.05$ ) higher  $\text{NH}_4^+\text{-N}$  concentrations than the Control

treatment. When other studies on the subject were examined, it was determined that DCD and DMPP treatments were effective in suppressing nitrification in soil for 21 days (Guardia et al., 2018) and 35 days (Gök & Pamiralan, 2020). On the 21st day of sampling, where significant differences were found between all three treatments, N+DCD had 1.3 and 51 times greater  $\text{NH}_4^+\text{-N}$  concentrations than N+DMPP and Control treatments, respectively. Although there were similar concentrations between N+DCD and N+DMPP treatments, nitrification could not be suppressed in the Control treatment due to the absence of any NI and  $\text{NH}_4^+$  was oxidized to  $\text{NO}_3^-$ . On this sampling day, 1%, 59% and 46% of the initially applied  $\text{NH}_4^+\text{-N}$  was retained for Control, N+DCD and N+DMPP treatments, respectively. Similarly, on the 51st day of the experiment, the highest  $\text{NH}_4^+\text{-N}$  value was obtained in the N+DCD treatment, with 6.7 and 6.3 times higher  $\text{NH}_4^+\text{-N}$  compared to N+DMPP and Control treatments, respectively. The results suggest that DCD is more effective than DMPP in suppressing nitrification in

alkaline and silty loam soils. This might be attributed to the high dose of DCD used (10 times higher than DMPP). In soils without aeration problems, the dominant N form as a result of nitrification is  $\text{NO}_3^-$  (Gök et al., 2004), while with NI application, the dominant form became  $\text{NH}_4^+$  for a certain period.

While N in the form of  $\text{NH}_4^+$  in soils is in a form that is available to plants, it is protected against leaching by adsorption on clay minerals and organic matter due to its positive charges (Zaman et al., 2009), and N loss is indirectly prevented by denitrification (Abalos et al., 2014; Akiyama et al., 2010).

On the contrary, while there is concern that  $\text{NH}_4^+$  preserved in the soil with NI applications may be lost as  $\text{NH}_3$ , especially in alkaline soil conditions (Kim et al., 2012), Yang et al. (2016) and Cui et al. (2021) reported in their studies that DCD and DMPP did not affect  $\text{NH}_3$  loss.

### Changes in the $\text{NO}_3^-\text{-N}$ concentration

The determined  $\text{NO}_3^-\text{-N}$  concentrations were presented in Figure 2.

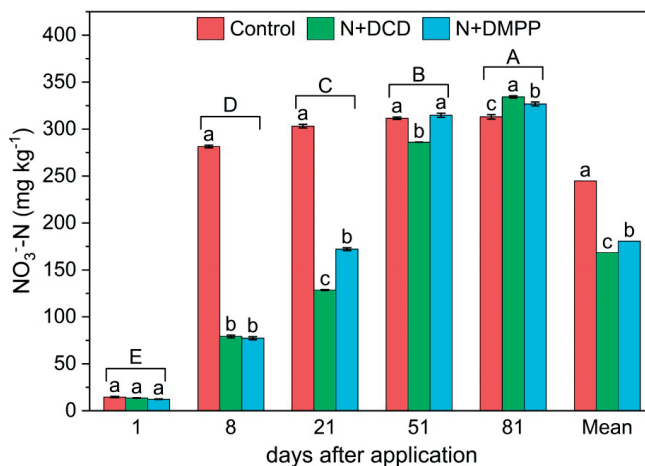


Figure 2. Effect of nitrification inhibitors on  $\text{NO}_3^-\text{-N}$  concentration in soil (According to the Tukey test, lowercase letters in the figure represent differences between treatments, whereas uppercase letters show differences between sampling days at a significance level of  $p < 0.05$ )

When the  $\text{NO}_3^-\text{-N}$  averages of the soils for 5 sampling days were examined, the Control treatment had 34% and 24% higher  $\text{NO}_3^-\text{-N}$  concentration than N+DCD and N+DMPP treatments, respectively, and it was found to be statistically significant among the treatments

( $p < 0.05$ ). According to the data obtained, the highest  $\text{NO}_3^-\text{-N}$  was determined as 334 mg  $\text{NO}_3^-\text{-N kg}^{-1}$  in the N+DCD treatment on the 81st day sampling and the lowest was determined as 12.2 mg  $\text{NO}_3^-\text{-N kg}^{-1}$  in the N+DMPP treatment on the 1st day sampling.

