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How did the Addition of Indaziflam Affect on Carbon and Nitrogen Mineralizations in a Vineyard Soil?

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ABSTRACT

Indaziflam is a herbicide used for weed control in vineyards, apple, peach and orange orchards that inhibit cellulose biosynthesis in plants. The objective of this study was to evaluate the effects of recommended field dose of herbicide Indaziflam (10 ml/ da, RD) and its 2 (RD x2), 4 (RD x4), 8 (RD x8) and 16 (RD x16) folds of RD on carbon and nitrogen mineralizations in a vineyard soil. Herbicide+soil mixtures were humidified at 80% of soil field capacity and then incubated for 42 days at 28ºC . Effects of RD and RD x2 doses on soil carbon mineralization were similar to control and no significant difference was found between them. Higher doses of indaziflam (RD x4, RD x8 and RD x16) stimulated mineralization of soil carbon and there were found significant differences between control and these doses ($P<0.05$). All application doses of herbicide showed variability in ammonium (NH_4-N) and nitrate (NO_3-N) contents while there were generally found no significant differences between control and RD. In general, contents of soil NH_4-N and NO_3-N were increased in all applications as time passed and there were significant differences between days that were measured of these contents ($P<0.05$). Results of soil nitrogen mineralization rate were as following: 1) It was significantly decreased by only RD x2 on 11th day ($P<0.05$) 2) Higher doses of Indaziflam (RD x4, RD x8 and RD x16) significantly stimulated it on 26th day ($P<0.05$) 3) All doses of this herbicide significantly decreased it on 42nd day ($P<0.05$). In conclusion, the recommended field dose of Indaziflam had no negative effect on microorganisms that play an active role in soil carbon and nitrogen mineralization.

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Introduction

The global area under vines and total production of grapevine were reported as 7.400.000 ha and 77.800.000 tonnes in world in 2018 [1]. Turkey takes place as fifth in world countries in the production area of vineyard (4.170.410 da) and total grape

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(3.933.000 tonnes) [1, 2]. It was noted that grape export is an important income source for the economy of a country [3].

Weed control and elimination in and between grapevine rows by chemicals were more widespread applied as the intensification of viticulture was increased [4]. Vines were competed by weeds for water and nutrients in soils and many pathogens causing diseases in weeds by hosting can cause loss in productivity in grapes at 10.1 % [3]. Prevention of trunk damage by mechanical weeding machines and decrease of working time spent in the vineyard were provided by herbicides [5]. Herbicides have become the most important control method for weed while these chemicals were more considered than other methods due to their easy application and being effective and reliable in weed control [6]. Amount of herbicides that was applied in agricultural fields was reported as 12.644 tonnes and this was 24.6% of total pesticides that were applied in Turkey in 2019 [7]. While the impacts of fungicides and insecticides on soil organisms that were applied in vineyards, it was reported that there is a little knowledge about the effects of herbicides on these organisms [8]. In addition to that, various non-target effects of herbicide on soil microorganisms were found in laboratory and pot studies [9, 10].

Indaziflam is a pre-emergent herbicide that inhibits cellulose biosynthesis and used for perennial grasses and broadleaf weeds [11]. This herbicide has been mainly applied in perennial plants (orange, grape, apple and drupe trees), settlements and areas that were not used for plant production, public domains and forests [12]. Indaziflam can remain as a residue for a long time even in low doses as well as it is resistant to decompose for much longer (150 days) [13]. Researches in four soil samples in Europe and two soil samples in the United States of America (U.S.A) showed that this duration could be in between 22 and 176 days [12].

It was reported that indaziflam showed weak acid ability over soil pH 5.4 [11, 13]. In addition, it was highlighted that indaziflam was non-volatile and could be fade away through decomposition and leaching [11]. It was found that mobility of indaziflam in soil was low and mid-level and sorption of this herbicide in six Brazil oxisol and in three U.S.A. mollisol soils showed positive correlation with organic carbon content [11]. It was indicated that the phytotoxic effects of indaziflam increased in soils containing low organic carbon [14]. Furthermore, it was pointed out that damage of indaziflam to hybrid bermuda grass grown in sandy soil was higher than in silt loam soil

[15]. Finally, it was suggested that the effects of increasing doses of Indaziflam on soil microbial activity should be determined [12].

