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The Effects of the Co-Application of MCPA Herbicide and Urea on Grass Rhizosphere Microcosms

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Abstract: Background: Urea fertilizer and MCPA herbicide are widely used agrochemicals in pastures. Even though urea hydrolysis impacts soil pH, potentially affecting MCPA dissipation, little is known about the effects of their co-application into the rhizosphere. Hence, we aimed to analyze the dynamics of urea transformation and MCPA dissipation when both are co-applied to the soil. Methods: A greenhouse experiment was conducted with a planted control and treatments incorporating urea and/or MCPA. Subsequently, pH changes, urea transformation into $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$, the enzymatic activity of urease and dehydrogenase, and MCPA dissipation were monitored for 30 d. Results: Urea application induced a significant ($p < 0.05$) pH change, production of $N\text{-}NH_4^+$ (from 50 and 250 mg kg^{-1}) and $N\text{-}NO_3^-$ (from 206 to 347 mg kg^{-1}), and urease (from 12 to 35 μmol $N\text{-}NH_4^+ g^{-1}$ h^{-1}) and dehydrogenase (from 0.5 to 2.5 mg TPF g^{-1} h^{-1}) activities. Urea also decelerated MCPA dissipation in the latter half of the experiment, whereas MCPA reduced urease activity when urea and herbicide were co-applied. Conclusions: Urea was the primary factor modifying the properties of the rhizosphere by stimulating the activity of microbial enzymes, shaping the pH changes during its mineralization, and decelerating MCPA dissipation. MCPA did not reduce urea mineralization but slowed urease activity, constituting an insight that requires further study.

Keywords: herbicide; urea; MCPA; rhizosphere

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1. Introduction

Intensive agronomical practices are conducted in southern Chile, where permanent pastures for feeding cattle are cultivated in volcanic soils [1,2]. This extensive farming system requires agrochemicals such as herbicides and fertilizers to maintain high productivity and remain sustainable [3]. The postemergence herbicide 4-chloro-2-methylphenoxyacetic acid (MCPA) has been widely accepted by farmers to control broadleaf weeds due to its low cost and high selectivity [4]. MCPA is a weak carboxylic acidic herbicide with a pKa value of 3.07, meaning it will mostly be in its anionic form in soils with a pH from 5.0 to 8.0 and sorbed by interactions between its carboxylic groups and ionizable groups of soil organic matter (OM) [4,5]. Since pH affects the protonation–deprotonation reaction of the ionizable groups of soil OM, it has been postulated that pH is the critical factor driving the transport, transformation, and bioaccumulation of MCPA in the environment [6]. Consequently, the sorption of MCPA and other acidic pesticides increases in soils with high OM contents but falls dramatically with increasing soil pH [7].

Pasturing in volcanic soils needs a suitable supply of nitrogen (N) fertilizers to cover the requirements of different plant species [8]. From the vast range of N-based fertilizers, urea has historically been one of the most used inorganic fertilizers on pastures due to its favorable cost–benefit properties; indeed, constant research is being performed to improve efficiency [9]. Despite this, up to 30% of applied urea can be lost after its hydrolyzation into volatilizing ammonia ($N\text{-}NH_3^+$) or leaching after being transformed into ammonium ($N\text{-}NH_4^+$) or nitrate ($N\text{-}NO_3^-$). These undesirable features require repeated urea applications to maintain fertility, which brings about negative environmental consequences [10]. These losses of N are mainly attributed to the stimulation of the hydrolytic action of plant and microbial ureases, for which the application of urease inhibitors is a common agricultural practice [11]. Urea hydrolysis consumes H^+ from water and releases vast amounts of OH^-, commonly causing a pH increase in soils soon after application [12]. However, continuous exposure and hydrolysis of urea usually leads to soil acidification when its natural buffering capacity is eventually exceeded by $N\text{-}NO_3^-$ availability [8]. These pH shifts directly and significantly disturb the soil microbial community and, thus, the entire cycling of N since microbial enzymes drive N gains and losses [13]. However, it also leads to the mobilization of soil xenobiotics such as MCPA and other acid herbicides [5] with recognized toxic effects on the metabolism and biomass of nitrifying, denitrifying, and N-fixing microbial groups [14,15]. Unfortunately, few reports have described the effects of the co-application of MCPA and urea in volcanic soils. Nonetheless, current insights from other acid herbicides have stated that their relationship depends on shifts in soil pH, which arise from the mineralization of urea, possibly by microbes [5,7].

