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How the environmental fate of clomazone in rice fields is influenced by amendment with olive-mill waste under different regimes of irrigation and tillage

Running title: Behaviour of clomazone in rice-growing conditions

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Abstract

BACKGROUND: Irrigation and tillage systems alone or in combination with organic amendments can strongly influence soil properties, which in turn may also modify the environmental fate of any pesticides applied. The present study was aimed at determining how amendment with composted olive-mill waste (W) influenced the herbicide clomazone's leaching, sorption, and persistence in rice field soils under different tillage and irrigation management practices. The field trial conducted covered three years in succession, with six treatments: irrigation by sprinkler and conventional tillage without (ST) or with W application (80 Mg ha^{-1}) (STW), irrigation by sprinkler but no tillage (SNT), irrigation by sprinkler but no tillage with W application (SNTW), and continuous flooding irrigation and tillage without (FT) and with W application (FTW).

RESULTS: The application of W significantly increased the adsorption of clomazone to soil in the first and third year. In the first year the persistence of clomazone under aerobic ($t_{1/2}$ = 33.1–36.3 d) and anaerobic incubation conditions ($t_{1/2}$ = 3.43–10.8 d) decreased after W application to $t_{1/2}$ values in the ranges 18.1–29.7 d and 3.06–5.44 d, respectively. However, in the third year while clomazone persistence increased significantly in SNT and ST when W was applied under both incubation conditions, it decreased significantly in FT management under anaerobic incubation conditions. The addition of W led to less leaching of clomazone, particularly for the FT case where the herbicide's leaching losses were 2.8 and 2.6 times lower in the first and third years after W addition, respectively.

CONCLUSIONS: Using W as an organic amendment could be regarded as an invaluable strategy to reduce water contamination by clomazone in rice-growing, especially under traditional tillage and flooding management.

Keywords: Aerobic rice; Anaerobic rice; Clomazone behaviour; Olive-mill waste.

1. Introduction

Traditionally, rice (*Oryza sativa* L.) has been grown worldwide under anaerobic (continuous flooding irrigation with tillage) conditions. These can contribute to increased soil degradation and water contamination, and decreased productivity of water use.[1-3] In the European Union, the area under rice cultivation is 475 000 ha, with 80% corresponding to two countries – Spain (30%) and Italy (50%). In both of these countries, limited water availability is leading to a steady decline in the area used for rice cultivation. For this reason, aerobic (non-flooding) rice production with and without conservation agriculture practices (i.e., no tillage) has recently been implemented in European countries as a productive and sustainable alternative to the traditional rice flooding system.[4,5] Indeed, Sánchez-Llerena et al.[2] found similar rice yields under aerobic and anaerobic management regimes using no-tillage, but with the advantage of on average 75% water savings in the former. However, lower short-term rice grain yields have been found under non-flooding than flooding regimes.[2] This has been described as due to lower soil organic carbon (SOC) in the soils of the former of these two management regimes at the beginning of its implementation.[6]

It is well established that soil properties can be strongly influenced by conservation agriculture practices[7] and irrigation method[2], affecting therefore the behaviour (sorption, leaching and persistence) of pesticides in the environment. However, the results often show contradictory trends. Thus, the implementation of no tillage generally increases water use efficiency, which is attributable to the greater microporosity observed in these systems.[8,9] Also, greater soil aggregate stability and lower penetration resistance have been reported for no-tillage soils relative to tilled soils.[10,11] Although the adoption of no-tillage has often been shown to raise the organic carbon content and lower the pH of a soil,[12,13] significantly lower pH's have been also reported in conventional than in no-tillage systems.[14-16] The main soil characteristics determining the influence of the tillage practice will have on SOC (and indeed other soil properties are the texture and the initial SOC level[17,18] Furthermore, the increase in SOC under no-tillage is usually accompanied by concomitant increases in microbial activity and community size, and these depend strongly on how anoxic the systems are.[19-21] Whereas significant reductions in both the organic matter content and the pH have often been reported in irrigated soils,[22] there are differences according to whether the irrigation regime is sprinkler or flooding. In particular, soil organic matter losses are found to be greater in the former case due to its higher rate of organic matter decomposition, which may lead to higher values of pH, reflecting the importance of which method of irrigation is used in determining the soil's properties.[2]