It was hypothesized that higher doses of Indaziflam would decrease soil microbial activity. The objective of this study was to evaluate the effects of recommended field dose (RD) and its 2 (RD x2), 4 (RD x4), 8 (RD x8) and 16 (RD x16) folds of indaziflam on carbon and nitrogen mineralizations in a vineyard soil.

Material and Methods

Material and study area

Soils used in this study were sampled at 0-10 cm depth from Cukurova University Faculty of Agriculture Vineyard (Adana, Turkey) in May 2018. Indaziflam containing herbicide ($C_{16}H_{20}FN_5$, molecular weight: 301.369 g/mol, 500 g/ 1000 ml active ingredient) was bought commercially and its recommended field dose was 10 ml/da.

Some soil physical and chemical properties

Soils were mixed homogenized and considered as a composite and representative sample and then sieved a 2 mm mesh sieve, plant debris was removed. Soil texture was determined by Bouyoucos hydrometer, field capacity (%, FC) by 1/3 atmospheric pressure with a vacuum pump, pH by a 1:2.5 soil-water suspension with pH-meter (inoLab pH/Cond 720, WTW GmbH, Weilheim, Germany) and $CaCO_3$ content (%) by a Scheibler calcimeter [16]. Organic carbon and total nitrogen (TN) contents of soils (%) were determined by the modified Walkley and Black method and Kjeldahl method, respectively [16]. The determination of soil organic carbon is based on the Walkley & Black chromic acid wet oxidation method. Oxidizable organic carbon in the soil is oxidised by potassium dichromate ($K_2Cr_2O_7$) solution in concentrated sulfuric acid. The determination of total nitrogen in soil is based on digestion of the dried and homogenised soil in a suitable Kjeldahl tube with sulfuric acid. To rise the temperature, potassium sulfate is added and copper sulfate is used as a catalyst. After adding sodium hydroxide to the digestion solution the produced ammonium from all nitrogen species is evaporated by distillation as ammonia. This is condensed in a conical flask with boric acid solution. The amount is titrated against Tashiro's indicator with sulfuric acid [16]. Three replicates were used for each analysis.

Soil carbon and nitrogen mineralizations

Based on the calculation of soil volume weight (1.28 g/cm^3) and introduction of herbicide to the soil at 1 mm depth, recommended field dose of herbicide containing Indaziflam (RD, 10 ml/da) and 2 (RD x2), 4 (RD x4), 8 (RD x8) and 16 (RD x16) folds of RD were mixed with soil. Soils untreated with Indaziflam were used as control.

Soil mixtures (100 g soil+herbicide) were placed in 750 ml incubation vessels and the final moisture content of soils was adjusted to 80% of their own field capacity before incubation at 28 °C over 42 days for carbon mineralization. CO_2 produced from the microbial activity was absorbed periodically in 10 ml saturated 1 M NaOH solution in beakers, which were placed on the top of the soil in incubation vessels. Microbial respiration was measured by titration with 1 M HCl in these closed vessels in the following days of incubation: 1, 3, 7, 14, 28 and 42 [17]. Three replicates were used for each treatment and control. Cumulative carbon mineralization (mg CO_2-C/100 g soil) was calculated by summing up all measured days CO_2 until the end of incubation period while their rates at 42^{nd} day were calculated by dividing cumulative mineralized carbon by its soil organic carbon [18].

