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Effect of controlled release formulations of diuron and alachlor herbicides on the biochemical activity of agricultural soils

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Abstract

The use of pesticides in agriculture is essential because it reduces the economic losses caused by pests, improving crop yields. In spite of the growing number of studies concerning the development and application of controlled release formulations (CRFs) of pesticides in agricultural soils, there are no studies about the effects of such formulations on the biochemical properties. In this paper the dissipation of diuron and alachlor in three agricultural soils for 127 days, applied either as commercial or CRFs, was determined as well as their concomitant effects on soil biochemical properties. Dehydrogenase, urease, β-glucosidase and phosphatase activities were measured thought the experimental period. The application of alachlor as CRF increases its half-life time in soils, whereas no differences were noticed between diuron formulations due to its slower degradation, which takes longer than its release from the CRF. At the end of the incubation period, the enzymatic activities were the same after the use of diuron either as commercial or CRF, recovering the soil previous status. For alachlor formulations, no differences in enzymatic activities were again observed between both formulations, but their levels in soils were enhanced. Therefore, the use of these CRFs does not adversely affect the soil biochemical properties.

Keywords: controlled release formulations; dissipation; herbicides; soil enzymatic activities

1. Introduction

The application of pesticides is a common agricultural practice that aims at minimizing the economic losses caused by weeds, insects, and pathogens, improving crop yields [1, 2, 3]. However, only a certain fraction of the applied pesticide reaches its target, whereas the rest is inactive due to bound to soil colloids, lost by water lixiviation with the subsequent groundwater contamination or is in the soil solution, being degraded by chemical

and/or biological processes [4, 5, 6, 7, 8, 9]. In recent years various controlled release formulations (CRFs) of herbicides have been developed to provide small amounts of the herbicide to the soil along the time, improving its efficiency and decreasing the risk of groundwater pollution [3, 10, 11, 12, 13, 14, 15].

The use of clay and surfactant for preparing formulations of herbicides is a very common practice. In the field of CRFs, these formulates have focused mostly on the use of the clay mineral montmorillonite and quaternary ammonium surfactants [16, 17]. In recent years new formulations have been developed by encapsulation of the herbicides in the structures formed by surfactants in solution (micelles or vesicles) and the subsequent, adsorption of micelles and vesicles containing the herbicide on montmorillonite. With the development of these new formulations, active substance content can reach high values, and in some cases, very close to those of the commercial products [11, 16]. In a variant of these formulations, quaternary ammonium surfactants that form vesicles and micelles involution have been replaced by the natural surfactant phosphatidylcholine (PC) [14, 18, 19, 20]. The advantage of these formulations is that the adjuvants used (PC and clay) are nontoxic, indicated by the EPA as approved substances of minimal toxicological risk. These authors showed reduced leaching and enhanced herbicidal activity from these CRFs.

In spite of the growing number of studies in the last decades concerning CRFs, these studies have focused mainly on their preparation and their use for sustained release in the field. As far as we know, there are no studies about the potential toxic effects of CRFs on the soil biochemical properties. The current literature indicates that soil biological factors react faster than physical variables after any chemical change in the soil [21]. Soil enzymatic activities have been suggested as potential indicators of soil use and management because of their relationship to soil biology, and it is generally assumed that the biological properties of

soil, such as enzymatic activities, are earlier indicators of soil degradation rather than chemical or physical parameters [22, 23, 24].

Dehydrogenase activity is an oxidoreductase enzyme which has been used as a measurement of overall microbial activity [25], since it is an intracellular enzyme related to oxidative phosphorylation processes [26]. Other hydrolytic enzymes involved in the cycling of principal nutrients such as β-glucosidase, urease and phosphate linked to C, N and P, are sensitive indicators of management induced changes in soil properties and provide rapid and accurate information on changes in soil quality [25].

There is abundant information indicating how herbicides influence on the soil biochemical properties. In this regard, several studies have shown that herbicides such as oxyfluorfen, MCPA, glyphosate or diflufenican cause negative effects on the biochemical properties of soils. Therefore, the study of enzyme activities is useful for understanding the potential toxicity of a particular herbicide on soil microorganisms [2, .27, 28, 29, 30].

Diuron and alachlor are herbicides widely used in order to minimize economic losses caused by weeds, and therefore to retain the current production and yield levels and maintain high quality [31]. Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) is a substituted urea herbicide not only used to control a wide variety of broad leaf and grassy weeds in many crop cultures but also employed on non-crop areas such as roads, gardens, and railways [32]. Alachlor (2-chloro-N-(2,6-diethylphenyl)-N-(methoxy-methyl)acetamide) is a preemergence chloroacetanilide herbicide that is widely used for the control of annual grasses and broad leaf weeds in corn, soybean, cotton, grain sorghum, peanut, and other cultures [33].

The aim of this work was to study the dissipation of diuron and alachlor herbicides in three agricultural soils applied either as commercial or CRFs (prepared by encapsulation of herbicides in vesicles formed by clay-PC systems), and their effects on soil biochemical properties as determined by their effect on enzymatic activities associated with the cycles of

C, N, and P in soil, which will inform us about the impact of such forms of these herbicides on soil biology.

2. Material and methods

2.1. Soil and herbicides characteristics

Three different agricultural soils from southwestern Spain were employed in this study (two of them, CR and AL, under conventional practices, and another one, LT, under organic farming). CR corresponds to a calcaric Fluvisol [34] with olive groves as principal crop and located in the CSIC experimental farm 'La Hampa' in Coria del Río (Seville, Spain) (37°16′59″ N, 06°04′03″ W). AL, corresponds to a dystric Cambisol [34] with gramineae and cork tree as principal crops and located in a farm in Alájar (Huelva, Spain) (37º 53´ 424´´ N / 6º 39´ 748´´ W). LT, corresponds to a calcaric Fluvisol [34] with vegetable farming as principal crops and located in the Guadalquivir River Valley (SW Spain), at the Centro de Investigación y Formación Agraria Las Torres-Tomejil farm in Alcalá del Rio (Seville, Spain) (37º 8´ 33´´ N; 5º 16´ 4´´ W).

The general properties of these soils (0-25 cm) are shown in Table 1. Soil samples were crushed to pass a 2 mm sieve, and were analyzed for pH in saturated paste [35], total $CaCO_3$ was measured by estimating the quantity of the CO_2 produced by HCl addition to the soil [36], and size particle distribution, by the hydrometer method [37]. Soil organic matter was determined by the method of Yeomans and Bremner [38]. N-Kjeldahl was measured by the digestion method [36].

The herbicides used in this experiment were diuron and alachlor. For diuron, the commercial formulation Diurokey 80% [WP] P/P was purchased from Industrial Química Key, S.A (Spain).The commercial formulation Alanex 48 EC (48% p v^{-1}, 480 g l^{-1}) was purchased from MakhteshimAgan España, S.A. (Spain).Analytical diuron (>98% purity) and

alachlor (99.2% purity) were purchased from Sigma-Aldrich (Sigma Chemical Co., St Louis, MO).

2.2. Preparation of herbicide–PC–clay formulations

The clay mineral used was Wyoming Na-montmorillonite (SWy-2, cation exchange capacity 0.8 $mmolg^{-1}$) provided by the Source Clays Repository of The Clay Minerals Society(Columbia, MO). The phosphatidylcholine (PC) used was SPC-3 (74% distearoyl-PC and 26% 1-palmitoyl-2stearoyl-PC) and was supplied by Lipoid GmbH (Ludwigshafen, Germany).

The preparation of herbicide-PC-clay formulations were made following the methodology described by Undabeytia et al. [14]. PC formulations of diuron and alachlor were prepared by dissolving the analytical herbicides in a solution of 6 mM PC via sonication and subsequently adding the suspension to montmorillonite. The concentrations of herbicides added were 8 mM, whereas the clay concentration was 5 g l^{-1}. After shaking for 24 h, the suspensions were centrifuged at 20,000 g for 20 min, and the supernatant was analyzed to quantify the remaining herbicide, determining the active substance content by difference. The pellets obtained from centrifugation were freeze-dried to yield PC-clay formulations. The nomenclature for PC–clay formulations was A-CRF for alachlor and D-CRF for diuron. The active substance content of the formulations was 13.9% for D-CRF and 15.0% for A-CRF. Similarly, PC-clay complexes were prepared in the absence of herbicide (nomenclature PC-clay).

2.3. Incubation procedure and analysis of herbicides

Two hundred fifty grams of dry soil was mixed with 7.69 mg of each herbicide (corresponding to a dose of 2 kg ha^{-1}) applied as either commercial or CRFs. In the case of

diuron, this is the usual rate for cotton, sorghum or winter wheat. We used the same rate for alachlor for the sake of comparison with diuron. The mixtures were vigorously stirred for 24 hours. Untreated soil was used as a control. Distilled water was added to each soil to reach 30-40% of its water-holding capacity. The incubation treatments are detailed as follows: C, control soil, soil without herbicides; Diurokey, soil with commercial diuron; Alanex, soil with commercial alachlor; PC-Clay, soil with PC-Clay complex; D-CRF, soil treated with D8/5; and A-CRF, soil treated with A8/5. The nomenclature for PC–clay formulations used referred to the herbicide (D or A, for diuron or alachlor, respectively), the first number denotes the initial herbicide concentration (8 mM), and the second number represents the clay concentration (5 g l^{-1}). Duplicate treatments were kept in semi-closed microcosms at 25 ± 1 °C for 127 days.