One of the principal processes used worldwide for olive oil extraction is two-phase centrifugation. In Mediterranean nations alone, it generates greater than 11 000 000 Mg yr^{-1} of waste. This waste can be an invaluable resource for use as organic amendment, improving crop productivity by impacting positively on the properties of the soil.[23] Because of its high content in organic matter (>85%), the use of composted olive-mill waste (W) as organic amendment could compensate the initial low organic matter levels observed under non-flooding conditions in rice soils, also increasing water and crop productivity,[24] enhancing therefore the sustainability of this crop in regions with limited water availability.

Changes in irrigation systems may greatly impact the persistence and environmental behaviour of pesticides frequently used in rice cropping.[25] Although sprinkler irrigation has been considered as a viable non-flooding option for rice cultivation in zones in which the limiting factor is water, this alternative may often be associated with very high yield losses as a consequence of the difficulty in controlling weeds. In this sense, the isoxazolane herbicide clomazone (2-[(2-chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone) is intensively applied worldwide in rice fields owing to its effectiveness in controlling broadleaf weeds and grasses. Due to its long dissipation half-life (28-84 d), minimal volatility (P_v=1.44×10^{-4} mm Hg), slow degradation by photolysis or hydrolysis, and great water solubility (1100 mg L^{-1}), this herbicide has a great potential to contaminate surface and ground waters. Indeed, it is one of the most frequently detected herbicides in water sampled in rice-growing areas,[26,27] with reported concentrations greater than 3.7 μg L^{-1} (e.g., Marchesan et al. [28]). Furthermore, several studies have shown that clomazone may negatively impact non-target organisms such as nitrogen-fixing bacteria[29] and invertebrates[30], some of which are essential for sustainable rice production.[31]

Various studies using W as organic amendment have highlighted its beneficial effects in that it increases the soil's adsorption of pesticides, and consequently reduces the risk of water contamination.[32, 33] Nonetheless, these effects depend on the compound's properties and the soil type.[34] Furthermore, the transformation of W in the field may modify the compound's future interactions with the amended soil. Although the implementation of conservation farming practices may enhance a soil's adsorption of pesticides because of the greater total organic carbon content, the results of studies of pesticide behaviour in soils under different tillage management regimes have often been contradictory.[35]

With respect to clomazone specifically, we could find no literature evaluating how its behaviour might be affected by different irrigation and tillage management regimes with and without organic amendment. The aim of this work therefore was to assess the persistence, sorption, and leaching of clomazone in rice-growing under different water and tillage management regimes influenced by W amendment. We also evaluated the residual and direct effects in years one and three after the W application.

2. Material and Methods

2.1. Herbicide assay

Clomazone (99.8% purity) came from Dr Ehrenstorfer, GmbH (Deutschland). It was subjected to HPLC assay as detailed in Text S1 of the Supplementary Material (SM).

