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ORIGINAL PAPER

EFFECTS OF NITROGEN FERTILIZERS WITH TWO DIFFERENT INHIBITORS (UREASE AND NITRIFICATION) ON THE SURVIVAL AND ACTIVITY OF EARTHWORMS (*OCTODRILUS COMPLANATUS*)

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Abstract

Earthworms are among the most important species of the soil macrofauna. However, they are very sensitive to inorganic fertilization, more specifically to urea, which makes them a well-known bio-indicator. The objective of this study was to test if a fertilizer with inhibitors could be less destructive to earthworms than urea. In two consecutive years, we studied how new type fertilizers with urea (urea + urease inhibitor, urea + nitrification inhibitor and urea + urease inhibitor + nitrification inhibitor) can differ from urea applied alone and be closer to control (no fertilizer) in terms of the impact on earthworms, verified on different days after application. The measurements concerned earthworms' mortality, casts and weight. Moreover, some soil properties which are affected by earthworms' activity, such as exchangeable Ca, cation exchange capacity (CEC) and soil CO_2 respiration, were studied. The inhibitors that were used comprised nitrification inhibitor, dicyandiamide (DCD), urease inhibitor, N-(n-butyl) thiophosphoric triamide (NBPT), and double inhibitors, both urease and nitrification inhibitor. The study revealed how a fertilizer with inhibitors could be less destructive to earthworms than urea. Urea with urease inhibitor and urea with urease inhibitor and nitrification inhibitor had the least negative impact on earthworms' activities. In contrast, urea and urea with nitrification inhibitor caused higher mortality among earthworms than the other fertilizers used.

Keywords: mortality of earthworms, urea, N inhibitors, CO_2 respiration, CEC.

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INTRODUCTION

Earthworms are among the most important organisms in the soil fauna. Aristotle was one of the first people who emphasized the role of earthworms in soil's turning over and called them "the intestines of the Earth" (Edwards 2004).

Octodrilus complanatus (Oligochaeta, *Lumbricidae*) is a large species of earthworms (Monroy et al. 2007) widely distributed around the Mediterranean basin (Pavlíček, Csuzdi 2006). Bilalis et al. (2013) pointed to the use of *Octodrilus* as a bio-indicator. Earthworms play an important role in determining soil composition and condition by mixing and aerating the soil, as well as improving water permeability (Lee 1983). They influence soil characteristics (pH, organic matter, nitrogen, granulometry, etc.) because they construct and destruct soil particles, and they participate in organic matter transfer (Lemtiri et al. 2014). Earthworms can accelerate nitrogen mineralization from organic matter, although this process is affected by the species involved as well as their interaction with soil characteristics, organic matter location and soil biota (Butenschoen et al. 2009). It is reported that earthworm casts have higher content of exchangeable potassium (K), calcium (Ca), and magnesium (Mg) than bulk soil does (Edwards, Bohlen 1996). An increased Ca-content in casts is probably due to the presence of an active calciferous gland in the esophagus that actively secretes mucus rich in calcium carbonates (Drake et al. 2007). This leads to the elimination of excess Ca ions through casting activity, and greatly increases Ca availability in soil.

Earthworms influence soil fertility, but fertilization influences their population (Bilalis et al. 2009). Moreover, these invertebrates break down organic waste substrates, stimulate microbial activity substantially, and increase rates of mineralization, turning waste into humus, including substances commonly called 'vermicomposts' with less structure than fertilizers but with greater and differentiated microbial activity (Atiyeh et al. 2000). Many inorganic fertilizers contribute indirectly to the buildup of earthworm populations because they increase crop yields and therefore amounts of crop residues added to the soil. Nitrogen is considered as a critical factor that affects earthworm populations in different ecosystems. However, these invertebrates are sensitive to ammonia, so fertilizers based on ammonia often have significant effects on earthworm populations, especially when these fertilizers are applied annually over several seasons (Edwards, Lofty 1982). Several studies demonstrated that the application of chemical fertilizers, in different forms such as pulverized or powdered, could have negative effects on earthworm populations. Catastrophic effects on earthworms can be caused by nitrogen fertilizers that create acidic conditions in soil (Reinecke, Reineke 2004). It was found that the type of fertilizer treatment had a significant effect on the abundance and biomass of grass communities in maize agro-ecosystems. The long-term use of inorganic nitrogen fertilizers, especially ammonia-based ones, may decrease the abundance and biomass of earthworms (Ma et al. 1990).