The exhaustive extraction of diuron and alachlor from the soils after different periods of time was performed using methanol (2g soil/10 ml methanol). To optimize the extraction method, soil samples were treated with $HgCl_2$ (200 mg kg^{-1}) to completely eliminate the activity of soil microorganisms and ensure that only abiotic conditions prevailed. Then they were spiked in triplicate with the pesticides solutions at two fortification levels (0.15 and 30 mg kg^{-1}). Extractions were performed after several incubation times. The recovery percentages were 97.5 ± 2.3 for alachlor and 96.6 ± 2.9 for diuron. The concentration of herbicide was determined by high performance liquid chromatography (HPLC) using a Kromasil C18 reversed phase column (15 x 0.40 cm), supplied by Teknokroma (Spain) and at a temperature of 30 °C. As mobile phase acetonitrile/water were used 55/45% for alachlor and 60/40% for diuron. The flow rate was 1 ml $min^{-1}$and the injection volume of 100 µl. Detection was performed using a diode-array detector at 220 nm with alachlor, and at 230 nm for diuron. The limit of quantification for the two herbicides was 0.15 mg l^{-1}. The dissipation processes of the herbicides in soil were adjusted to a first order equation [39].

$$LnC = LnC_0 - kt$$

where, C is the concentration of herbicide in the soil at time t, C_0 is the initial concentration of herbicide in the soil, and k is the dissipation constant. The time required to achieve a herbicidal dissipation of 50% is the half-life ($t_{1/2}$), which, for a first-order kinetics, is defined as:

$$t_{1/2} = 0.6932 / k$$

2.4. Soil enzymatic activities

The activity levels of four soil enzymes for each treatment were measured at days 2, 4, 7, 16, 25, 65, 90 and 127 during the incubation period. Dehydrogenase activity was determined by the method of Trevors et al. [40] as modified by García et al. [41]. In this procedure, 0.1 g of soil was exposed to 0.2 mL of 4% INT (2-p-iodo-3-nitrophenyl 5-phenyltetrazolium chloride) in distilled water for 20 h at 22 °C in darkness. The iodonitrotetrazaolium formazan (INTF) formed was extracted with 10 mL of a 1:1.5 mixture of ethylene chloride and acetone by shaking vigorously for 2 min. INTF was measured in a spectrophotometer at 490 nm. Controls were prepared without substrate.

Urease activity was determined by the buffered method of Kandeler and Gerber [42]. In this procedure, 0.5 mL of a solution of urea (0.48%) and 4 mL of borate buffer (pH 10) were added to 1 g of soil in hermetically sealed flasks, and then incubated for 2 h at 37 °C. The ammonium content of the centrifuged extracts was determined by a modified indophenol blue reaction. Controls were prepared without substrate to determine the ammonium produced in the absence of added urea. Ammonium was determined spectrophotometrically at 690 nm.

Alkaline phosphatase activity was measured by the method of Tabatabai and Bremner [43] except that incubation was at 30 °C in maleate buffer (2 mL, pH 6.5) for 90 min and 0.5

mL of substrate (0.115 p-nitrophenyl phosphate) added to 0.5 g to soil. Controls were prepared without substrate. *p*-nitrophenol was determined spectrophotometrically at 400 nm.

β-Glucosidase activity was determined using 2 mL of 0.1 M maleate buffer (pH 6.5) and 0.5 mL of 50 mM p-nitrophenyl-β-D-glucopyranoside (PNG) to 0.5 g of soil. The rest of method was the same as for alkaline phosphatase activity [44]. Also, *p*-nitrophenol was determined spectrophotometrically at 400 nm.

2.5. Statistical analysis

Data were submitted to two-way ANOVA considering the time of incubation and the treatments used as independent variables and each soil parameter as the dependent variable. For this, the Statgraphics Plus 2.1 software package was used. The means were separated by the Tukey's test, considering a significance level of $p<0.05$ throughout the study. For the ANOVA, duplicate data were used for each treatment and each day of incubation.

With the aim of integrating the data provided by the different methods, a Principal Component Analysis (PCA) with the varimax rotation was performed, to determine if these variables changed with the different treatments. The variables included were herbicides concentration and enzymatic activities (dehydrogenase, urease, β-glucosidase and phosphatase). PCA was conducted using the same software package previously described.

3. Results and Discussion

3.1. Diuron

Figure 1 showed diuron dissipation in the three experimental soils after the application of the commercial and the CRF. The herbicide dissipation in soil was very similar with both formulations. No statistical differences were noted in the remaining amounts with soils CR and LT, and only at 7 days after treatment (DAT) in AL soil.

The dissipation of diuron in the soils showed a good fitting to a first order kinetic (Table 2). In general, the application of diuron as CRF provided no difference in half-life times obtained compared to the commercial formulation, ranging between 28 and 41 days, well below the average of 75 days recorded by the IUPAC (IUPAC Pesticides Database Properties, http://sitem.herts.ac.uk/aeru/iupac/). Diuron soil dissipation half-life was reported to be from days, months or even years [45, 46]. In agreement with our results, Cullington and Walker [47] also found in an incubation experiment of 10 soils polluted with diuron dissipation from 29 to 43 days. The similar behavior between Diurokey and the D-CRF was due to the slow degradation of the herbicide which parallels the release from the CRF, yielding the practical coincidence of both dissipation patterns.

Dehydrogenase activity in soils provides correlative information on the biological activity and microbial populations in the soil. Measurements of dehydrogenase activity represent immediate metabolic activities of the soil microorganisms [48].

Compared with the control soil, the dehydrogenase activity decreased significantly ($p<0.05$) in soils with Diurokey (Figure 2). This decrease was observed 4 DAT and was markedly different along the time, until 65 DAT for CR and LT soils, and 25 DAT for AL soil. After these incubation times, no statistically differences in dehydrogenase activity were noticed between Diurokey and the control. According to El-Fantroussi et al. [49] and Prado and Airoldi [50], our results suggest that diuron has a toxic effect on the soil biochemical activity, despite the fact that the microorganisms tolerant to this herbicide are degrading it, and causing a gradual decrease of diuron in soil. The negative effect of Diurokey is possibly due to the resulting metabolites degradation of this herbicide. Tixier et al. [51] suggested that the metabolites resulting from the degradation of diuron have a more toxic character than the herbicide own.

Unlike Diurokey application, the use of D-CRF did not affect dehydrogenase activity with respect to the control. This is due to sustained release of the herbicide that reduces its toxic effect on soil microorganisms. In the case of application of PC-complex without herbicide, the soil dehydrogenase activity values were not modified, indicating that it has a neutral effect on soil microbial activity.

Soil β-glucosidase activity had a similar behavior to the dehydrogenase activity. The application of the Diurokey and D-CRF to the soil also inhibited the β-glucosidase enzymatic activity (Figure 3), indicating that the herbicide adversely affected microorganisms related to the C-cycle. The pattern of inhibition of this enzyme was identical with both formulations: a decrease from 7 to 25 DAT in AL soil, and from 4 to 65 DAT for CR and LT soils. At the end of the incubation experiment, β-glucosidase enzymatic activity was recovered to its initial value.

Unlike dehydrogenase and β-glucosidase activities, the urease and phosphatase activities were not inhibited by the application of diuron herbicide (Figures 4 and 5). Diuron molecule presents an urea structure, and it could be used as a substrate for the enzyme urease. However, low urease activity was observed in soils spiked with diuron [52], but, on the contrary, Liu et al. [53] observed inhibition of this enzymatic activity. Such difference may be explained by the different diuron rates used in these studies: 3 mg kg^{-1} in Fernandez-Bayo's study [52] and 5000 mg kg^{-1} in Liu`s [53].

In polluted soils with diuron, PCA showed clear discrimination between the different treatments (Figure 6). In this respect, the C and PC-clay treatments were grouped and differentiated from other treatments, which confirms that PC-clay is innocuous to soil microorganisms. Diuron (commercial and formulation), soil dehydrogenase and β-glucosidase activities are grouped, probably due to the decrease of these activities in the presence of the herbicide. These soil enzymatic activities along diuron are grouped in component 1, which is

showing greater specific weight in the explanation of the Figure. The direction shown by these enzyme activities and diuron was opposite, due to the negative correlation between these variables. In contrast, the urease and phosphatase showed a different behavior, grouping in component 2, which has a smaller specific weight in the explanation of the Figure. This suggests that both enzymatic activities are less important in explaining the relationships of these enzymes with the soil diuron contents.

3.2. Alachlor

Figure 7 shows the results of alachlor herbicide dissipation in the three experimental soils. Unlike the diuron herbicide, the alachlor dissipation applied as CRF was lower than that of the commercial formulation. In soils AL and CR the differences between both formulations were observed after 4 DAT, whereas in LT soil this occurred after 16 DAT. This was indicative of a longer acclimation period of the indigenous microflora in LT soil due to the absence of previous pesticides treatments. Alachlor dissipation in soils showed a good adjustment to the first order kinetics (Table 2). The half-life times corresponding to the dissipation of alachlor in the three soils suggest that there were differences between Alanex and the A-CRF, being higher the half-life when the herbicide was applied as CRF. In this respect, the alachlor half-life when was applied as Alanex was 68.5, 33.9 and 27.4 % lower in CR, AL and LT soils, respectively, that when alachlor was applied as CRF. This one, based on the PC-clay complex, offers greater protection than that reported adjuvants of the commercial formulation. Our results indicate that alachlor herbicide is relatively non-persistent in soil. These results are in agreement with those obtained with Peter and Weber [54] and Barbash et al. [55], who reported a range of biodegradative half-lives of about 14-21days for this herbicide.