Although there was no difference between the treatments on the 1st day of sampling, on the 8th day of sampling, the  $\text{NO}_3^-$ -N content in all treatments increased by 19.3, 5.9 and 6.3 times for Control, N+DCD and N+DMPP treatments, respectively. Despite the application of NI in N+DCD and N+DMPP treatments,  $\text{NO}_3^-$ -N contents increased by  $65 \text{ mg kg}^{-1}$  in the 8 days. The increase in the Control treatment was found to be  $266 \text{ mg kg}^{-1}$ , suggesting that NI's were successful in suppressing nitrification but were not entirely effective in preventing it. The results are compatible with research conducted by Gök and Pamiralan (2020), which found that both DCD and DMPP delay nitrification, and the impact is similar. On the 21st day, the Control treatment had 2.4 and 1.8 times greater  $\text{NO}_3^-$ -N than the N+DCD and N+DMPP treatments, respectively. This difference was statistically significant ( $p < 0.05$ ). On the 51st day of soil sampling,  $\text{NO}_3^-$ -N concentrations were similar for the N+DMPP and Control treatments, however, the N+DCD treatment had about 9% lower  $\text{NO}_3^-$ -N, representing an inhibition effect continued. The findings are consistent with the results of Chaves et al. (2006), who found that DCD lost its effectiveness after 50 days, but contradict the findings that DMPP prevented nitrification for at least 95 days. The main reason for this may be due to the pH difference of the soils between the two studies. The soil pH used in Chaves et al. (2006) was 6.5, whereas the pH in this experiment was 7.84. Similar to the data in this study, Cui et al. (2021) reported that the effectiveness of NI's lasted for more than 110 days in acid soil ( $\text{pH} = 5.44$ ), but this effectiveness decreased to 70 days in alkaline soil ( $\text{pH} = 7.66$ ). According to the researchers, this was caused by the weakening of nitrification in acidic soils. Furthermore, Guardia et al. (2018) found that the nitrification inhibition efficiency of DMPP decreased when compared to DCD in low organic carbon and alkaline soil pH conditions. While the highest  $\text{NO}_3^-$ -N concentrations in the 81st day sampling of the soils were determined in N+DCD and N+DMPP, these two treatments were determined to be statistically significantly higher ( $p < 0.05$ ) compared to the Control treatment. The low  $\text{NO}_3^-$ -N concentration obtained in the Control application at the end of

the experiment may be due to the fact that the dominant N form was  $\text{NO}_3^-$  as of the 8th day sampling and this  $\text{NO}_3^-$  was lost by denitrification (Abalos et al., 2014). Although denitrification is a reduction reaction that occurs in anaerobic conditions (Gök et al., 1998), N loss in the form of  $\text{N}_2\text{O}$  can also occur at water contents below 60% of the total pore volume (Blume et al., 2016). This could have occurred in N+DCD and N+DMPP, but since their  $\text{NO}_3^-$  concentration is smaller than in the Control treatment, there would likely be less denitrification loss.

### **Changes in the mineral nitrogen ( $\text{N}_{\text{min}}$ ) concentration**

The total mineral nitrogen as the sum of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N concentration values are given in Figure 3. According to the data obtained, the lowest and highest  $\text{N}_{\text{min}}$  concentrations were determined as  $295 \text{ mg N}_{\text{min}} \text{ kg}^{-1}$  in the 1st day sampling and  $349 \text{ mg N}_{\text{min}} \text{ kg}^{-1}$  in the 81st day sampling in the N+DCD treatments, respectively. There was no statistically significant difference between the  $\text{N}_{\text{min}}$  concentrations of the treatments until the 81st day of the study. On the 81st day,  $\text{N}_{\text{min}}$  concentrations increased significantly by 9% and 7% in N+DCD and N+DMPP treatments, respectively, compared to the Control treatment ( $p < 0.05$ ). This difference may have occurred due to possible mineralization of organic matter in favorable moisture content and temperature. The fact that both NI applications have higher  $\text{N}_{\text{min}}$  concentrations than the Control treatment suggests that  $\text{N}_{\text{min}}$  losses may have occurred in the Control treatment. This finding is consistent with the results of Linquist et al. (2013) that N losses in the pH range of 6-8 are reduced by the use of NI. In the Control treatment, the dominant  $\text{N}_{\text{min}}$  form being  $\text{NO}_3^-$  may have caused the difference between the treatments, resulting in N loss by denitrification. In a study conducted by Menéndez et al. (2012), dual application of fertilizer and DMPP reduced  $\text{N}_2\text{O}$  loss by 3 to 45% depending on incubation conditions compared to sole fertilizer application. In a meta-analysis conducted by Yang et al. (2016), it was stated that DCD and DMPP reduced  $\text{N}_2\text{O}$  at a similar rate (45% and 48%, respectively) and that this was due to the decrease in the  $\text{NO}_3^-$ -N concentration of the

soil. During the experiment period, the  $N_{min}$  concentration of the soils increased in all three treatments. With the application of N+DCD, 67% of the added DCD contains N and as a result of the mineralization of 30 mg DCD  $kg^{-1}$ , an increase of 20 mg N  $kg^{-1}$  is expected in the soil. This increase may be the main reason why N+DCD has the highest  $N_{min}$  concentration.