The rhizosphere is a hot-spot zone supporting countless interactions between plants and microorganisms, including beneficial and unbeneficial ones [16]. Therefore, studies have analyzed the contribution of rhizosphere microorganisms in transforming N-fertilizers into molecules available for plant uptake and their active role in accelerating the losses of N from agricultural soils [17]. On the other hand, pesticide dissipation is often faster in the rhizosphere, as adapted microbes with suitable catabolic capacities can metabolize them [18]. Nonetheless, it has been shown that herbicides can affect the functionality of sensitive, non-targeted microorganisms involved in critical biochemical processes of soils by shifting their abundance and activity [19–21]. In this respect, the persistence of pesticides in soils has been well associated with fertilizers, with the complexity and variety of interactions between them constituting a challenging field for developing new approaches to agricultural practices [22]. In the case of urea, the evidence suggests a controversial effect on the sorption and toxicity of several pesticides, which depends on the nature of the pesticide's chemical structure and the soil's buffering capacity [23,24]. However, in the case of the co-application of urea and MCPA, little is currently known regarding the specific dynamics between both chemicals. Hence, a better understanding of the dynamics between these two agrochemicals and their contribution to the rhizosphere could contribute to developing novel strategies to improve N efficiency for plant uptake. For this purpose, the objective of the present study was to analyze the dynamics of the transformation of urea and the dissipation of MCPA when both are co-applied to the rhizosphere of grass-planted volcanic soil.

2. Materials and Methods

2.1. Chemicals

Analytical standards of MCPA (≥99% purity), triphenyl tetrazolium chloride (TTC), and triphenyl formazan (TPF) were purchased from Sigma Aldrich (St Louis, MO, USA). All other chemicals and solvents employed for preparing stock solutions were of analytical reagent grade and purchased from Merck (St Louis, MO, USA).

2.2. Soil Properties

An Andisol topsoil (0–10 cm) belonging to the Freire series was collected from the Agricultural Station Maquehue, Universidad de La Frontera (38°50′ LS and 72°42′ LW). The soil sample was air-dried at room temperature, passed through a 2 mm sieve, and characterized as described by Sadzawka et al. [25] for this kind of soil. The soil contained 15% OM, 36 mg kg^{-1} available N, 46 mg kg^{-1} available phosphorus (P), 0.49 mg kg^{-1} available potassium (K), and a pH value of 5.9. The pH was measured in a soil suspension with deionized water (1:2.5 *v*/*v* ratio) and 0.01 M $CaCl_2$. The cation exchange capacity (CEC) was calculated from the total exchangeable base (Mg, Ca, K, and Na) extracted with 1 M NH_4OAc at pH 7.0, as analyzed by flame atomic absorption spectrophotometry. Sieved soil was stored at 20 °C in the dark for 7 d before setting up the greenhouse experiment.

2.3. Greenhouse Experiment

The experiment was conducted in a greenhouse at the Department of Chemical Science and Natural Resources, Universidad de La Frontera. The soil was fertilized with commercial P-K fertilizer at a rate of 35 kg ha^{-1} in 96 flowerpots (1 kg soil each) sown with ryegrass (*Lolium perenne*, Banquet II Endo 5) and adjusted to 60% of its water-holding capacity (WHC). To reach 0.8 mg MCPA per kg of soil, 80 mg MCPA was dissolved completely in 0.01 L distilled water to obtain a solution of 80 mg/L, which was then sprayed onto the soil of each flowerpot. Sown flowerpots were kept under a 16 h photoperiod at 20–28 °C at a constant WHC (60%). After 30 d, flowerpots were divided into 4 different sets ($n = 24$), each pot with 50–200 g of soil mass rhizosphere. One set served as the control and contained untreated plants (Plant), whilst the other three were treated only with urea fertilizer (Plant + Urea), only with MCPA (Plant + MCPA), or with both chemicals (Plant + MCPA + Urea). To carry this out, urea (46% N fertilizer) was applied at a nitrogen rate of 200 kg N ha^{-1}, whereas MCPA (≥99% purity) was diluted 2-fold into acetonitrile (ACN) and resuspended in water, with 10 mL applied to the corresponding flowerpots at a final concentration of 0.8 mg kg^{-1} soil dry weight (DW). After the application of urea and MCPA, the WHC of control and treated flowerpots was revised periodically and adjusted to 60% until the end of the experiment. Triplicate flowerpots from each control and treatment were removed after 0, 2, 3, 4, 5, 10, 20, and 30 d of the experiment and used for further analysis. Rhizosphere soil was obtained by vigorously shaking roots from the samples taken at a depth of 10 cm. Strongly adhered rhizosphere soil samples were used to determine pH changes, the dynamics of $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ production, and the activities of urease and dehydrogenase enzymes and for the estimation of the residual concentration of MCPA.

2.4. pH Determination in Rhizosphere Soils

The soil pH was determined in a 1:2.5 soil:deionized water suspension and 0.01 M $CaCl_2$ using a glass electrode following the same procedure described in Section 2.2 [25].