During the first 16 days of incubation, the application of alachlor did not affect the dehydrogenase activity in the experimental soils (Figure 8). From day 16 until the end of the experiment, the application of this herbicide significantly ($p<0.05$) stimulated this enzymatic activity, independently of the formula that the alachlor was applied. In this sense and compared to the control treatment, the dehydrogenase activity increased at the end of the incubation period by 26.1%, 28.6% and 26.1% in AL, CR and LT soils when the herbicide was applied as Alanex. When applied as controlled release formulation (A-FLC), the dehydrogenase activity increased by 22.7%, 25% and 26.1%, respectively.

The results of biochemical analysis indicate that soil dehydrogenase activity showed no change during the first weeks of incubation. Felsot and Dzantor [56] found that alachlor inhibited soil dehydrogenase in soil at concentrations about 250 mg kg^{-1}, with prolonged inhibition through at least 21 d occurring at concentrations ≥750 mg kg^{-1}. In this study, the much lower amount of herbicide used (38.5 mg kg^{-1}) explain this discrepancy. It should be noted that the toxic effect of pesticides on microorganisms not only depends on its chemical composition, but also the concentration of this organic compound applied to the soil [8, 57]. After 16-25 DAT, dehydrogenase activity was enhanced with the use of Alanex and A-CRF relative to the control. An enhancement of this microbial activity was also observed by Saha et al. [58] with three chloroacetanilide herbicides at rates close to those used here (25-50 mg kg^{-1}). Given that these enhancements correspond to the times when most of the herbicide was degraded, these features point out that the forming metabolites were more bioavailable for further degradation and mineralization and lesser toxic than the parent compound.

β-glucosidase activity showed a similar behavior to dehydrogenase, as also in Saha et al. [58] (Figure 9). After 7-16 DAT, this enzymatic activity significantly ($p<0.05$) increased independently of the formulation used. Compared with the control treatment, the β-glucosidase activity increased at the end of the incubation period by 19.3%, 29.3% and 21.4%

in AL, CR and LT soils when the herbicide was applied as Alanex. As A-CRF, the β-glucosidase activity increased by 19.3%, 23.8% and 15.4%, respectively.

Phosphatase activity showed an enhancement similar to those of dehydrogenase and β-glucosidase activities with both alachlor formulations (Figure 10). This was as a result of a high exacerbation of the microbial activity due to the fast degradation of the herbicide metabolites, unlike the parent compound. Although alachlor molecule does not pose any phosphate moiety, the enhancement of this activity reflects an increase in the signal transduction and cell division, since the role of phosphatase enzymes is not only limited to simple phosphate scavenging functions [59].

Unlike dehydrogenase, β-glucosidase and phosphatase activities, the soil urease activity was not stimulated after the application of alachlor as any of the formulations (Figure 11). These results are in agreement with those obtained by Saha et al [58] who found that the urease activity is not affected by the presence of low concentrations of alachlor in the soil.

In polluted soils with alachlor, PCA again indicated that the C and PC-clay treatments were grouped and differentiated from other treatments (Figure 12). Alachlor herbicide (commercial and formulation), soil dehydrogenase, β-glucosidase and phosphatase activities are grouped, probably due to these enzymatic activities increase from the middle of the experimental period until the end of the experiment. These enzymatic activities have a higher specific weight in component 1. The alachlor variable also an important specific weight in component 2. However, whereas LT has a positive value, in AL and CR has a negative value, which makes us think that the behavior of this herbicide is strongly dependent on the soil physicochemical properties. As seen in the above Figures, the behavior of dehydrogenase, β-glucosidase and phosphatase activities began to increase in mid-experimental period to 127 days of incubation. For this reason, the distribution of alachlor in the Figure is not grouped in the same component that these enzymatic activities. The urease activity is not grouped with

the other enzyme activities, probably because to this enzymatic activity was not changed throughout the experiment in the presence of alachlor.

4. Conclusions

The novelty of the present paper lies in that, to the best of the authors' knowledge, it is the first study in which tests of microbial activity are performed so far for the examined compounds. From this study, it can be concluded that (i) the PC-clay complex is innocuous to soil microorganisms and therefore is optimal for use in formulating herbicidal slow release; (ii) the application of alachlor as CRF using a complex herbicide-PC-clay increases the half-life time of this herbicide in soils, whereas no differences were noticed between diuron formulations due to its slower degradation, which takes longer than its release from the CRF; (iii) the enzymatic activities except urease were enhanced when using alachlor formulations because the forming metabolites were lesser toxic than the parent compound and more bioavailable for further degradation and mineralization; on the contrary, diuron formulations adversely affected dehydrogenase and β-glucosidase activities due to the higher toxicity of its main metabolite; (iv) urease activity was not affected by the application of alachlor and diuron formulations due to the low concentrations used in the current study; (v) at the end of the incubation period, the enzymatic activities were the same after the use of diuron either as commercial or CRF, recovering their initial values, and consequently, the soil previous status; in the case of alachlor formulations, no differences in enzymatic activities were again observed between both formulations, but their levels in soils were enhanced.

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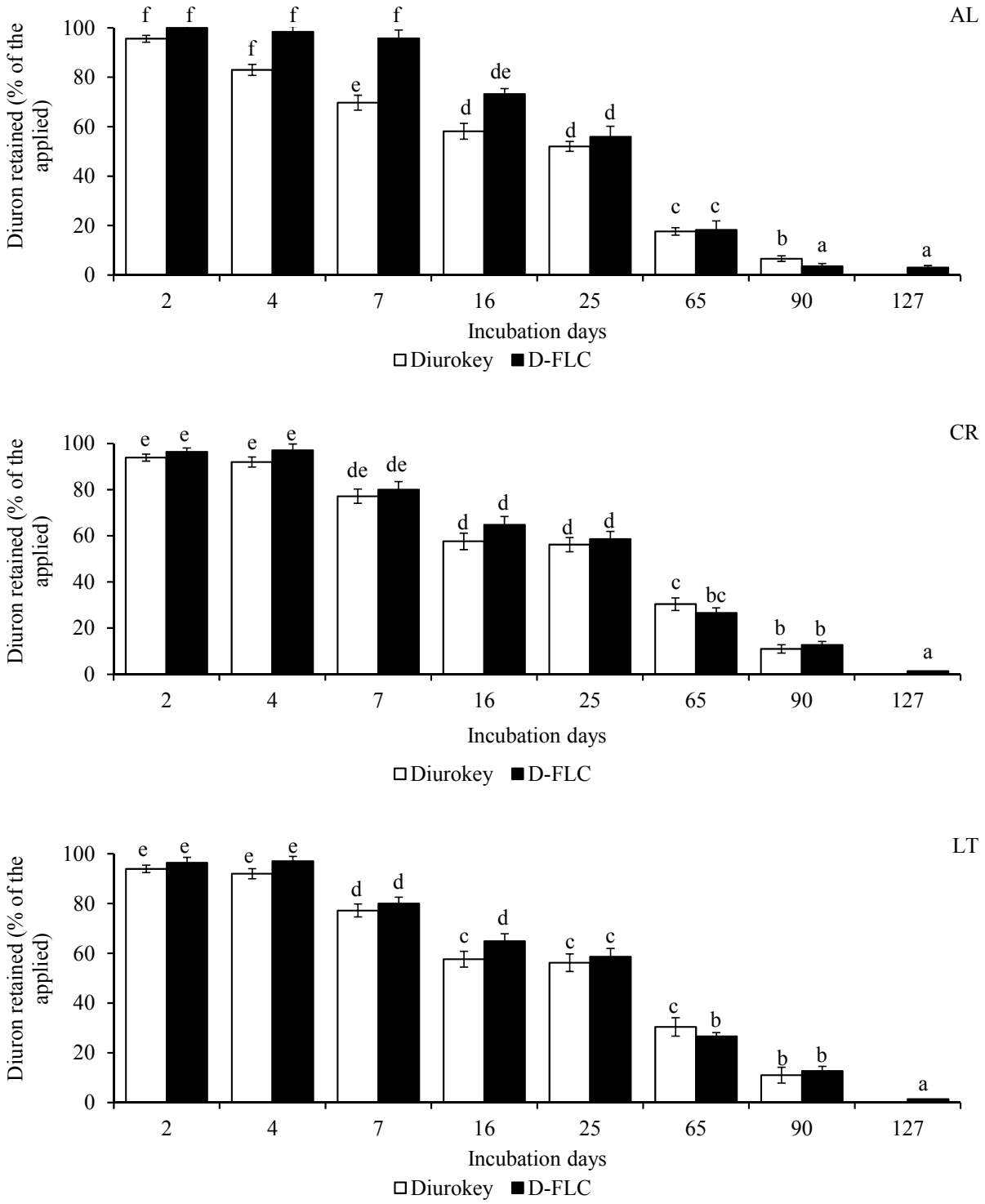