Soil samples (100 g) mixed with herbicide were humidified at 80% of soil field capacity, placed in 750 ml incubation vessels and incubated for 42 days at 28°C for nitrogen mineralization. Three replicates were used for each treatment and control. These vessels covered with gauze for aeration were weighed three times every week to determine any weight loss. Distilled water was added when necessary to maintain soil moisture for 42 days. Ammonium and nitrate contents (NH_4-N + NO_3-N) were measured in soils to calculate nitrogen mineralization rate at 11^{th}, 26^{th} and 42^{nd} days of incubation. All soil samples were mixed separately with 200 ml 1 N $CaCl_2$ solution and shaken for 1 h strained samples were distilled to measure mineral nitrogen by the Parnas-Wagner method [19, 20]. Nitrogen mineralization rate was calculated by dividing the total amount of mineral nitrogen by total nitrogen of soil [21].

Statistical analysis

Statistical analyses were performed by the software SPSS v.20. The data were submitted to ANOVA to assess the differences among treatments and incubation days. The separation of means was made according to the Tukey honestly significant difference (HSD). Differences between the data were declared as significant at $P < 0.05$.

Results

Soil analysis

Some physical and chemical properties of soil sampled from the vineyard were summarized in Table 1 below. The soil was loamy and slightly alkaline while field capacity and CaCO3 contents of soil were 24.48% and 43.38%, respectively. Soil organic carbon and nitrogen contents were 1.97% and 0.15% respectively while C/N was determined 12.86.

Table 1 Some physical and chemical properties of soil samples

Clay (%)	25.10 ± 0.06
Silt (%)	30.83 ± 0.09
Sand (%)	44.07 ± 0.03
Texture	Loam
FC (%)	24.48 ± 0.16
pH	7.98 ± 0.03
$CaCO_3$ (%)	43.38 ± 0.24
C (%)	1.97 ± 0.02
N (%)	0.153 ± 0.001
C/N	12.86 ± 0.11

Results are presented as means ± standard error of triplicate analysis (FC: field capacity)

Soil CO_2 evolution

Cumulative carbon mineralization of soils (mg C(CO_2)/100 g soil) for 42 days were as following: 55.57 for control, 55.16 for RD, 54.91 for RD x2, 58.02 for RD x4, 58.85 for RD x8 and 66.85 for RD x16 (Figure 1). There were found no significant differences between control, RD and RD x2. RD x4, RD x8 and RD x16 significantly increased soil microbial respiration compared to control ($P<0.05$).

Carbon mineralization rates (%) were determined as at the end of incubation period as following: 2.82 for control, 2.80 for RD, 2.79 for RD x2, 2.95 for RD x4, 2.99 for RD x8 and 3.40 for RD x16 (Figure 2). No significant differences were found between control and both RD and RD x2. There were only found significant differences between control and higher doses of herbicide (RD x4, RD x8 and RD x16) in rate of carbon mineralization.