2.5. Estimation of $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$

The nitrification and ammonification rates of urea in the rhizosphere soil samples were estimated by measuring the $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ soluble content, respectively. Briefly, $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ contents were assessed by mixing 2.5 g of soil with 12.5 mL 1 M KCl, shaking for 30 min, centrifuging at 3000 rpm, and filtering. Then, $N\text{-}NH_4^+$ concentration was determined by mixing 1 mL of a water dilution of the filtered solution (1:10 *v*/*v*) with 2 mL 0.1% sodium dichloroisocyanurate and 5 mL sodium salicylate, incubating the resulting solution at room temperature for 30 min, and measuring absorbance at 620 nm [26]. On the other hand, the $N\text{-}NO_3^-$ concentration was determined by mixing 400 µL of the filtered samples with 2 mL of a solution of Griess reagent plus vanadium (III) chloride, incubating the resulting solution at 60 °C for 2 h, and measuring absorbance at 540

nm [27,28]. All results were expressed in mg N-NH_4^+ or N-NO_3^- per kg of rhizosphere soil (mg kg^{-1}).

2.6. Enzymatic Activity of Rhizosphere Soil

Urease activity was measured using the method described by Alef & Nannipieri [29]. Briefly, 12 mL of a 0.1 M phosphate buffer (pH 6.0), 3 mL of distilled water, and 1 mL of 1.067 M urea were added to 3 g of rhizosphere soil. The samples were incubated at 37 °C for 2 h, 15 mL of 2 M KCl was added, and samples were incubated for 60 min with shaking. Subsequently, samples were filtrated using Whatman filter paper, and the released N-NH_4^+ was measured using an ion-selective electrode. Three sub-replicates of each replicate were tested, as well as a control sample (without urea). All results were expressed as µmol N-NH_4^+ per g of rhizosphere soil (µmol N-$NH_4^+ g^{-1}$ h^{-1}).

Dehydrogenase activity was determined using the method described by Garcia et al. [30]. Briefly, 5 g of rhizosphere soil was treated with 0.4% *v*/*v* TTC in Tris buffer (pH 7.6) for 24 h at 30 °C. On reduction, TTC forms TPF, which was detected at 485 nm after incubation for 24 h at 37 °C. A blank sample was prepared with TPF as an extraction solution. Each sample was measured in triplicate, and all results were expressed in mg TPF per g of rhizosphere soil (mg TPF g^{-1} h^{-1}).

2.7. Residual MCPA Analysis

The residual MCPA concentration contained in the rhizosphere samples was extracted by mixing an aliquot of 5.0 g DW with 50 mL of a methanol–water (60:40 *v*/*v*) solution acidified with 0.1% H_3PO_4, then shaking for 1 h at 250 rpm, and ultrasonicating for 30 min. After centrifugation at 10,000 rpm, 5 mL of the supernatant was collected, filtered through a PTFE membrane (0.2 µm pore size), evaporated to dryness under a continuous flow of N_2, and resuspended in 1 mL ACN. The recovery rate of MCPA was >85%. The residual MCPA concentration was determined using an HPLC-DAD system (Shimadzu Prominence HPLC chromatograph LC-20AT, Kyoto, Japan) equipped with a diode array detector (SPD-M20A) and a ProntoSIL RP-C18 column (250 mm × 4.6 mm). The mobile phase was 50:50 (*v*/*v*) ACN–water adjusted to pH 2.0 with H_3PO_4. Then, 20 µL of the extracted solution was injected into the HPLC system with a flow rate of 1.0 mL min^{-1} in an oven at 30 ± 1 °C, and MCPA residues were detected at 225 nm. The limit of detection was 0.006 mg kg^{-1} soil and the limit of quantification was 0.03 mg kg^{-1} soil. A calibration curve was constructed using the concentrations 0.03, 0.05, 0.075, and 0.1 mg L^{-1} of pure MCPA (≥99% purity). Results were expressed as mg residual MCPA kg^{-1} soil.

2.8. Statistical Analysis

Three independent replicates (n = 3) were conducted for each measurement, statistical analyses were performed using one-way ANOVA, and the averages were compared with Tukey's range test ($p < 0.05$) using the IBM SPSS software version 25.

3. Results

3.1. pH of Rhizosphere Soils

In the case of the pH of the rhizosphere soil (Figure 1), significant differences ($p < 0.05$) were detected between treatments and control. Urea application induced a significant increase in the pH of the rhizosphere of Plant + Urea and Plant + MCPA + Urea treatments compared with the Plant control. In the Plant control and the Plant + MCPA treatment, the pH was similar (5.9–6.1) throughout the experiment. However, the pH of the Plant + Urea treatment increased to 7.3 after 2 d and to 6.9 in the Plant + MCPA + Urea treatment. However, the pH of the Plant + Urea treatment was significantly higher than that of the Plant + MCPA + Urea samples during the first 3 days of the experiment. The pH of both these treatments fell to 6.5 after 5 d, declining to ~5.6 at 30 d, which tended to be lower than the pH of the control and Plant + MCPA treatment (~6.0).