By examining the effect of N fertilizer on soil microbial respiration, several studies have demonstrated that N applications can reduce respiratory rates (Bowden et al. 2004, Craine et al. 2007, Ramirez et al. 2012). According to others, application of all inorganic N forms was followed by some reduction in microbial respiration, and the magnitude of the observed response (up to 60% reduction) in all soils was negatively correlated with the N concentration (Ramirez et al. 2012). Urea also reduced respiratory rates in nearly all cases, but the effect was attenuated by the associated input of labile organic carbon.

In recent years, new types of fertilizers containing inhibitors that can replace pure urea have been developed. The most widely used inhibitors are the urease inhibitor, thiophosphorictriamide (NBPT) and the nitrification inhibitor, dicyandiamide (DCD). They reduce formation NH_4^+ and NO_3^-. Urease inhibitors delay urea hydrolysis in soil. This way, the toxic effect of a high ammonia concentration on seed germination is reduced. No literature is available about the effect of fertilizers with inhibitors on the abundance and activity in earthworms.

The aim of this study was to determine effects of different inhibitors, urea with urease inhibitor (UI), urea with nitrification inhibitor (NI) and urea with double inhibitors (urease and nitrification inhibitors) compared to urea and control (no fertilizer use), on the mortality, abundance and activity of *Octodrilus complanatus,* and on selected soil properties affected by earthworms.

MATERIALS AND METHODS

Experimental design

Two experiments were conducted at the Agricultural University of Athens, AUA, (39°59′N, 23°42′E, 29 m a.s.l.) in 2019 and 2020. A randomized complete block design (RCBD) with factorial arrangement (taking urea combinations and application methods as factors with equal importance) was followed, with 3 replications and 5 fertilizer treatments. In particular, the following treatments were examined: 1) control (without fertilizer), 2) urea (46-0-0), 3) urea + nitrification inhibitor (NI) + urease inhibitor (UI), 4) urea + nitrification inhibitor (NI), 5) urea + urease inhibitor (UI). The nitrification inhibitor was dicyandiamide (DCD) and urease inhibitor was N-(n-butyl) thiophosphoric triamide (NBPT).

Collection of earthworm and establishment of the experiment

For the first experiment (experiment A), earthworms (*Octodrilus complanatus*) were collected from an organic experimental field of the AUA in November 2019. They were collected manually from soil samples (50 cm x 50 cm; 10-20 cm depth) and transferred in the soil samples to the laboratory. Then, the earthworms were washed to remove the soil from their bodies. Only healthy adult earthworms were used for the experiment, with a well-developed clitellum, same as in Xiao et al. (2006), and of similar weight, as suggested by Bilalis et al. (2013), with the average weight of 2.000±0.900 g. In the second experiment (experiment B), earthworms were collected in January 2020, the same way as in experiment A.

Earthworms were cultured in a substrate of peat soil with high organic matter content and the following characteristics pH-value H_2O=5-6.5, N=16.6 mg $100L^{-1}$.

Earthworms were placed in plastic containers, size 20 x 14 x 10 cm (Travlos et al. 2017). Every test container was filled with 500 g peat, 3 earthworms, 20 g of crushed leaves of mulberry (*Morus alba* L.) on the surface, 1 g oatmeal and 150 ml water. In addition, a 5 g dose of different fertilizer was added to each container, so 500 g of soil contained 2.3 g N. Finally, the containers were covered with a net screen to prevent the earthworms from escaping. The containers were kept at 20°C with 12 h of light per day. The weight of the containers was monitored weekly to maintain the right moisture content, and additional food was added when required. The experiments received exactly the same treatments. Each experiment lasted 24 days. Experiment A was set up on 27 November 2019 and ended on 21 December 2019, and experiment B was carried out from 15 January to 8 February 2020. After this period, surviving earthworms were left to emerge to the surface, where they were collected.

Measurements

On four different days after application (DAA), earthworms were washed with distilled water and weighed. In addition, on ten DAAs, mortality was determined by counting how many of the earthworms were dead. The earthworm casts were counted with the line interest method (Newman 1996) on three different days after application (DAA).