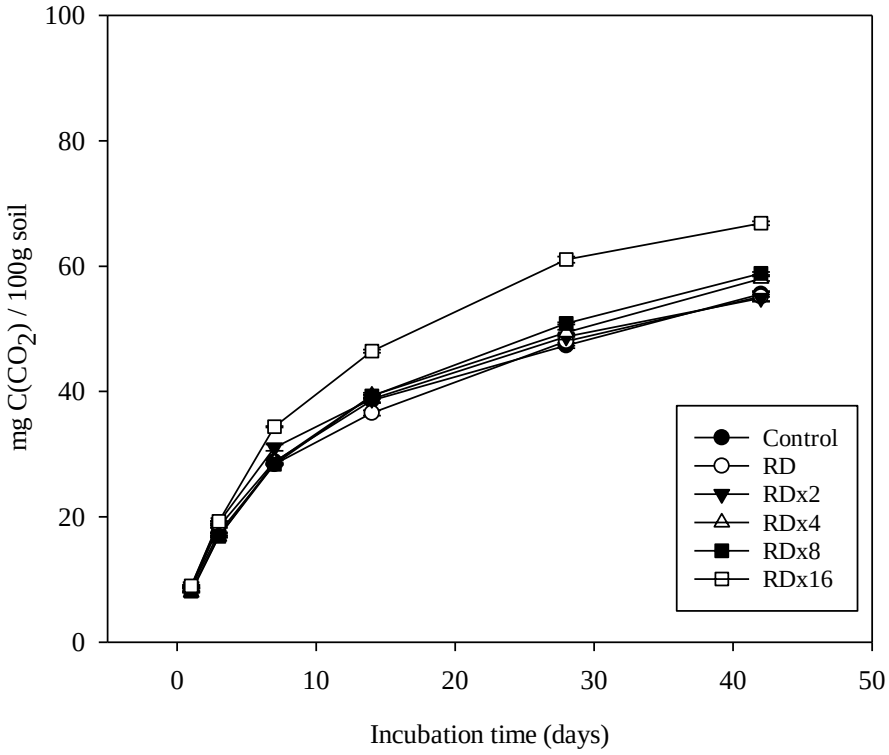

Fig 1 Cumulative carbon mineralization of soils during 42 days at 28ºC (mean ± standard error, mg $C(CO_2)$ /100 g soil, n=3)

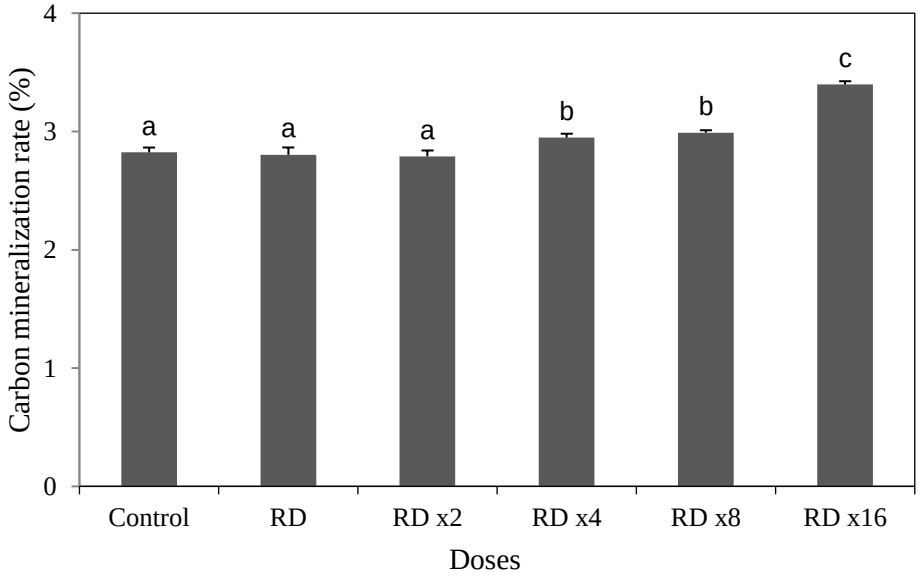

Fig 2 Soil carbon mineralization rates (mean ± standard error, %, n=3, a, b and c indicate significant differences between means)

Soil NH_4-N and NO_3-N contents and nitrogen mineralization rates

Soil NH_4-N and NO_3-N contents (mg/kg) were summarized in Table 2 and were as following: NH_4-N contents were in between 7.92 (RD x16) and 23.97 (Control) while NO_3-N contents were in between 3.92 (Control) and 15.07 (Control) in all measured days.

NH_4-N content results were on 11^{th} day as following: Control was significantly higher than higher doses of herbicide except RD ($P<0.05$). The highest decrease was determined in RD x16 dose and this dose decreased NH_4-N content for 38.1% compared to control. In contrast, NO_3-N contents on 11^{th} day in all herbicide doses were higher than control and there were found only significant differences between control and higher doses (RD x4, RD x8 and RD x16, $P<0.05$). The highest increases in NO_3-N contents were obtained at RD x4 and RD x16 for 106.2% and 97.2% compared to control, respectively (Table 2).

Herbicide doses generally increased mineral nitrogen contents (NH_4-N and NO_3-N) on 26^{th} day as following: there was an only significant difference between control and RD x8 in NH_4-N contents while this increase was 33.3% compared to control ($P<0.05$). On the other hand, RD x4, RD x8 and RD x16 significantly increased NO_3-N contents ($P<0.05$) while these contents were highest increased by RD x16 for 165.5% compared to control (Table 2).