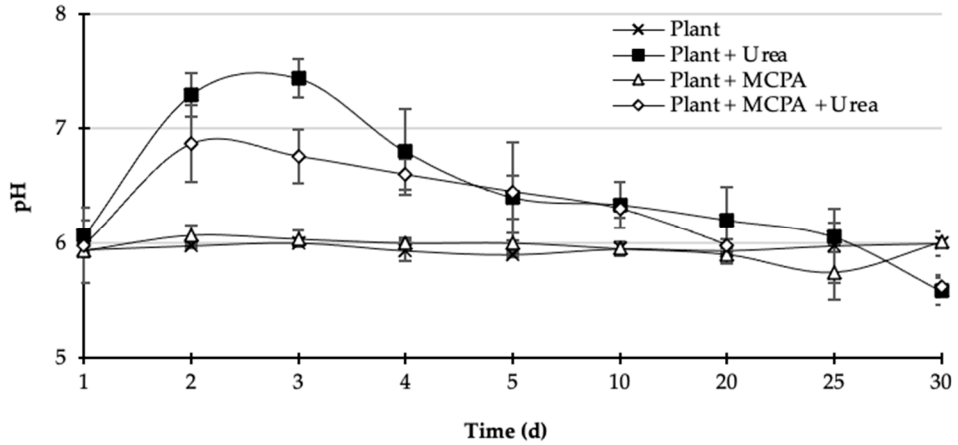
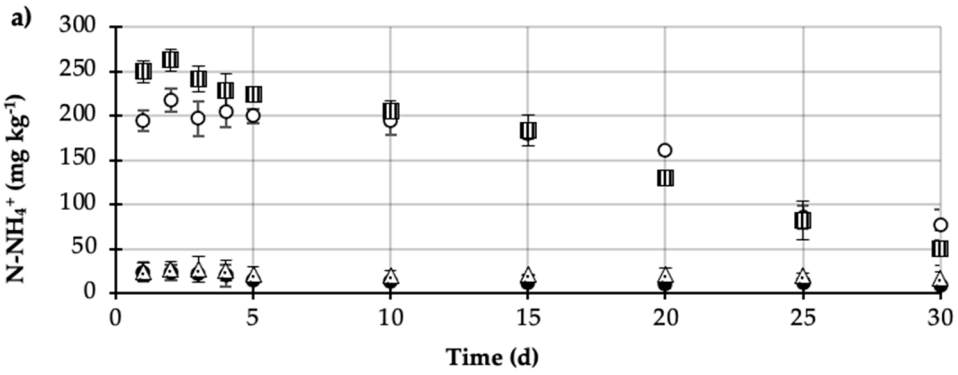


Figure 1. Determination of pH in rhizosphere soil samples over 30 d. The pH was evaluated in control (× = Plant) and three planted treatments, one incorporating only urea (■ = Plant + Urea), a second incorporating only MCPA herbicide (△ = Plant + MCPA), and a third incorporating MCPA and urea (◊ = Plant + MCPA + Urea). Values represent the mean of three replicates (n = 3) ± standard deviation.

3.2. Mineralization of Urea into N-NH_4^+ and N-NO_3^- in Rhizosphere Soil Samples

The dynamics of urea transformation into N-NH_4^+ (ammonification) and N-NO_3^- (nitrification) were monitored in the rhizosphere soil samples from control and treated samples for 30 d (Figure 2). In general terms, N-NH_4^+ production was significantly higher ($p < 0.05$) in the treatments that incorporated urea alone or in combination with MCPA than in the control and treatment without urea (Figure 2a). Specifically, urea incorporation stimulated a production rate of N-NH_4^+ close to 200–250 mg kg^{-1} compared to 10–25 mg kg^{-1} in the Plant control and Plant + MCPA treatment. Comparing the treatments incorporating urea, a higher production of N-NH_4^+ was observed in the Plant + Urea (~250 mg kg^{-1}) treatment than in the Plant + MCPA + Urea (~200 mg kg^{-1}) during the first 2 d of incubation, values which decreased progressively to ~75 mg kg^{-1} in both treatments at the end of monitoring.

Regarding the production of N-NO_3^-, this was also significantly higher ($p < 0.05$) in the treatments that incorporated urea alone or combined with MCPA than in the treatment that only incorporated MCPA and the control plant (Figure 2b). Specifically, the treatments that received urea had a higher rate of N-NO_3^- production (bordering 347 mg kg^{-1}) compared to the control (115–181 mg kg^{-1}). Additionally, differences between Plant + Urea and Plant + MCPA + Urea treatments were noticeable from day 1 until day 10, with N-NO_3^- production higher in the Plant + Urea treatment (~350 mg kg^{-1}) than in Plant + MCPA + Urea samples (~260 mg kg^{-1}). After this period, differences between all treatments and the control were negligible, and a final concentration of N-NO_3^- close to 200 mg kg^{-1} was reached.