The soil samples were air-dried for almost 48 h, and then sieved through 2 mm mesh. Afterwards, the soil samples were analyzed to determine different soil properties: exchangeable Ca, cation exchange capacity (CEC) and soil respiration CO_2. The method used to calculate exchangeable Ca was the Drouineau-Galet one, with the use of a spectrometer. Calcium cations were exported with ammonium acetate solution. Cation exchange capacity was determined according to the ammonium acetate method (Chapman 1965). Basal soil respiration (CO_2-C) was determined on five different days after application (DAA) using the titration method (Isermeyer 1952).

Statistical analysis

Analysis of variance was carried out on data using the Statistica esign. The significance of differences between treatments was estimated using the LSD test and probabilities equal to or less than 0.05 considered significant.

RESULTS AND DISCUSSION

The earthworms' weight was reduced by between 8.13% and 100% in experiment A and between 10.06% and 100% in experiment B (Table 1). In both experiments, the highest reduction was 100% in the urea with nitrification inhibitor and in the urea treatments, while the lowest weight loss

Table 1

The earthworm dry weight reduction (%), casts of earthworms, exchangeable Ca (mg kg^{-1}) and cation exchange capacity (meq.100 g^{-1}) as effected by fertilizer treatments (urea with nitrification inhibitor, urea with urease inhibitor, urea with double inhibitors, urea and control)

Specification	Experiment A					
	earthworm dry weight reduction (%)	**2 DAA**	**casts 7 DAA**	**22 DAA**	**exchangeable Ca (mg kg^{-1})**	**cation exchange capacity (meq.100 g^{-1})**
Urea + NI	100.0^{a}	0.340^{ns}	0.330^{ns}	0^{d}	1321.0^{e}	27.490^{e}
Urea + UI	41.99^{b}	0.330^{ns}	0.250^{ns}	0.220^{c}	1211.0^{b}	24.770^{b}
Urea + NI +UI	46.82^{c}	0.370^{ns}	0.350^{ns}	0.350^{a}	1285.0^{c}	26.550^{c}
Urea	100.0^{a}	0.370^{ns}	0.370^{ns}	0^{a}	1012.0^{d}	22.320^{d}
Control	8.130^{d}	0.340^{ns}	0.300^{ns}	0.300^{b}	1472.0^{a}	28.360^{a}
Experiment B						
Urea + NI	100.0^{a}	0.440^{ns}	0.580^{ns}	0^{d}	1340^{e}	27.450^{e}
Urea + UI	44.80^{b}	0.520^{ns}	0.600^{ns}	0.450^{b}	1219^{b}	24.640^{b}
Urea + NI +UI	22.83^{c}	0.470^{ns}	0.480^{ns}	0.590^{a}	1268^{c}	25.960^{c}
Urea	100.0^{a}	0.520^{ns}	0.550^{ns}	0^{d}	1029^{d}	22.340^{d}
Control	10.06^{d}	0.570^{ns}	0.480^{ns}	0.420^{c}	1458^{a}	28.340^{a}
$F_{fertilization}$	50.96***	0.569^{ns}	0.880^{ns}	83.59***	679.138***	113.2***
F_{exper}	0.034^{ns}	1.142^{ns}	15.62***	8.507**	0.587^{ns}	0.566^{ns}
$F_{fertilization\ x\ exper}$	1.433^{ns}	0.650^{ns}	1.492^{ns}	2.116^{ns}	1.912^{ns}	0.313^{ns}

ns – not statistically significant; * statistically significant at a significance level of p<0.05, ** statistically significant at a significance level of p<0.01, *** statistically significant at a significance level of p<0.001, DAA – days after application, NI – nitrification inhibitor, UI – urease inhibitor was in control. In experiments A and B, the urea + NI and the urea treatments were statistically different from the other treatments. Concerning the casts, on 2*nd* and 7th day after application (DAA), there were no statistically significant differences between the treatments in both experiments. On 22*nd* DAA, the values ranged from 0 to 0.35 in experiment A and from 0 to 0.59 in experiment B. The highest value was in the treatment composed of urea with double inhibitors, 0.35 in experiment A and 0.59 in experiment B, while the lowest was in the treatments with urea and urea + NI. Urea + NI + UI was statistically significantly different from the other treatments in both experiments. As for exchangeable Ca, the values ranged from 1012 to 1472 mg kg^{-1} in experiment A and from 1029 to 1458 mg kg^{-1} in experiment B. As shown in Table 1, exchangeable Ca revealed statistically significant differences between all treatments. The control reached the highest value in both experiments, 1472 mg kg^{-1} in experiment A and 1458 mg kg^{-1} in experiment B. The cation exchange capacity (CEC) values ranged from 22.32 to 27.49 meq.100 g^{-1} in experiment A and from 22.34 to 27.45 meq.100 g^{-1} in experiment B. The highest value was 27.49 meq.100 g^{-1} in urea with nitrification inhibitor and the lowest was 22.32 meq.100 g^{-1} in urea. In experiment A (Table 1), all treatments were statistically significantly different from one another.