2.2. Experimental design, soil sampling, and analysis

An experiment in the field was carried out from 2015 to 2017 in southern Spain (38°55'N; 6°57'W) on a 20.8% clay, 28.9% silt, and 50.3% sand soil, in a semi-arid region of the Mediterranean with mean annual rainfall and temperature of 460 mm and 16.2 °C, respectively. This experimental area had already been cropped for eleven years with rice (*O. sativa* L.) with deep ploughing and flooding, however clomazone had not been previously applied in this area. The experiment involved eighteen plots (18 m × 10 m) with six treatments in triplicate: conventional tillage and sprinkler irrigation without (ST) or with first-year of W application (STW), no-tillage and sprinkler irrigation without (SNT) or with first-year of W application (SNTW), and continuous flooding irrigation and tillage without (FT) or with first-year of W application (FTW). The W was obtained from a mixture of 10% olive leaves and 90% two-phase olive-mill waste. It had the following properties: total organic carbon (TOC) 382 g kg^{-1}; pH 7.71; total nitrogen 21.7. g kg^{-1}; and electrical conductivity (EC) 2.32 dS m^{-1}. The W application rate in the STW, SNTW, and FTW treatments was 80 Mg ha^{-1}. After harvest in November 2015 and 2017, four subsamples of soil were taken from each of the plots (20 cm depth) for sorption-desorption, leaching, and dissipation determinations in laboratory experiments. The sub-samples from each plot were mixed and homogenized to get a composite sample for every plot. The measurements done in 2015 and 2017 constituted the "direct" and "residual" effects, respectively. Measurements were also made of the soil pH, EC, TOC, and water soluble organic carbon (WSOC), fulvic acids (FA), and humic acids (HA) contents as described by López-Piñeiro et al.[36] and briefly in Text S2. Table 1, which is adapted from Gómez et al.[37], presents the values of selected soil properties for the direct and residual years.

2.3. Adsorption-desorption experiments

The technique used to determine clomazone adsorption isotherms was batch equilibration in accordance with OECD[38] as described by López-Piñeiro et al.[36]. The adsorption-desorption data were fitted to a Freundlich equation. Detailed information is given in Text S3 of the SM.

2.4. Dissipation studies

The clomazone dissipation studies were conducted under non-flooded (aerobic) and flooded (anaerobic) incubation conditions. Clomazone additions were applied so as to reach an initial concentration of 3.3 μg g^{-1} soil (equivalent to 1 kg ha^{-1}). For each treatment, triplicate samples were periodically removed to determine the herbicide's residual concentration. Clomazone was extracted from the soil samples with methanol (10 mL). After centrifuging, residues of clomazone were analysed in the supernatants by HPLC. A kinetics equation of first order was fitted to the data corresponding to dissipation (Fig. S1), followed by calculation of the respective half-lives ($t_{1/2}$). Further information can be found in Text S4 of SM.

Dehydrogenase activity (DA) determinations were made under aerobic and anaerobic incubation conditions following García et al.[39] as detailed in SM (Text S4).

2.5. Leaching studies

For each treatment, PVC disturbed-soil columns (5-cm i.d. × 30-cm length) in triplicate were used to measure clomazone leaching in accordance with OECD[40]. After saturating with a solution of 0.01 M $CaCl_2$, the columns were allowed to drain for 24 h. Clomazone was then applied at a 1 kg ha^{-1} dose to the top of each column. Each day, an additional 50 mL 0.01 M $CaCl_2$ was poured into the top of the columns, and the leachates were recovered as long as the herbicide was detected. These leachates were analysed by HPLC to determine their clomazone concentration. When the monitoring period concluded, each soil column was partitioned into sections of 5-cm depth to determine the clomazone remaining at four different depths. The extraction procedure followed was that described above in the Dissipation studies subsection. Further information about these leaching experiments is given in SM (Text S5).

2.6. Statistical study

The statistical analysis was carried out using the SPSS statistics software package version 22.0. After having verified the normality distribution (Shapiro-Wilk method) and homoscedasticity (Levene test) of the data, these were subjected to a one-way ANOVA. Duncan test was applied to compare inter-treatment differences ($p<0.05$). A Pearson's correlation analysis was applied to measure relationships between selected variables.