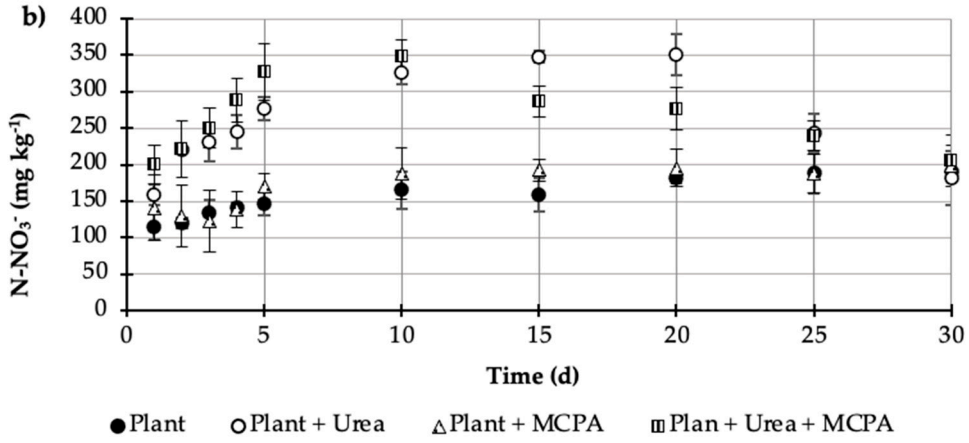
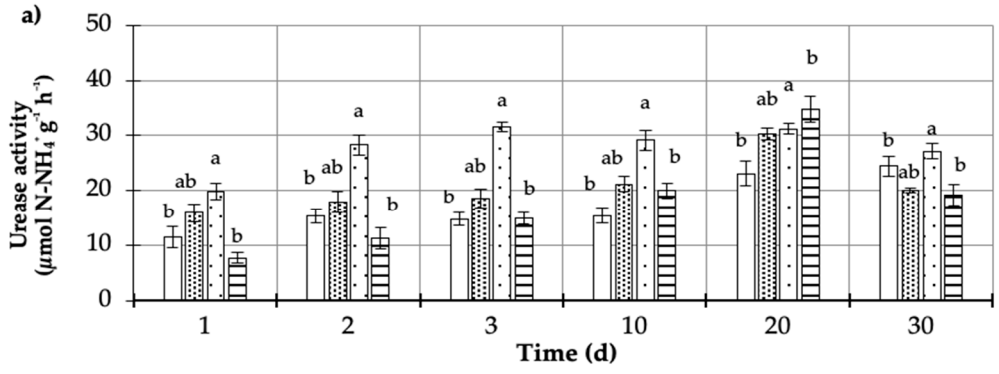


Figure 2. Production of (**a**) N-NH_4^+ and (**b**) N-NO_3^- in rhizosphere soil samples over 30 d. Contents were determined in control (• = Plant) and three planted treatments, one incorporating only urea (o = Plant + Urea), a second incorporating only MCPA herbicide (△ = Plant + MCPA), and a third incorporating MCPA and urea (▥ = Plant + MCPA + Urea). Values represent the mean of three replicates (n = 3) ± standard deviation.

3.3. Enzymatic Activities of Rhizosphere Soil Samples

Important enzymatic activities involved in N-recycling (urease and dehydrogenase) were tested in rhizosphere soil samples (Figure 3). In general, urease activity (Figure 3a) was significantly lower ($p < 0.05$) for all control and treatments at the beginning of the experiment (~12 to ~20 μmol N-$NH_4^+ g^{-1} h^{-1}$) than at the final stages of the incubation (20 d to 30 d; ~20 to ~35 μmol N-$NH_4^+ g^{-1} h^{-1}$). On the first day of treatment, no significant differences in urease activity were observed between the control and treatments (from ~7 to ~20 μmol N-$NH_4^+ g^{-1} h^{-1}$). Subsequently, the activity in the Plant + MCPA samples was significantly higher (~30 μmol N-$NH_4^+ g^{-1} h^{-1}$) than in other treatments and the control (from ~15 to ~20 μmol N-$NH_4^+ g^{-1} h^{-1}$). However, after 20 d of incubation, the three treatments presented similar urease activities (from ~30 to ~35 μmol N-$NH_4^+ g^{-1} h^{-1}$), all of which were significantly higher than the control (~22 μmol N-$NH_4^+ g^{-1} h^{-1}$). Finally, this activity decreased similarly after day 20 in all three treatments.

Regarding dehydrogenase activity (Figure 3b), values were similar for all control and treatments during the first 20 d of the experiment (from ~0.5 to ~1.5 mg TPF $g^{-1} h^{-1}$), although a significant ($p < 0.05$) increase after 30 d was observed for all soil samples (from ~1.8 to ~2.5 mg TPF $g^{-1} h^{-1}$). Interestingly, the values for the Plant + Urea treatment showed higher values from ~0.7 to ~2.5 mg TPF $g^{-1} h^{-1}$).