Figure 1. Diuron dissipation in soils from commercial formulation (Diurokey) and controlled release formulation (D-CRF). Error bars represent the SE of means (n = 2). Column (mean ± SE) followed by the same letter(s) are not significantly different ($p>0.05$). AL: dystric Cambisol; CR: calcaric Fluvisol; LT: calcaric Fluvisol

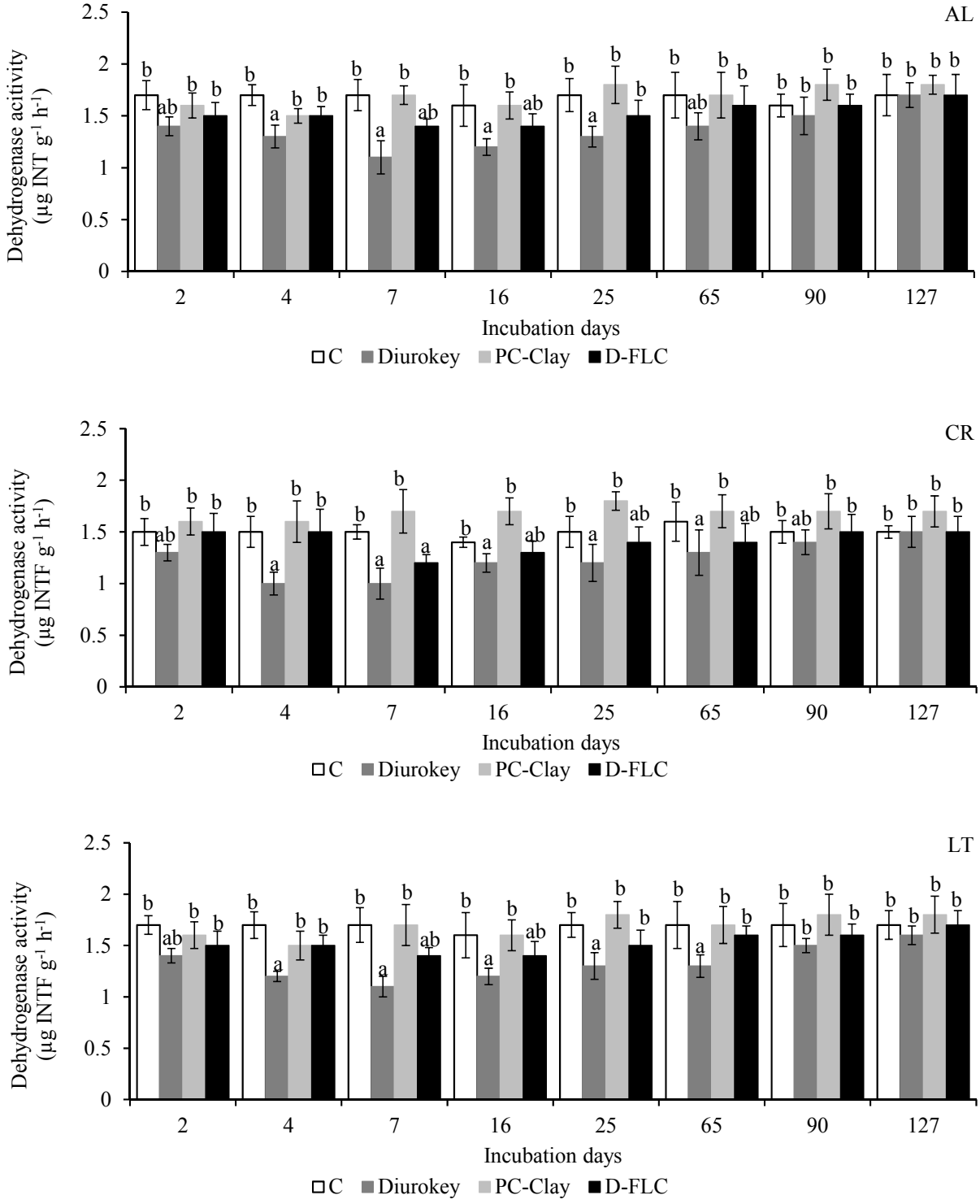

Figure 2. Evolution of dehydrogenase activity in the three experimental soils affected by the application of different diuron formulations and the complex PC-Clay without herbicide. Error bars represent the SE of means (n = 2). INTF = 2-piodo-3-nitrophenyl formazan. Column (mean ± SE) followed by the same letter(s) are not significantly different ($p>0.05$). AL: dystric Cambisol; CR: calcaric Fluvisol; LT: calcaric Fluvisol

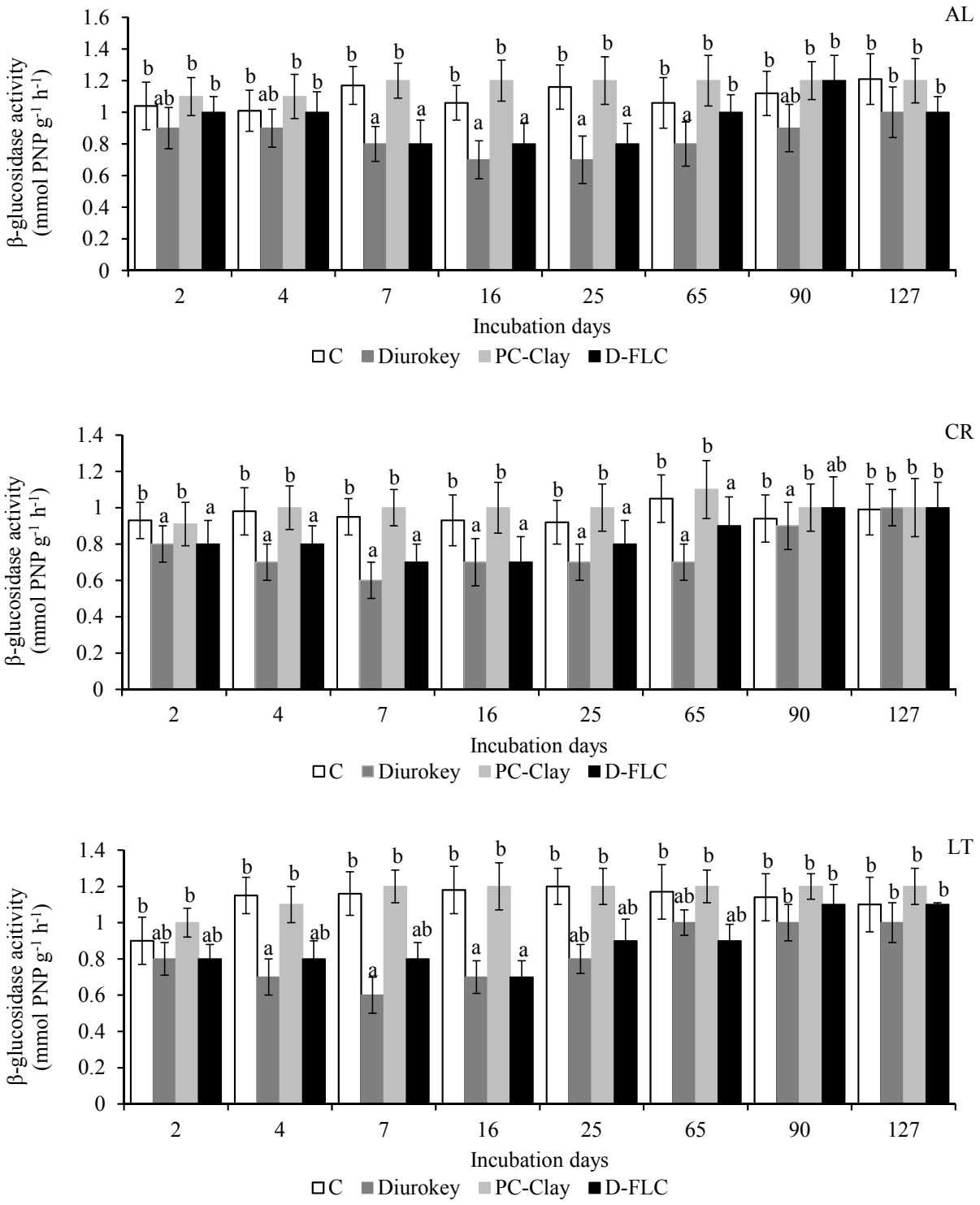

Figure 3. Evolution of β-glucosidase activity in the three experimental soils affected by the application of different diuron formulations and the complex PC-Clay without herbicide. Error bars represent the SE of means (n = 2). INTF = 2-piodo-3-nitrophenyl formazan. Column (mean ± SE) followed by the same letter(s) are not significantly different (p>0.05). AL: dystric Cambisol; CR: calcaric Fluvisol; LT: calcaric Fluvisol