Table 2 shows percentages of mortality among earthworms on different dates after application. The control had no mortality cases from the beginning until the end of the experiment. On 2*nd* and 5th DAA, none of the treatments was statistically significant in either of the experiments. On 7th DAA, the values ranged from 0% to 33% in the two experiments. The urea with double inhibitors and the U + UI treatments had the lowest value, i.e. zero mortality, in both experiments (A and B). The urea treatment was statistically significantly different from the other fertilizers on 9th DAA in experiment B. urea + NI had the highest mortality, 33% in experiment A. On 12th DAA, urea + NI continued to reach the highest mortality. The urea and the urea + NI + UI treatments had zero mortality in both experiments. On 14th DAA, mortality in the urea with urease inhibitor treatment occurred in experiment B. The urea and the Urea + NI treatments were statistically significant different from the other treatments. Mortality in the treatment composed of urea with double inhibitors occurred in experiment A on 16th DAA. In experiment B, urea + NI + UI was statistically significantly different from the other fertilizers. From 14th to 19th DAA, the mortality percentage ranged from 0% to 66%. In experiment A, urea + NI + UI was statistically significantly different from the other treatments. On 21st DAA, the value ranged from 0% to 100% in both experiments, the highest value of 100% was in the urea + NI and in the urea treatments in experiments A and B. On 24th DAA mortality reached 100% in all the treatments with fertilizers in both experiments and there were no statistically significant differences between them (Table 2). Mortality in Urea + NI was first observed on 5th DAA, in urea on 7th DAA, in urea + UI on 14th DAA and finally in urea + NI + UI on 16th DAA.

Table 2

The earthworm mortality percentage (%) as effected by fertilizer treatments (urea with nitrification inhibitor, urea with urease inhibitor, urea with double inhibitors, urea and control).

Specification	2 DAA (%)	5 DAA (%)	7 DAA (%)	9 DAA (%)	12 DAA (%)	14 DAA (%)	16 DAA (%)	19 DAA (%)	21 DAA (%)	24 DAA (%)
Experiment A										
Urea + NI	0^{ns}	33^{ns}	33^{a}	66^{a}	66^{a}	66^{a}	66^{a}	66^{a}	100^{a}	100^{a}
Urea + UI	0^{ns}	0^{ns}	0^{b}	0^{b}	0^{b}	0^{b}	33^{b}	66^{a}	66^{b}	100^{a}
Urea + NI +UI	0^{ns}	0^{ns}	0^{b}	0^{b}	0^{b}	0^{b}	33^{b}	33^{b}	33^{b}	100^{a}
Urea	0^{ns}	0^{ns}	30^{a}	33^{a}	33^{a}	66^{a}	66^{a}	66^{a}	100^{a}	100^{a}
Control	0^{ns}	0^{ns}	0^{b}	0^{b}	0^{b}	0^{b}	0^{c}	0^{c}	0^{c}	0^{b}
Experiment B										
Urea + NI	0^{ns}	0^{ns}	0^{b}	0^{b}	33^{a}	66^{a}	66^{a}	66^{a}	100^{a}	100^{a}
Urea + UI	0^{ns}	0^{ns}	0^{b}	0^{b}	0^{b}	33^{b}	33^{b}	33^{b}	33^{b}	100^{a}
Urea + NI +UI	0^{ns}	0^{ns}	0^{b}	0^{b}	0^{b}	0^{b}	0^{c}	33^{b}	33^{b}	100^{a}
Urea	0^{ns}	0^{ns}	33^{a}	33^{a}	33^{a}	66^{a}	66^{a}	66^{a}	100^{a}	100^{a}
Control	0^{ns}	0^{ns}	0^{b}	0^{b}	0^{b}	0^{b}	0^{c}	0^{c}	0^{c}	0^{b}
$F_{fertilization}$	ns	0.576^{ns}	4.263^{**}	8.088^{***}	9.293^{***}	23.36^{***}	20.16^{***}	11.242^{***}	39.021^{***}	639.0***
F_{exper}	ns	0.953^{ns}	2.257^{ns}	5.965^{*}	0.0196^{ns}	0.471^{ns}	2.941^{ns}	0.741^{ns}	1.128^{ns}	ns
$F_{fertilization\ x\ exper}$	ns	0.576^{ns}	1.383^{ns}	2.712^{ns}	0.760^{ns}	1.200^{ns}	1.040^{ns}	1.368^{ns}	0.579^{ns}	ns