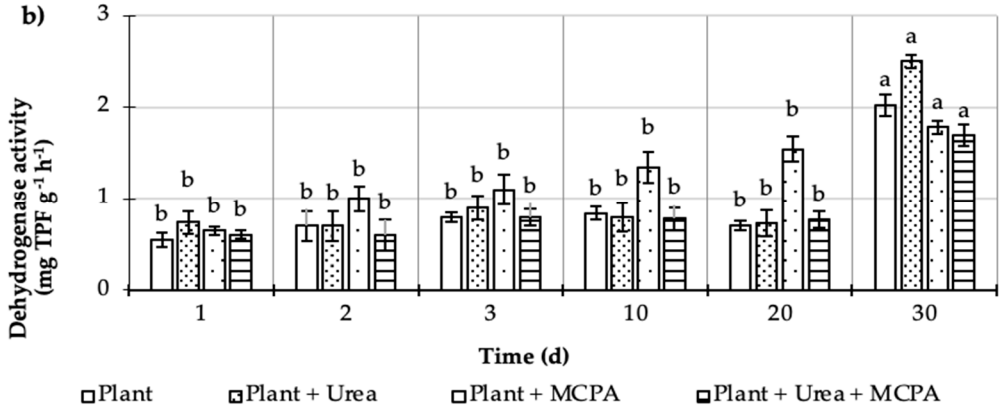


Figure 3. Activities of urease (**a**). and (**b**). dehydrogenase enzymes in rhizosphere soils over 30 d. Activities were determined in control (Plant) and three planted treatments, one incorporating only urea (Plant + Urea), a second incorporating only MCPA herbicide (Plant + MCPA), and a third incorporating MCPA and urea (Plant + MCPA + Urea). Values represent the mean of three replicates (n = 3) ± standard deviation. Different letters indicate a significant difference among treatments.

3.4. Dissipation of MCPA in Rhizosphere Soil Samples

The dissipation of MCPA was best described by the following first-order model $C = C_0 e^{-kt}$, where "C" was the soil concentration of MCPA at time T (d), C_0 0.8 mg Kg^{-1} was the initial concentration for both treatments at "time 0", and, as a first-order dissipation rate constant, the k value was 0.134 for the Plant + MCPA samples and 0.094 for the Plant + MCPA + Urea treatment. In general terms, the half-life ($t_{1/2}$) of MCPA was not significantly different between the Plant + MCPA + Urea ($t_{1/2}$ = 7.35 d) and Plant + MCPA ($t_{1/2}$ = 5.16 d) samples (Figure 4). However, the dissipating behavior was substantially different between both treatments as the experiment progressed. Specifically, the MCPA dissipation rate was significantly higher during the first 5 d in Plant + MCPA + Urea samples (~57%) compared to the Plant + MCPA treatment (~35%), a difference that was maintained until day 10. However, these differences inverted after 10 d, where dissipation rates for the Plant + MCPA (~93%) treatment surpassed those of the Plant + MCPA + Urea samples (~76%). At the end of the experiment, the Plant + MCPA treatment possessed ~100% MCPA dissipation, whereas that of the Plant + MCPA + Urea samples was only ~85%.

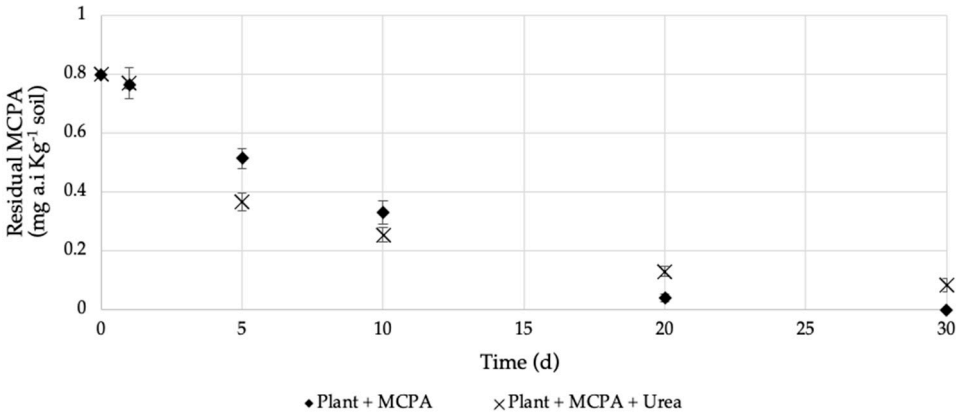


Figure 4. Determination of residual MCPA in rhizosphere soil treated with MCPA and urea (× = Plant + MCPA + Urea) or without urea (♦ = Plant + MCPA) over 30 d. Values represent the mean of three replicates (n = 3) ± standard deviation.