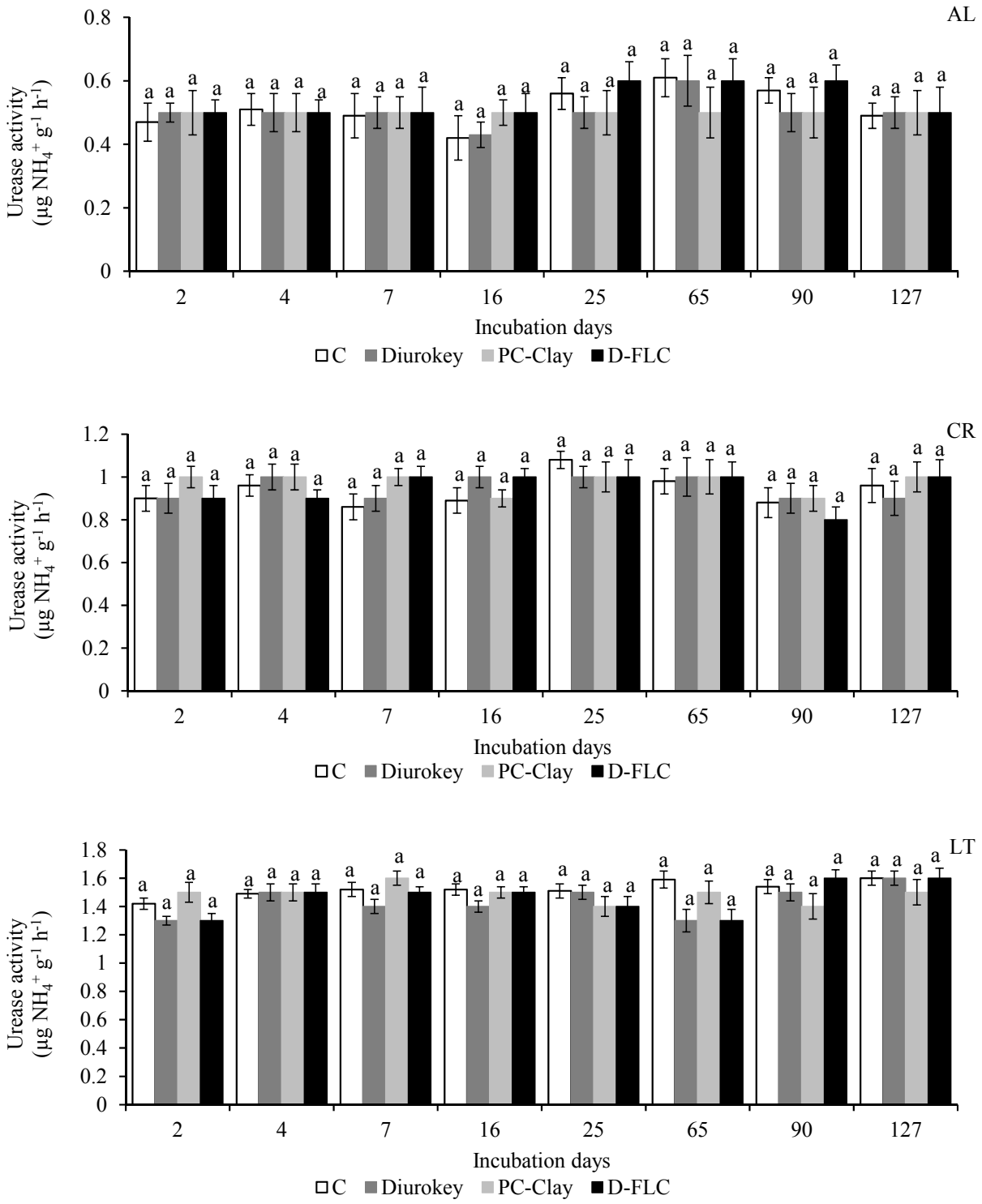

Figure 4. Evolution of urease activity in the three experimental soils affected by the application of different diuron formulations and the complex PC-Clay without herbicide. Error bars represent the SE of means (n = 2). Column (mean ± SE) followed by the same letter(s) are not significantly different ($p>0.05$). AL: dystric Cambisol; CR: calcaric Fluvisol; LT: calcaric Fluvisol

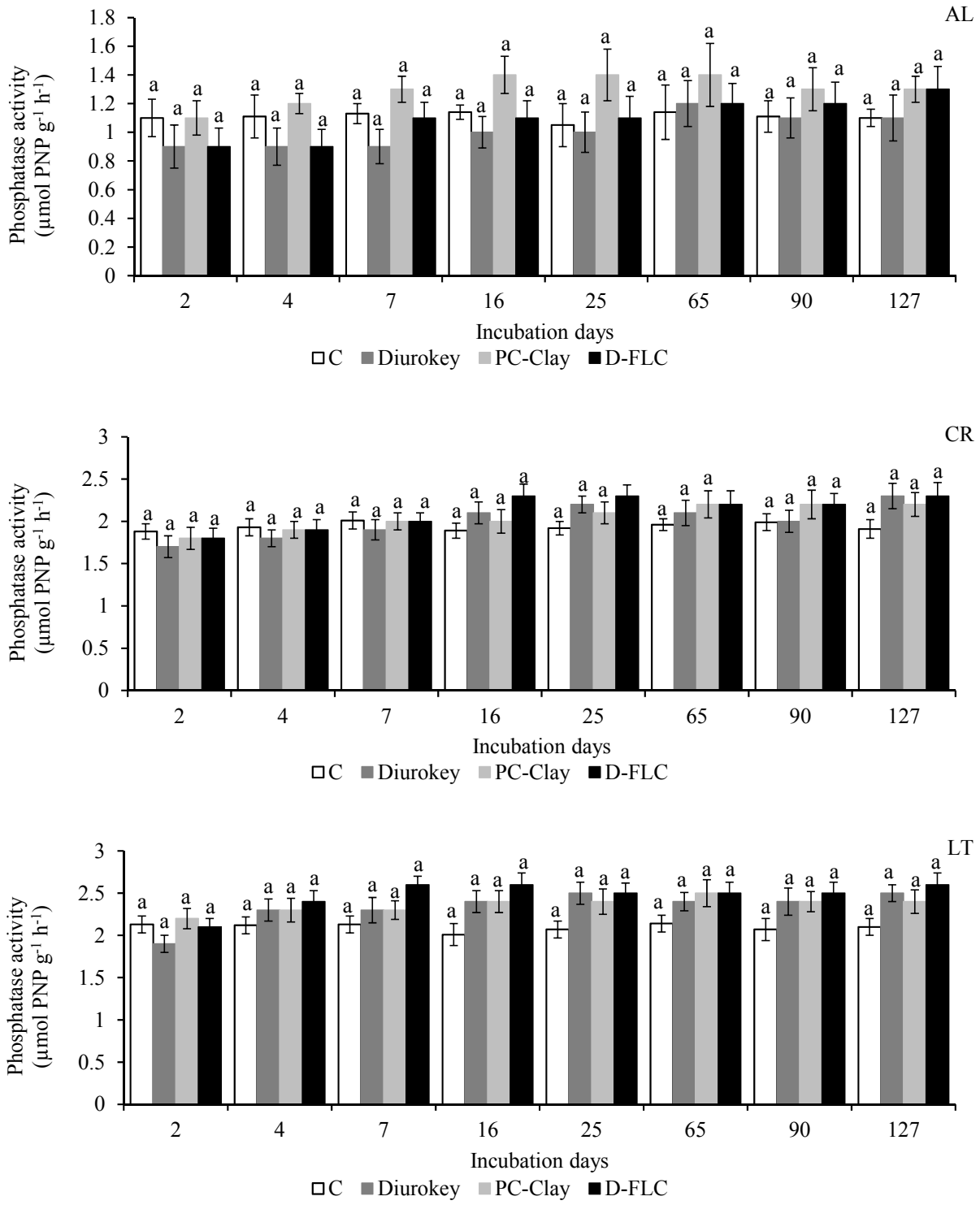

Figure 5. Evolution of phosphatase activity in the three experimental soils affected by the application of different diuron formulations and the complex PC-Clay without herbicide. Error bars represent the SE of means (n = 2). PNP = p-nitrophenol. Column (mean ± SE) followed by the same letter(s) are not significantly different ($p>0.05$). AL: dystric Cambisol; CR: calcaric Fluvisol; LT: calcaric Fluvisol