ns – not statistically significant, * statistically significant at a significance level of $p<0.05$, ** statistically significant at a significance level of $p<0.01$, *** statistically significant at a significance level of $p<0.001$, DAA – days after application, NI – nitrification inhibitor, UI – urease inhibitor

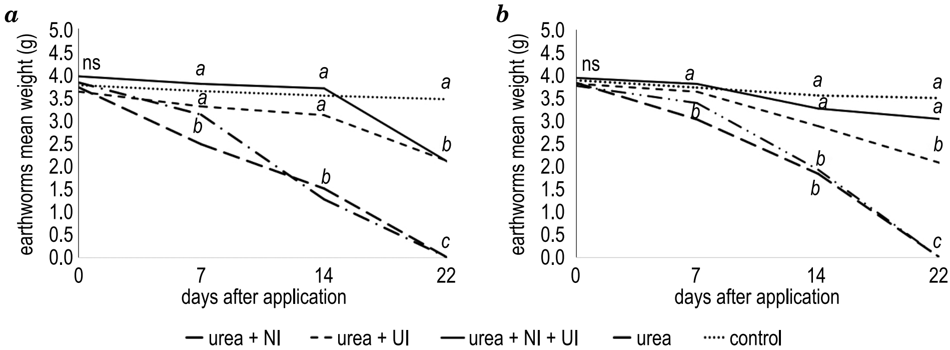

Fig. 1. Changes in mean earthworms' weight effected by treatments (urea with nitrification inhibitor, urea with urease inhibitor, urea with double inhibitors, urea and control) on different days after application (DAA), in the two experiments, A and B: ns – not statistically significant at p=0.05. NI – nitrification inhibitor, UI – urease inhibitor

Figure 1 shows changes in the mean weight of earthworms exposed to different fertilizer applications on different DAA in the two experiments.

More specifically, in experiment A (Figure 1*a*), no statistically significant differences in the weight of earthworms between the treatments appeared on day 0 after application, that is the first day of the experiment, since the earthworms selected for the study weighed nearly the same. Then, on 7th DAA, no statistically significant differences were observed between urea + NI + UI with control and urea + NI + UI with urea + UI, as well as between urea with urea + NI. On the other hand, statistically significant differences were shown between urea + NI + UI with urea and urea + NI, control with urea and urea + NI, and also urea + UI with urea and urea + NI. The same was true on 14th DAA. As for 22nd DAA, statistically significant differences appeared between control and all the other applications. There were also differences between urea + NI + UI and urea + UI with urea + NI and urea. However, no statistically significant differences appeared between urea + NI + UI and urea + UI, and on urea + NI and urea.

The only difference between experiment A and experiment B (Figure 1*b*) was on 22nd DAA, when no statistically significant difference was determined between control with urea + NI + UI, while statistically significant differences appeared between control and urea + NI + UI with urea + UI.

Furthermore, there was a relative reduction in experiment A in the weight of earthworms from the treatments with urea with urease inhibitor and urea with nitrification inhibitor and urease inhibitor, while the biggest reduction was observed in the treatments with urea and with urea plus nitrification inhibitor. In the other experiment, the weight in the control and in urea + NI + UI decreased slightly. The other applications had the same effects as experiment A.