3. Results and Discussion

3.1. Studies of the sorption and desorption

The clomazone sorption-desorption isotherms for direct and residual years are presented in SM (Fig. S2). For both years, the sorption isotherms fitted appropriately the

Freundlich equation ($R^2 > 0.956$; Table 2), showing that the herbicide's sorption was concentration-dependent (n_f values <1; Table 2). The clomazone K_d values for the direct (1.54-3.05) and residual (1.24-2.95) years are of the same order as those found by Xu et al.[41] of 1.11-3.26 for soils from China with and without burned rice straw amendment. However, a wider range of K_d values (2.3-11.0) was found by Gunasekara et al.[42] for rice soils from California (USA). The different treatments significantly (p<0.001) influenced the clomazone sorption, with different effects in the direct and residual years since the treatment × year interaction was found to be significant (Table 2). For the unamended soils, in the direct year the K_d values were 1.2 times lower in ST than in SNT and FT. In the residual year, however, these values were significantly greater in FT than in SNT and ST by factors of 1.4 and 1.2, respectively, indicating that the timing of the implementation of different water and tillage management regimes is of major importance for the sorption behaviour of this herbicide. These results are contrary in part than those reported by Gómez et al.[37] which found sorption values greater in FT than in SNT and ST in both the direct and residual years, although for bispyribac-sodium (BS) herbicide.

For all treatments, clomazone sorption was influenced significantly by W field application in each of the two years (Table 2). Thus, the K_d values were greater by factors of 1.65 and 1.47 in SNTW and STW, respectively, than in the corresponding unamended soils (SNT and ST) in the direct and residual years, and by factors of 1.56 and 1.75 in FTW than in FT in the direct and residual years, respectively (Table 2). One explanation for the above results could be the increase in the soil's organic matter as a consequence of the W addition. Indeed, K_d was correlated significantly with TOC (r= 0.754, p <0.01), HA (r=0.549, p <0.01), and WSOC (r=0.516, p <0.01), indicating that both transformed and fresh soil organic matter could provide active sites for clomazone sorption. This is coherent with several published studies that also report more sorption of clomazone and other non-ionic pesticides as soil organic matter content increases (e.g., Benoit et al., Li et al., Pereira et al,[43-45]). However, these results contrast with Gómez et al.[37] who found that soil pH was the major contributor to BS sorption in the same rice soils used in this study.

The data of clomazone desorption were also appropriately fitted by a Freundlich equation, with R^2>0.968 (Table 2). The values of H were influenced significantly by the different treatments in the direct year and in the residual year. In the unamended soils, the lowest H values observed corresponded to the FT treatment, indicating lower reversibility of sorbed clomazone under non-flooding than flooding management, especially when no-tillage is implemented (Table 2). These results are consistent with those of various workers who report lower reversibility of different pesticides under no-tillage management (e.g., Reddy and Locke[46]). Furthermore, for both years the H values were significantly lower after W application, with again the lowest values corresponding to FTW (Table 2). These results show that sorbed clomazone may be retained more strongly in soils that are under non-flooding management, especially when W has been applied as organic amendment. Greater reversibility of clomazone in soils under flooding conditions may be a result of the significant falls in their HA values, especially for the amended soils whose HA values were up to 1.5 and 1.3 times lower under flooding than non-flooding conditions in the direct and residual years, respectively (Table 1). This is consistent with Gunasekara et al.[42] who also reported that HA compounds limited clomazone desorption in rice soils from California (USA).

Dissipation studies

The clomazone dissipation curves and the dehydrogenase activity (DA) are presented in Figure 1. The data fit first-order kinetics under anaerobic and aerobic experimental conditions, giving values of $R^2 > 0.858$ and $R^2 > 0.824$ in the direct and residual years, respectively (Table 3). For both years, the values of DA determined before (DA_B) and two hours after (DA_A) clomazone application and by considering all 50 d of incubation time (DA_T) were much lower under aerobic than anaerobic conditions (Table 3). This agrees with Gómez et al.[37] who also found values of DA much lower under aerobic than anaerobic conditions in the same unamended and amended soils of the present study, but after application of BS herbicide. Similar results have been also reported for MCPA and bensulfuron-methyl herbicides in different unamended rice-field soils,[47] which also agreed with a study finding a reduction in DA with increasing soil aeration and redox potential.[48]

The treatments significantly ($p<0.001$) influenced clomazone dissipation, with differences between the two years, as indicated by the significant ($p<0.001$) treatment × year interaction (Table 3). When only unamended soils are considered, the clomazone $t_{1/2}$ values ranged from 33.1 to 62.6 d for aerobic and from 3.43 to 20.5 d for anaerobic incubation conditions (Table 3), which agrees with the U.S. Environmental Protection Agency[49] report of also faster degradation of clomazone under anaerobic than aerobic conditions. Our values under aerobic conditions are similar to those reported for aerobic incubation conditions by Tomco et al.[27] of 47 d, but slightly higher than those found by Gámiz et al.[50] of 29 d, although in a soil with a sorption value lower than those observed in our study.