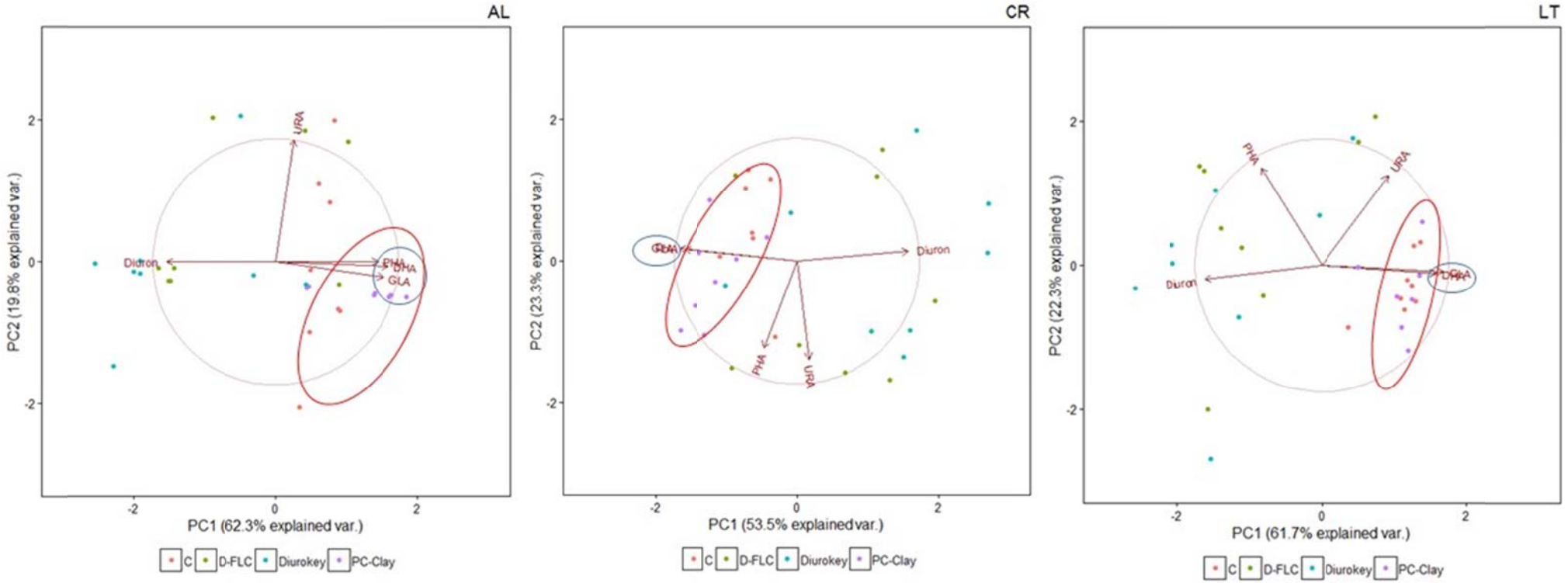

Figure 6. Principal components analysis in soils affected by the application of different diuron formulations and the complex PC-Clay without herbicide. Values on axes 1 and 2 represent the percent of total variance explained by the axes. DHA: dehydrogenase activity; URA: urease activity GLA: β-glucosidase activity PHA: phosphatase activity; AL: dystric Cambisol; CR: calcaric Fluvisol; LT: calcaric Fluvisol

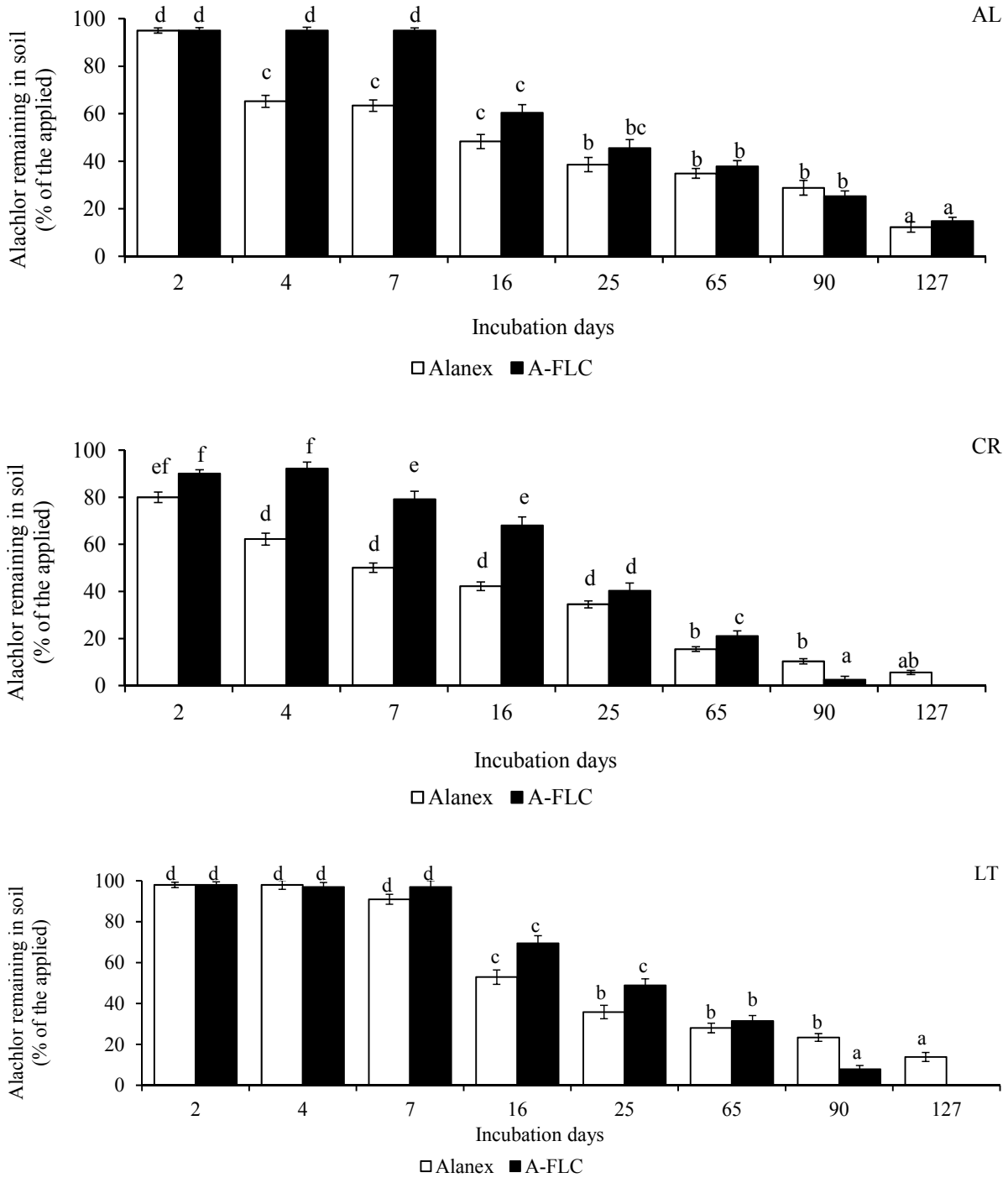

Figure 7. Alachlor dissipation in soils from commercial formulation (Alanex) and controlled release formulation (A-CRF). Error bars represent the SE of means (n = 2). Column (mean ± SE) followed by the same letter(s) are not significantly different ($p>0.05$). AL: dystric Cambisol; CR: calcaric Fluvisol; LT: calcaric Fluvisol

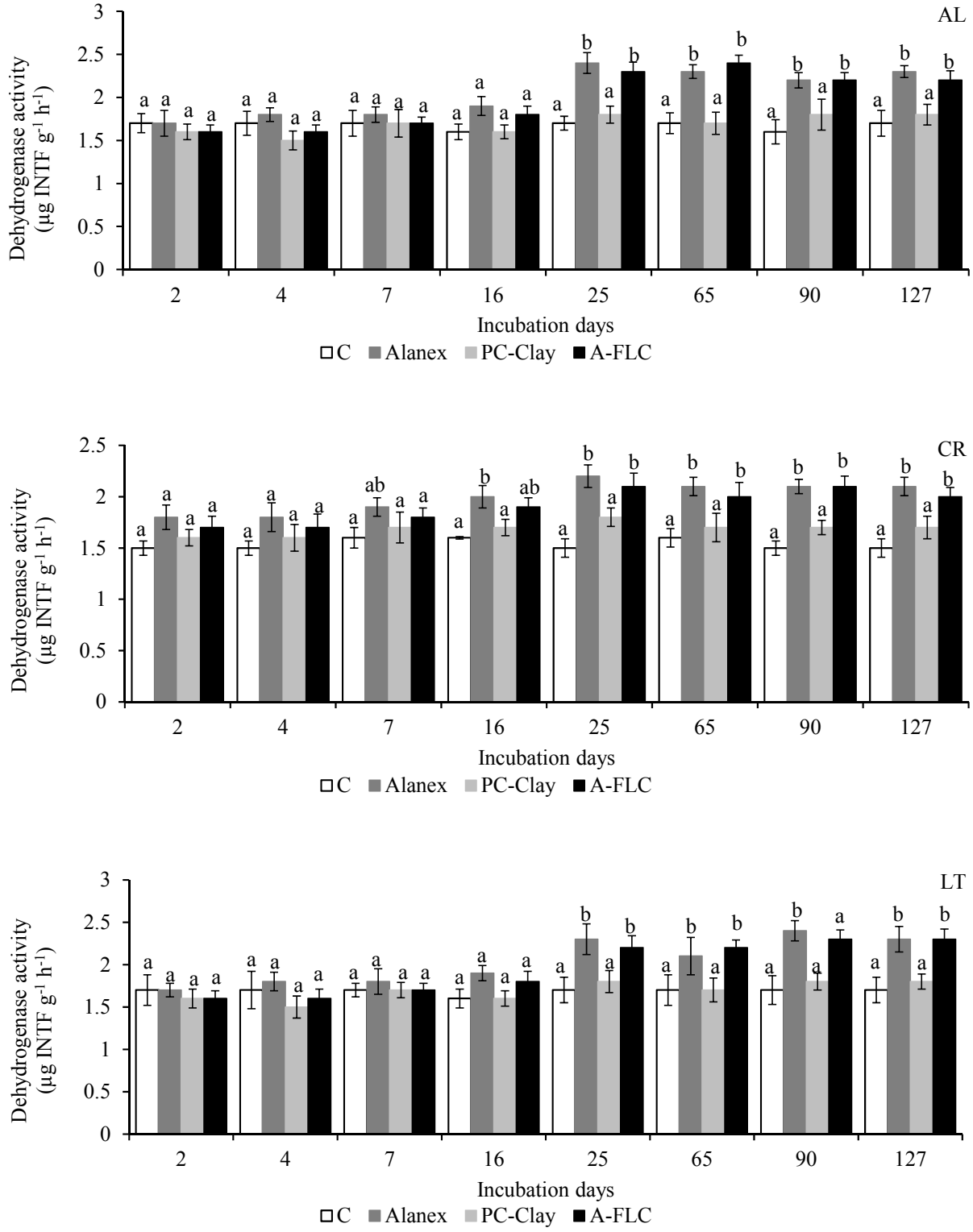

Figure 8. Evolution of dehydrogenase activity in the three experimental soils affected by the application of different alachlor formulations and the complex PC-Clay without herbicide. Error bars represent the SE of means (n = 2). INTF = 2-piodo-3-nitrophenyl formazan. Column (mean ± SE) followed by the same letter(s) are not significantly different ($p>0.05$). AL: dystric Cambisol; CR: calcaric Fluvisol; LT: calcaric Fluvisol