Figure 2 illustrates the course of soil respiration (CO_2-C) on different days before and after application in the two experiments. In both experi-

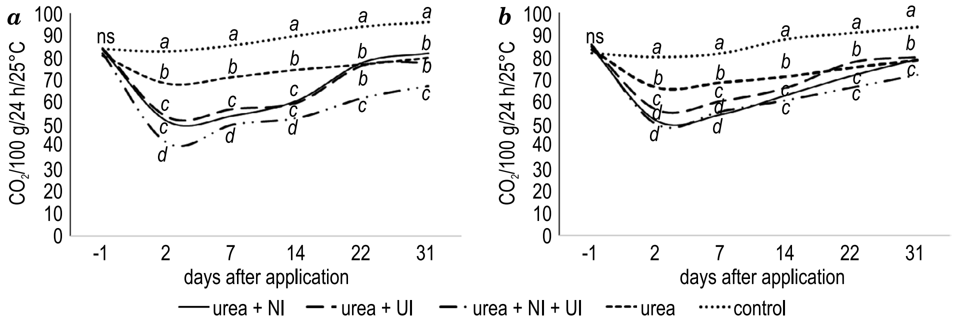

Fig. 2. Course of CO_2 evolution on different days after application (DAA) in the two experiments – A and B – effected by treatments (urea with nitrification inhibitor, urea with urease inhibitor, urea with double inhibitors, urea and control): ns – not statistically significant at p=0.05), NI – nitrification inhibitor, UI – urease inhibitor

ments before the application of fertilizers, soil CO_2 respiration was not statistically significantly different between the treatments. However, in experiment A (Figure 2*a*), statistically significant differences appeared on 2nd DAA and on 7th DAA and 14th DAA between all the applications beside urea + UI and urea + NI, where there were no significant differences between these treatments. As for 22nd DAA and 31st DAA, statistically significant differences were determined between control and the other applications, as well as urea + NI + UI and the other applications. Furthermore, there were no statistically significant differences between urea with urea + NI and with urea + UI.

In addition, in the second experiment, all the applications showed statistically significant differences between one another on 2nd, 7th and 14th DAA, beside urea + UI and urea + NI, which were not statistically significantly different. As for 22nd and 31st DAA, no statistically significant differences appeared between urea with urea + NI and with urea + UI. On the other hand, statistically significant differences were determined between control with urea + NI + UI, control with urea, urea + NI and urea + UI, and finally urea + NI + UI with urea + NI and urea + UI. In addition, there was a decrease in CO_2 respiration in both experiments, except for control applications, on 2nd DAA, which was followed by a gradual increase. Concerning the percentage of earthworm dry weight reduction (%), the largest change occurred in the urea with the nitrification inhibitor as well as in the urea treatments. The smallest change occurred in the urea with double inhibitors and in the urea with the urease inhibitor applications. This is because of the two latter fertilizers delay the release of ammonia, which is destructive to earthworms, hence the weight of these invertebrates is not reduced as dramatically as in the treatments with urea alone and urea with nitrification inhibitor. In addition, their population is not reduced. Ma et al. (1990) reported similar results, in which the earthworm biomass decreased with ammonium fertilizers. Reduction in the weight of earthworms also occurs under the influence of herbicides, as reported in other studies (TRAVLOS et al. 2017). Furthermore, there were statistically significant differences in the exchangeable Ca content between the treatments in both experiments. BILALIS et al. (2013) found the same effects in a study into the influence of aluminum on earthworms. Lower values occurred in the urea treatment, which was related to the activity of urea and the earthworm population.

The cation exchange capacity was statistically significant different in experiments A and B. The highest value was in the control and in the urea with nitrification inhibitor treatments. This was contrary to the results of BILALIS et al. (2013), where there were no statistical differences observed under the influence of aluminum.

Moreover, the casts showed significant differences on 22*nd* DAA, with urea and urea + NI treatments presenting the zero value in both experiments. The presence of casts is negatively correlated with mortality (Figure 3). The treatments that had the least mortality were urea with double inhibitors and urea with urease inhibitor. In particular, it was observed that mortality in urea + UI and urea + NI + UI occurred on 14th DAA and 19th DAA, respectively, in experiment B and on 16th DAA in both treatments in experiment A. From the onset of the experiments to the day of mortality occurred, the urea +NI + UI and urea + UI treatments behaved the same as the control, which