Furthermore, N mineralization in soils is expected to cause an increase in  $N_{min}$  concentration. In an incubation study conducted by Kaleem Abbasi et al. (2007), there was an increase of 24.4 mg  $kg^{-1}$  in the  $N_{min}$  concentration of the soil after 90 days in a soil with 0.85% organic matter.

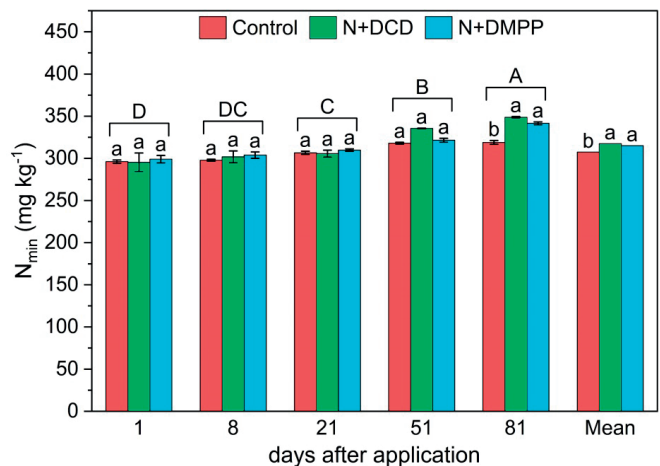


Figure 3. Effect of nitrification inhibitors on  $N_{min}$  concentration in soil (According to the Tukey test, lowercase letters in the figure represent differences between treatments, whereas uppercase letters show differences between sampling days at a significance level of  $p < 0.05$ )

### $NH_4^+$ -N and $NO_3^-$ -N distribution among 5 sampling days

The distribution of  $NH_4^+$ -N and  $NO_3^-$ -N in soils on 5 sampling days is given in Figure 4. These data show that both  $NH_4^+$ -N and  $NO_3^-$ -N are distributed homogeneously in NI treatments, but in the Control treatments, the distribution is predominantly in the direction of  $NO_3^-$ -N. This unbalanced distribution has the potential to cause  $NO_3^-$ -N losses in soils. On the contrary, the use of NI will ensure that  $NH_4^+$  in the soil is converted to  $NO_3^-$  over time, thus keeping N stable in the soil and increasing N use efficiency (Akiyama et al., 2010).

### CONCLUSIONS

Applications of NI's combined with fertilizer inhibited nitrification and maintained the soil's  $NH_4^+$  content for over 21 days. This study shows that NI's are a significant tool to increase N utilization efficiency, particularly in soils with a risk of  $NO_3^-$  leaching. According to the findings, DCD is more advantageous than DMPP since it persists for longer than 51 days under alkaline soil conditions, is less expensive, and provides N to the soil through mineralization. Testing the real effectiveness of NI's in field conditions and examining their effects on plant parameters will be beneficial for farmers in the region.



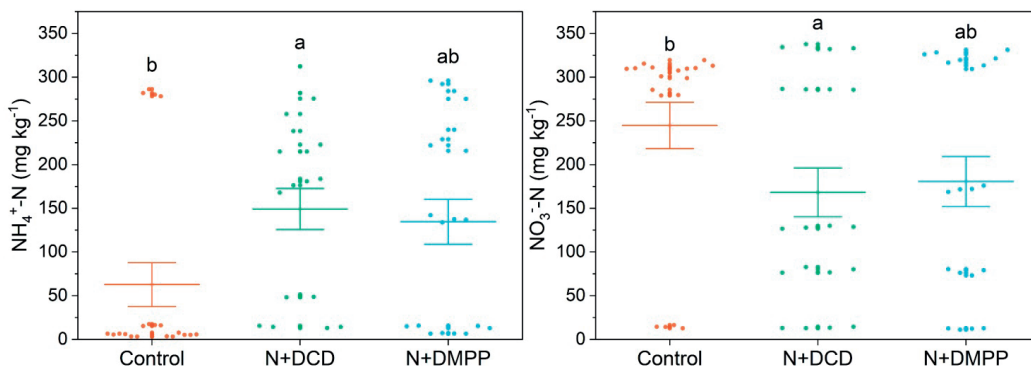


Figure 4. The 81-day distribution of the soil's  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  concentrations (Letters in the figure indicate differences at  $p < 0.05$  significance level according to Tukey test)

## ACKNOWLEDGEMENTS

We are grateful to Prof. Dr. Ali COŞKAN for his thorough review of the manuscript, his constructive suggestions and improving the language.

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