All doses of herbicide decreased soil NH_4-N and NO_3-N contents at the end of incubation (42^{nd} day) as following: Significant differences were found between control and all doses except RD in NH_4-N contents while there were between control and RD and RD x4, separately ($P<0.05$). The highest decrease rates were obtained at RD x16 for 26.4% in NH_4-N contents and at RD for 61.1% in NO_3-N contents compared to control on 42^{nd} day.

Soil NH_4-N and NO_3-N were generally increased in all treatments as time progressed as following: 11^{th} day$<26^{th}$ day$<42^{nd}$ day. There were significant differences in NH_4-N contents of all treatments between 11^{th} and 26^{th} days ($P<0.05$). However, only significant differences were found between 26^{th} and 42^{nd} day in NH_4-N contents of control, RD x4 and RD x8. In contrast, differences between 11^{th} and 26^{th} days in nitrate contents of all treatments except RD x16 were non-significant while differences between 26^{th} and 42^{nd} days in nitrate contents of all treatments except RD x4 were found

significant (P<0.05). There were significant differences between 11th and 42nd days in ammonium contents of all treatments and nitrate contents of all treatments except RD x4 (P<0.05).

Table 2 Soil NH_4-N and NO_3-N contents (mg/kg) on 11th, 26th and 42nd days

	NH_4-N (mg/kg)			NO_3-N (mg/kg)		
	11. Day	26. Day	42. Day	11. Day	26. Day	42. Day
Control	12.79 abz	17.72 by	23.97 ax	3.92 cy	4.05 dy	15.07 ax
	(0.71)	(1.07)	(0.18)	(0.03)	(0.37)	(0.33)
RD	14.97 ay	18.15 bxy	23.96 ax	4.20 cy	5.03 dy	9.21 bx
	(0.97)	(0.07)	(1.48)	(0.01)	(0.33)	(0.30)
RD x2	8.53 cy	16.99 bx	19.51 bcx	4.54 cy	6.01 cdy	13.71 ax
	(0.40)	(0.23)	(1.19)	(0.12)	(0.80)	(0.83)
RD x4	9.75 bcz	18.01 by	19.44 bcx	8.09 ax	8.60 bcx	9.22 bx
	(0.25)	(0.07)	(0.22)	(0.22)	(0.35)	(0.02)
RD x8	9.54 cz	23.62 ay	19.07 bcx	6.09 by	7.99 aby	14.16 ax
	(0.04)	(0.33)	(0.05)	(0.09)	(0.47)	(0.49)
RD x16	7.92 cy	18.75 bx	17.65 cx	7.74 az	10.74 ay	13.12 ax
	(0.44)	(0.23)	(0.07)	(0.42)	(0.26)	(0.43)

Results are presented as means ± standard error of triplicate analysis. Significant differences between measurement days were indicated in the same column as x, y and z and in the same line as a, b, c and d between treatments (P<0.05)

Rates of nitrogen mineralization in all treatments were given in Figure 3. These rates were between 0.85 % (RD x2 on 11th day) and 2.55% (Control on 42nd day). Only RD x2 significantly decreased this rate on 11th day compared to control (P<0.05). All treatments except RD and RD x2 doses significantly increased this rate on 26th day (P<0.05). At the end of incubation period, all herbicide doses significantly reduced nitrogen mineralization rate (P<0.05).