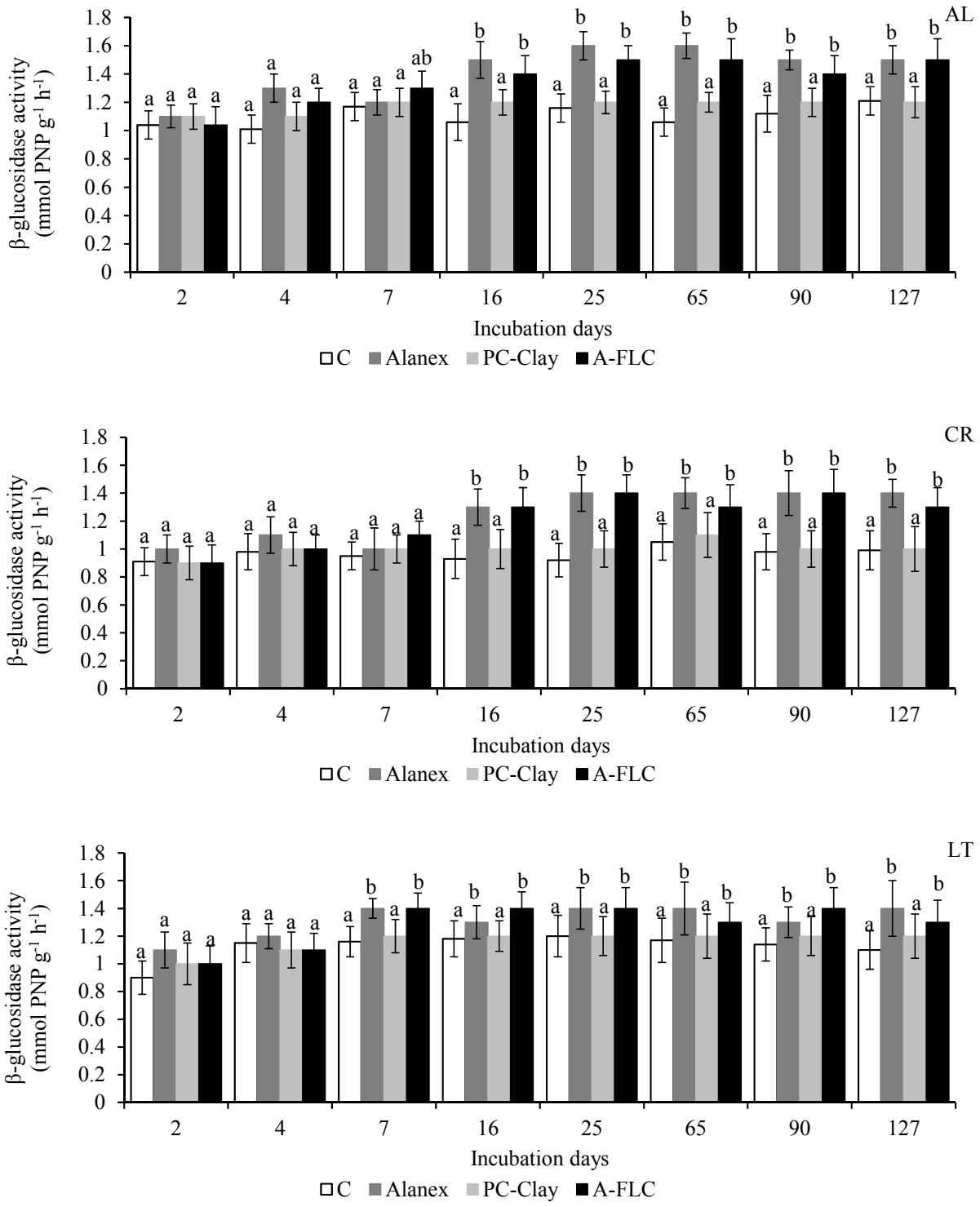

Figure 9. Evolution of β-glucosidase activity in the three experimental soils affected by the application of different alachlor formulations and the complex PC-Clay without herbicide. Error bars represent the SE of means (n = 2). PNP = p-nitrophenol. Column (mean ± SE) followed by the same letter(s) are not significantly different ($p>0.05$). AL: dystric Cambisol; CR: calcaric Fluvisol; LT: calcaric Fluvisol

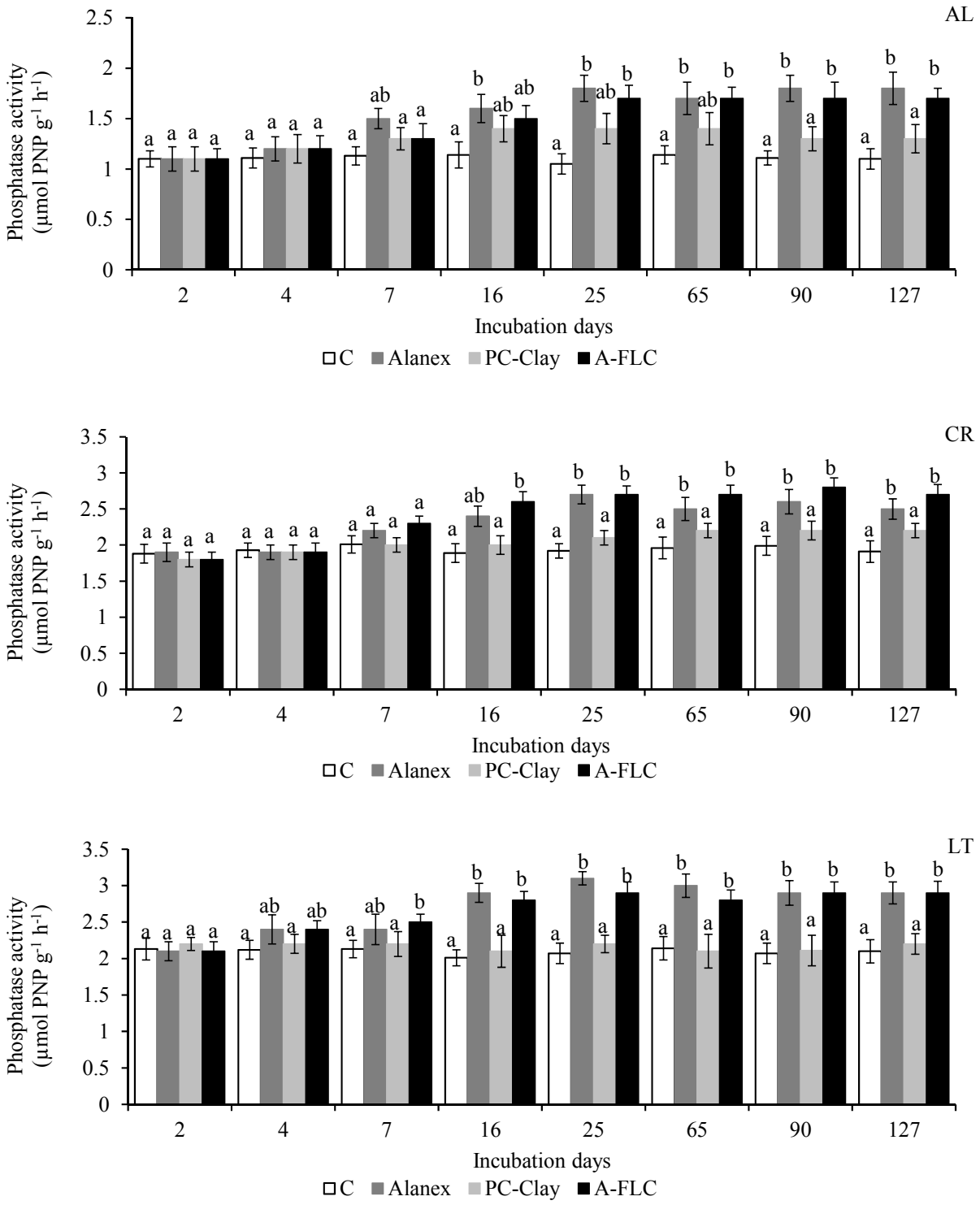

Figure 10. Evolution of phosphatase activity in the three experimental soils affected by the application of different alachlor formulations and the complex PC-Clay without herbicide. Error bars represent the SE of means (n = 2). PNP = p-nitrophenol. Column (mean ± SE) followed by the same letter(s) are not significantly different ($p>0.05$). AL: dystric Cambisol; CR: calcaric Fluvisol; LT: calcaric Fluvisol