4. Discussion

The purpose of the present study was to evaluate the effect of the joint application of MCPA herbicide and urea fertilizer on a rhizosphere microcosm by simulating the doses and application methods commonly adopted in grasslands in southern Chile in order to improve the current strategies for weed control and plant nutrition. In this context, it is well known that the long-term application of herbicides and fertilizers significantly changes the physicochemical and biotic properties of soils, including a reduction in their OM content [31], acidification [22], and perturbance of the microbial communities involved in nutrient cycles [20,32].

According to our results, the application of MCPA and urea had different effects on rhizosphere soil properties in terms of pH, urea mineralization, and the microbial activity of N-cycling enzymes. Indeed, the pH in the treatments incorporating urea increased significantly from ~5.9 to 7.3 compared to the control and those treated with MCPA alone, which kept closer to basic pH values. In the urea mineralization process, it is known that this molecule is initially transformed into $N\text{-}NH_4^+$ and then oxidized into $N\text{-}NO_3^-$. Subsequently, since urea hydrolysis produces $N\text{-}NH_4^+$ and a considerable amount of OH^- [12], the pH increase in the early days of the experiment could be in line with this first step of urea mineralization. This suggestion was confirmed, as $N\text{-}NH_4^+$ production was indeed significantly higher during these treatments than in the remaining soils without exposure to urea. During the later stages of the experiment, the pH fell dramatically in soils treated with urea, becoming even more acidic than in the control and in soil samples without fertilizer. In this context, the acidification of these soil samples could be related to the oxidation of excessive $N\text{-}NH_4^+$ into $N\text{-}NO_3^-$, a process mainly catalyzed by ammonia-oxidizing microbes that produce twice as much H^+ for each mole of $N\text{-}NH_4^+$ [33]. In our study, this may be responsible for the higher production of $N\text{-}NO_3^-$ in the urea-treated soils, a finding supported by previous studies describing enhanced N-mineralization into easily leachable molecules such as $N\text{-}NO_3^-$ after urea incorporation into Andisol soils [34].

Interestingly, differences in the pH and conversion rates of $N\text{-}NH_4^+$ to $N\text{-}NO_3^-$ varied in the samples in the presence or absence of the MCPA herbicide, a factor indicative of a gradual increase in $N\text{-}NH_4^+$ and a decrease in $N\text{-}NO_3^-$. Therefore, Palma et al. [5], in comparing the effect of the acidic herbicides MCPA and flumetsulam on the hydrolysis of urea in soils, demonstrated that more acidic MCPA produced a two-fold rise in pH compared to flumetsulam as a result of higher production of soluble $N\text{-}NH_4^+$. Unfortunately, neither measurements of the individual effects of herbicides and urea application nor measurements of $N\text{-}NO_3^-$ production to determine whether it correlated with subsequent soil acidification were conducted in said study [5]. Similarly, Rose et al. [35] reported that the application of the acidic herbicide 2,4-D, from the same phenoxyacetic family, increased $N\text{-}NH_4^+$ production and decreased the concentration of $N\text{-}NO_3^-$ in an acid soil microcosm but without adding urea. However, more recently, Palma et al. [7] noticed that the application of urea and acidic 2,4-D increased the release of $N\text{-}NH_4^+$ by 12.5–23% and decreased the production of $N\text{-}NO_3^-$ by 20% in an Andisol soil. Thus, even though information about the rates of production of $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ from urea in the presence of MCPA is scarce, it is well known that several pre- and postemergence herbicides can temporarily retard the hydrolysis and nitrification of urea in soil [36] as they are potential ecotoxic agents that act upon microbial communities involved in N-cycling of soils [21].

The rhizosphere offers favorable environmental conditions for optimal microbial metabolism due to the mutualistic interaction with plant roots. However, the rhizosphere microbial community of agricultural soils is constantly prone to being exposed to herbicides, with subsequent effects on key nutrient cycling enzymes [21,37,38]. In the present study, we analyzed the activities of ureases in order to evaluate urea mineralization and dehydrogenases as indicators of soil microbial performance. Our results show that urease activity was significantly higher in the presence of urea, and dehydrogenase activity gradually increased until the end of the experiment, suggesting induction by urea. In this sense, the increase in urease could be explained by the stimulation of urease-harboring