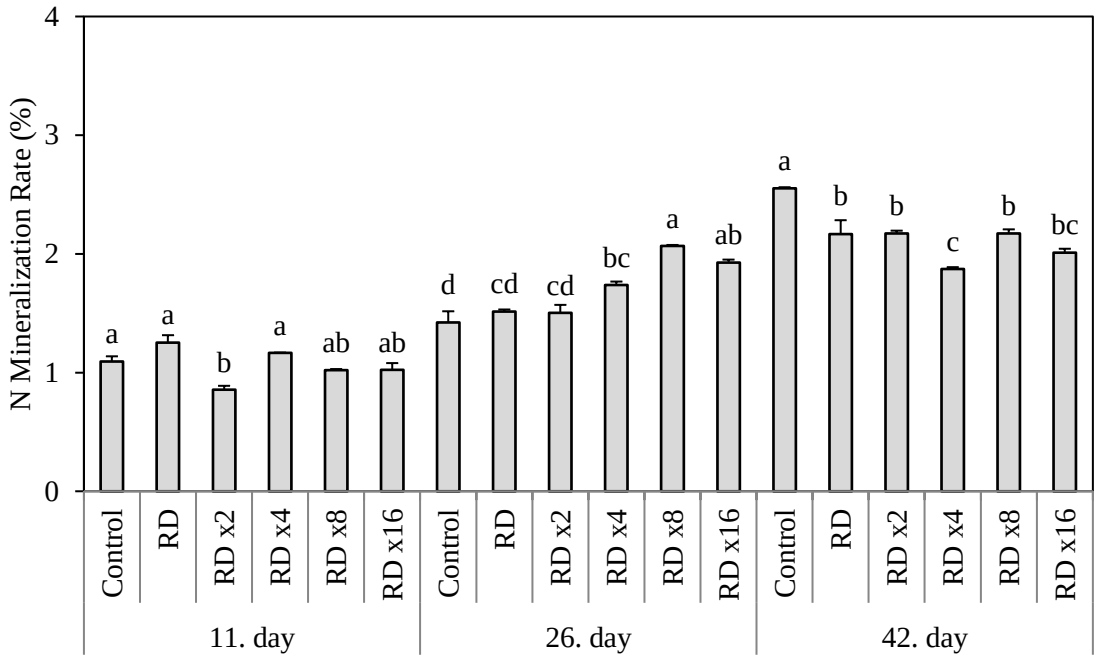

Fig 3 Soil nitrogen mineralization rates (%, means ± standard error, a, b and c indicate significant differences between means for each day separately, n=3)

Discussions

It was known that microbial activity stimulate the decomposition of soil organic matter and influence the soil nutrient dynamics. Therefore, this process in the solid phase of soil has been called nutrient mineralization [22]. Microorganisms in soil have a key role in ecosystem functions like suppression of pathogens, production of phytohormones, decomposition of pesticides including herbicides, bioremediation, application of wastewater, stimulation of plant growth [23, 24]. In general, adaptations of microorganisms in soil to exposition of agricultural herbicides occur in 3 ways: 1) increase in the metabolization of these pesticides by microorganisms [25, 26], 2) increase in negative effects on soil biota, 3) no effect on vital processes in soil biota [27-29].

Recommended field dose of herbicide containing indaziflam was similar with control in soil carbon mineralization and differences between control and these doses were non-significant. These results indicated that these doses of herbicide had no positive or negative effect on soil microbes. In contrast, higher doses of indaziflam (RD x4, RD x8 and RD x16) clearly and significantly increased soil carbon mineralization. It was

possible to conclude that soil microorganisms could use these herbicide doses for energy source and their activity.

Soil microorganisms can decompose natural and synthetic organic compounds while their decomposition products may increase or decrease microbial activity [30, 31]. As a result, when any environmental change like the application of herbicides for weed control occurred, this can affect soil carbon cycling by altering metabolic activity and community structure [22]. It was reported that soil microorganisms that were not affected by different soil moistures could use imazamox (a herbicide) as both carbon and nitrogen source and recommended field dose and its 2 fold dose of this herbicide were found similar with control in soil carbon mineralization [32]. In another study, it was noted that recommended field dose, its 2 and 4 folds of another herbicide named glyphosate-amine salt had no negative or positive effect on soil carbon mineralization compared to its non-herbicide exposed soil [33].