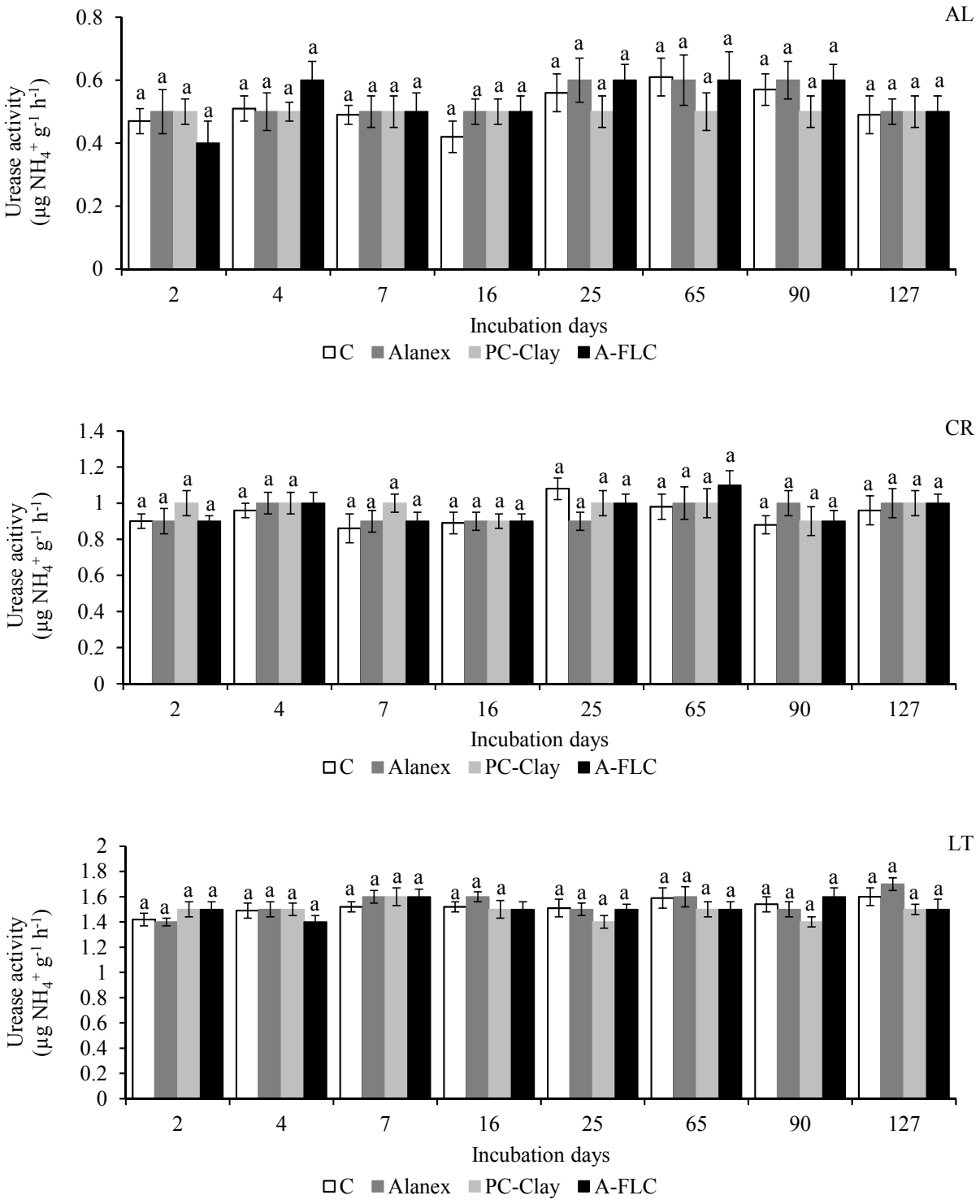

Figure 11. Evolution of urease activity in the three experimental soils affected by the application of different alachlor formulations and the complex PC-Clay without herbicide. Error bars represent the SE of means (n = 2). Column (mean ± SE) followed by the same letter(s) are not significantly different ($p>0.05$). AL: dystric Cambisol; CR: calcaric Fluvisol; LT: calcaric Fluvisol

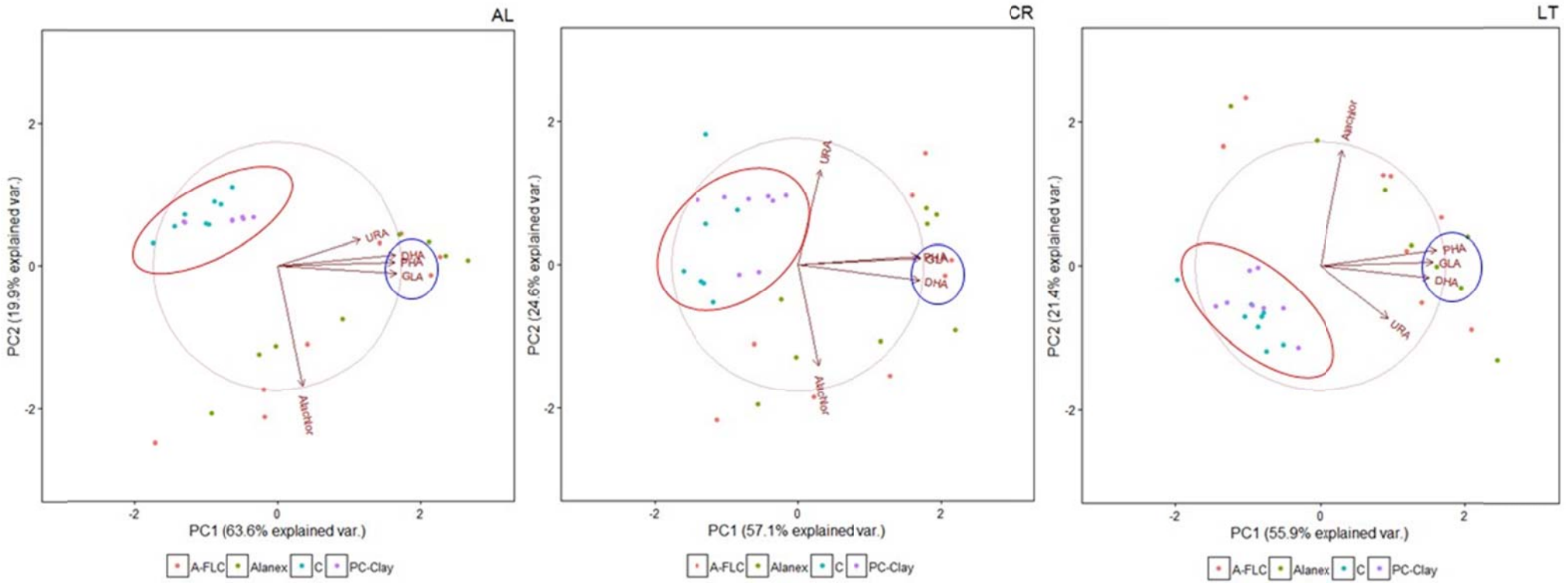

Figure 12. Principal components analysis in soils affected by the application of different alachlor formulations and the complex PC-Clay without herbicide. Values on axes 1 and 2 represent the percent of total variance explained by the axes. DHA: dehydrogenase activity; URA: urease activity GLA: β-glucosidase activity PHA: phosphatase activity; AL: dystric Cambisol; CR: calcaric Fluvisol; LT: calcaric Fluvisol

Table 1

Characteristics of the experimental soils. Data are the means of two samples

	AL	CR	LT
pH (H_2O)	6.2 ± 0.06	8.7 ± 0.11	8.2 ± 0.08
CO_3^{2-} (%)	1.6 ± 0.3	17 ± 2.2	21.8 ± 1.5
Sand (%)	24.2 ± 3.0	59.6 ± 4.5	28.5 ± 2.2
Silt (%)	59.4 ± 4.1	19.4 ± 2.0	45.8 ± 4.1
Clay (%)	16.4 ± 1.6	21.0 ± 2.3	25.7 ± 1.8
Organic matter (%)	3.4 ± 0.5	1.1 ± 0.1	1.3 ± 0.2
Kjeldahl-N (%)	0.083 ± 0.009	0.049 ± 0.006	0.079 ± 0.008

AL: dystric Cambisol; CR: calcaric Fluvisol; LT: calcaric Fluvisol

Table 2

Kinetic parameters corresponding to Alachlor and Diuron dissipation in soils affected by the application of their commercial and CRFs.

Formulations	Soil	$t_{1/2}$ (days)	K ($days^{-1}$)
Diurokey	CR	32.2 ± 1.1	0.02155
	AL	27.9 ± 1.6	0.02483
	LT	40.8 ± 0.2	0.01697
D-SRF	CR	33.6 ± 0.7	0.02060
	AL	31.5 ± 0.6	0.02199
	LT	41.5 ± 0.7	0.01671
Alanex	CR	7.81 ± 0.2	0.00887
	AL	14.4 ± 0.9	0.04820
	LT	18.0 ± 1.0	0.03855
A-SRF	CR	24.8 ± 1.2	0.02798
	AL	21.8 ± 1.4	0.03180
	LT	24.8 ± 1.7	0.02798

AL: dystric Cambisol; CR: calcaric Fluvisol; LT: calcaric Fluvisol