bacterial communities responsible for the transformation of urea into $N\text{-}NH_3^+$ and its rapid transformation into $N\text{-}NH_4^+$, a typical effect observed in acid soils with an excess of H^+, such as the one employed in our study [39]. Here, we link the rise in urease activity with our samples' higher loads of $N\text{-}NH_4^+$. In parallel, the stimulation of the urea-mineralizing microbes could suggest a rise in the biomass of microbes involved in this process, responsible for the gradual increase in dehydrogenase activity [40]. Additionally, urea increased the pH close to the optimum level for urease activity (pH = 7.2) in the treatments receiving urea fertilization in our study, a physicochemical association that has been previously reported for this kind of soil [34]. However, the stimulation of urease or the negligible change in dehydrogenase activity when MCPA was applied alone with the control or in the presence of urea should be interpreted carefully. In general, the effect of pesticides on the activity of soil enzymes is controversial, with the most common effect being negative or neutral [19]. For example, Tejada et al. [41] indicated that MCPA application in non-organic amended soils reduced urease and dehydrogenase activities by 20% and 39.9%, respectively, compared to soil amended with OM, reflecting the noxious effect of MCPA, which may have been attenuated by the protective actions of OM on microbial performance. On the other hand, in a different study, as the MCPA dose rose, dehydrogenase activity fell [42], mainly by affecting the microbial biomass [43], which subsequently recovered [44], as observed in our results. Furthermore, Tejada et al. [45] demonstrated that urease and dehydrogenase were less affected by MCPA in soil containing higher contents of OM due to enhanced herbicide sorption and, therefore, reduced exposure of microbes to this ecotoxic molecule. The positive effect on urease activity after the incorporation of MCPA could result from the organic nature of the soil employed in the study (15% OM), or possibly the adaptation of rhizospheric microbes to similar compounds, as the soil sample was collected from a pasture that receives regular applications of the herbicide 2,4-D [9]. However, the lower urease activity observed in the co-application of urea and MCPA could be related to the enhanced ecotoxicity of the herbicide on more sensitive microbial populations that are involved in N-cycling, as observed previously for other pesticides [41,45,46].

Regarding the dissipation of MCPA, a similar half-life was obtained for the treatments with herbicide alone or in combination with urea. However, a notable slowing in the MCPA dissipation rate in the presence of urea was observed as the experiment progressed. This effect could be attributed to the increasing mineralization of urea and the resulting rise in pH in the treatment that combined both chemicals compared to the soils exposed only to MCPA. As discussed above, adsorption rates of MCPA depend mainly on the OM content of soils but are highly influenced by pH; as pH rises, rates fall [47]. This relationship has been attributed to the pH alteration in the percentage of dissociation for acidic herbicides and the dissolved fulvic acid and humic acid content of soil OM [8]. Consequently, as urea mineralization resulted in a progressive increment in pH in our study, an increment in the desorption of MCPA in the initial stages of the experiment was expected. Other studies have chemically modeled that increasing MCPA desorption is directly related to the release of $N\text{-}NH_4^+$ from urea, coupled with the higher pH, as these changes also stimulate the release of dissolved organic carbon [5]. In this study, the mechanism by which MCPA could be desorbed during the first steps of urea mineralization was established, but the association between acidification by $N\text{-}NO_3^-$ and MCPA adsorption into OM was not evaluated. Whilst our study did not evaluate the transformation of soil OM to associate urea mineralization and pH shifts with the sorption of MCPA residues, our findings concerning enzymatic activities, particularly urease, provide evidence on how microbial urea mineralization and soil acidification can increase MCPA adsorption and slow its dissipation in organic soils in the long term. In this context, a recent study linked the active participation of ammonia-oxidizing bacteria with significant reductions in the dissipation of 2,4-D (>28%) in the presence of urea, highlighting the contribution of soil microbes in the interaction between urea fertilizers and acidic herbicides in soils [9]. On the other hand, increasing the bioavailability of MCPA to degrading microorganisms

resulting from urea mineralization appears to be desirable. Nevertheless, considering that urease is the main enzyme involved in N losses from grasslands [40] and that the MCPA desorption rate can surpass the degrading capacity of microorganisms, leading to its leaching [38], the joint application of urea fertilizer and acidic herbicides must be evaluated. Further research centered on the real participation of microbes is necessary for estimating the magnitude of the effect of herbicides on N-cycling communities and how this affects the availability of this nutrient for plant growth in pastures.

5. Conclusions

The presence of urea was the primary factor modifying the physicochemical and biological parameters of rhizosphere soils. When present, the activity of urease-harboring microbes was significantly stimulated, resulting in high loads of $N\text{-}NH_4^+$ and an increase in pH during the initial stages of the study. After several days, urease was still active, while $N\text{-}NH_4^+$ gradually gave way to the formation of $N\text{-}NO_3^-$ accompanied by acidification. The presence of MCPA alone did not significantly intensify or reduce these shifts. However, the co-application of urea and MCPA resulted in an initial accelerated dissipation of MCPA (until day 15), followed by a slowdown as soil acidification intensified. The presence of MCPA did not reduce the mineralization of urea but slowed microbial urease activity temporarily and increased MCPA adsorption. Hence, although reduced urease activity benefited plant nutrition by avoiding undesirable losses of N from urea fertilizer, the accumulation of MCPA would be a challenge for the long-term performance of soil microbes, constituting a topic that requires further study for the development of truly sustainable agronomic practices.

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