Diversity of microorganisms is in a wide range in soil ecosystem and therefore they were classified as photolithotrophic, photoorganotrophic, chemolithotrophic and chemoorganotrophic based on nutritional status. Phototrophic microorganisms (photolithotrophic and photoorganotrophic) were found rare in soil but chemotrophic microorganisms can be found in a wide range and numbers. All fungi species are chemoorganotrophic and can use organic molecules for carbon and energy source while bacterial and actinomycetes are originated from different groups. Nitrification (conversion of NH_4 to NO_3) and biological nitrogen fixation were maintained by chemolithotrophic organisms and these organisms can use CO_2 as a carbon source and obtain energy from the oxidation of inorganic compounds [34].

Most microorganisms play an active role in nitrification are chemolithotrophic and they can show the metabolic decomposition of herbicides [35]. It was indicated that when glyphosate and glufosinate (herbicides) applied in two different soils (haplustox and quartsmanet), NO_3-N contents were decreased in quartpsament soil but had no change in haplustox soil [36]. The reason of this decrease was explained by the higher amounts of clay and organic matter in quartpsament soil than haplustox soil [36]. Same authors reported that the avaliability of NH_4-N to NO_3-N microorganisms were lower in haplustox soil than quatzpsament and this decrease was caused by higher ion absorption in soil colloidal fraction [36]. In addition, it was found that glyphosate had a great

interest in iron and aluminium oxides that are common in oxisol soils [37]. Therefore, it was reported that this strong connection between glyphosate and these compounds decreased the bioavailability of glyphosate for microorganisms [35]. A decrease in nitrification may indicate a damaging factor for soil microbiota while an increase in conversion of ammonium to nitrate may cause nitrate contamination in soil and underground waters. It is important to indicate that these events can decrease the amount and availability of this nutrient [35].

Effects of recommended field dose of indaziflam (RD) on soil ammonium and nitrate contents with nitrogen mineralization rates were generally similar with control for 42 days of laboratory incubation in this study. It was possible to conclude that RD dose had no negative or positive effect on soil microorganisms that play an active role in ammonification and nitrification. In contrast, higher doses of indaziflam generally decreased ammonium contents but increased nitrate contents in this study. Mineral nitrogen contents were generally increased in all treatments as time progressed (11^{th} day$<26^{th}$ day$<42^{nd}$ day). In general, indaziflam had no negative or positive effects on the soil nitrogen mineralization rate on 11th day. In contrast, higher doses of indaziflam (RD x4, RD x8 and RD x16) on 26th day significantly increased but all treatments on 42^{nd} day decreased nitrogen mineralization rate ($P<0.05$).

It was reported that applications of recommended field dose and its 2 and 4 folds of imazamox (a herbicide) showed similarities with control in ammonium contents while increasing doses of imazamox decreased soil nitrate production [32]. Authors in the same research indicated that nitrate producing bacteria in soils were sensitive to addition of imazamox into soil [32].

Temporal results in nitrogen mineralization can be differed based on soil type. It was noted that mixation of a soil sampled from Adana (Turkey) with potassium bichromate at soil Cr levels increased in NH_4-N and NO_3-N contents after 42 days of incubation as following: 11^{th} day$< 26^{th}$ day $< 42^{nd}$ day [38]. It was suggested that chromium affected bacteria that take role in nitrogen mineralization [38]. In contrast, it was indicated that NH_4-N contents and nitrogen mineralization of gypsum, marl and serpentine soils were higher on 11^{th} day than 26^{th} and 42^{nd} days becuase of the availability of biodecomposable organic matter [39].

Conclusions

Recommended field dose of herbicide containing indaziflam had generally no negative effect on soil carbon and nitrogen mineralizations in this study. Higher doses of this herbicide (RD x4, RD x8 and RD x16) stimulated carbon mineralization after 42 days of incubation. In general, all herbicide doses had generally no negative or positive effect on soil nitrogen mineralization rate on 11^{th} day but it was positive on 26^{th} day. In contrast, all doses of herbicide had negative effect on soil ammonium and nitrate producing microorganims on 42^{nd} day.

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