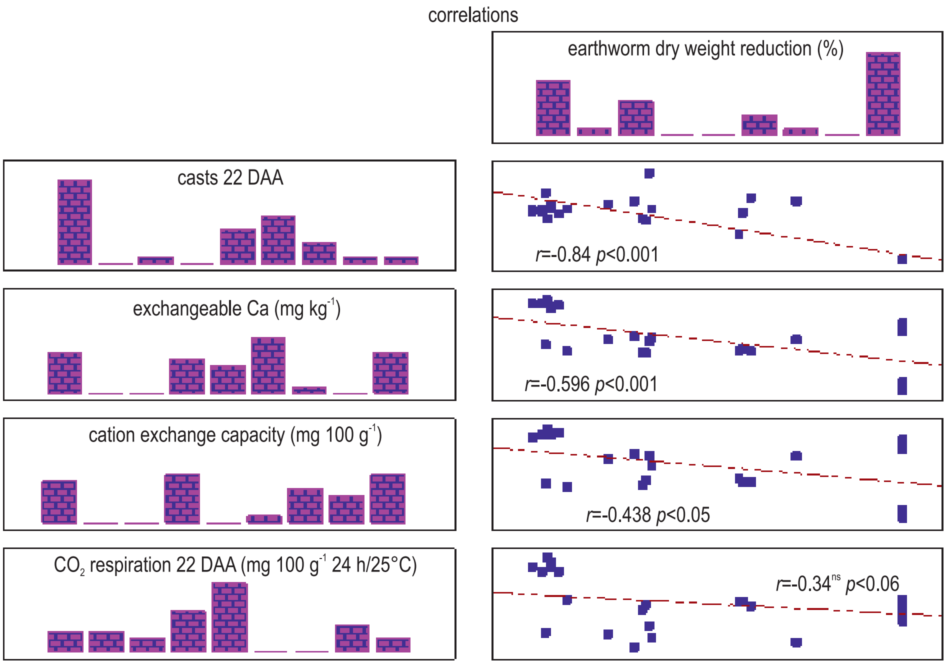

Fig. 3. Correlation matrix between earthworms' dry weight reduction and cast, exchangeable Ca, cation exchange capacity meq.100 g^{-1}, soil respiration CO_2: ns – not statistically significant, DAA – days after application

had no mortality cases. This happened because the urease inhibitor is a substance that inhibits the hydrolytic activity of the urease enzyme in urea, delaying its degradation in ammonia and carbon dioxide for approximately 12-20 days.

Intervention mortality occurred from 5th DAA in the urea with nitrification inhibitor and from 7th DAA in the urea treatments. In these treatments, there was mortality detected on the first measurement event due to ammonia, which has been reported to be deadly for earthworms. Various studies reported that there is a decrease in earthworm populations when chemical fertilization is applied (Xiang et al. 2006, Yang et al. 2007).

As for the weight of earthworms in the present experiment, there were significant differences between the treatments. Urea with urease inhibitor as well as urea with double inhibitors had the same effect as the control until the 14th day after application. This is because no urea release had taken place in these applications until 14th DAA. Then, however, the urease inhibitor gradually stopped acting so that on 22nd DAA it stopped completely, and the weight reduced rapidly as the mortality rate increased. On the other hand, urea and urea with nitrification inhibitor had almost the same effects on earthworms' weight as urea released instantly after the application. There were positive correlations, from the beginning of the experiment, between weight reduction and days after application in these two treatments. Xiao et al. (2004) reported that the weight change rate in earthworms that were exposed to urea was a sensitive indicator of ecological toxicity.

Jumadi et al. (2019) reported that microbial respiration in soil contributes to the production of CO_2 gas. Inorganic fertilizers kill soil's microbial load. This is the reason for the CO_2 reduction since the 4th DAA. In addition, hydrolysis of urea takes place 1-4 days after application of urea. During this process, CO_2 is released. The presence of urease is responsible for this process. However, through their activities such as breaking down soil matter, feeding and producing casts, earthworms increase the population of soil microbes thereby reducing the CO_2 emission (Caravaca et al. 2005, Chapuis-Lardy et al. 2010). In the treatments where the number of earthworms was higher, earthworms were more active for a longer time, there were more casts, and the microbial activity was more intensive, hence the CO_2 emission was reduced.

Exchangeable Ca and CEC were positively correlated with mass reduction given the reduced activity of earthworms and the negative effect of fertilizers on them (Figure 3).

CONCLUSION

To sum up, the research results show that the most positive effects on the survival, the lowest mortality rate, as well as the activity of the earthworm *Octodrilus complanatus*, were noted after the application of urea with the addition of the urease inhibitor as well as with the addition of the double inhibitor. Earthworms' activity depends on the characteristics of soil, such as exchangeable Ca and CEC, which in turn are affected by the intensity of earthworms' activity. In contrast, pure urea had the most negative effect on earthworms.

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