Except for aerobic experimental conditions in the direct year, the clomazone dissipation rates were significantly influenced by the tillage and irrigation regime. This was particularly evident under anaerobic experimental conditions for which clomazone persistence was significantly shorter in FT, with $t_{1/2}$ values being lower than those corresponding to SNT and ST by factors of 1.6 and 3.1 for the direct year, and 1.8 and

2.5 for the residual year, both respectively (Table 3). The longer persistence of clomazone observed in ST compared to SNT for both direct and residual years under anaerobic conditions may be attributable to these soils' lower microbial activity as indicated by their DA values. Indeed, the DA_T values in ST were 1.7 times lower than those corresponding to SNT for both years (Table 3). This is consistent with Dalal et al.[51] and Biederbeck et al.[52] who also found that microbial biomasses were smaller under conventional tillage management than under no-tillage.

Clomazone dissipation was significantly influenced by field application of W under both anaerobic and aerobic experimental conditions (Table 3). The significant treatment × year interactions for aerobic (p<0.01) and anaerobic (p<0.001) incubation conditions (Table 3) show that the effect differed between the direct year and the residual year. As in the unamended case, in the W field amended soils, clomazone was more persistent under aerobic than anaerobic incubation conditions, with the respective ranges of the $t_{1/2}$ values being 18.1 to 64.1 d and 2.5 to 23.2 d (Table 3). This is consistent with previous studies reporting a faster dissipation of this herbicide in flooded than in non-flooded soil conditions (e.g., Van Scoy and Tjeerdema[53]). Similarly, Tomco et al.[27] found shorter persistence of clomazone in rice-field soils under anaerobic than aerobic conditions. They attributed this to faster biotransformation of the compound to the open-ring form as a consequence of the lower soil redox potential under anoxic conditions. However, our results contrast with those finding longer persistence of BS[37,54] and bentazon[55] under anaerobic than aerobic conditions, indicating that the dissipation of herbicides in soils may also be strongly conditioned by these compounds' particular physical and chemical characteristics. With regard to the determinations in the direct year, clomazone persistence significantly decreased after W addition in both aerobic and anaerobic conditions, although for FT under anaerobic condition this decrease was not significant ($p > 0.05$). In particular, the $t_{1/2}$ values were smaller by factors of 1.7, 1.1, and 1.8 in SNTW, STW, and FTW, respectively, than in the corresponding unamended soils (SNT, ST, and FT) under aerobic incubation conditions, and by factors of 1.5 and 2.0 in SNTW and STW, respectively, than in their corresponding unamended soils under anaerobic incubation conditions (Table 3). These results could be attributable to the differences in their DA values (Table 3). In particular, DA_T values were much higher in the amended than in unamended soils in aerobic (up to 3.7 times higher), and anaerobic (up to 4.2 times higher), indicating that clomazone was degraded preferably by biotic processes. This is in concordance with Tomco and Tjeerdema[56] and Van Scoy and Tjeerdema[53] who also described biological processes as constituting the major pathway of clomazone degradation in soils.

In the residual year, DA_T values were also much higher in the amended than in unamended soils in anaerobic and aerobic incubation conditions (Table 3). However, contrary to the results for the direct year, for the residual year, clomazone persistence was significantly increased in SNT and ST when W was applied under both anaerobic and aerobic incubation conditions, but it was decreased in FT management, although only this decrease was significant under anaerobic incubation conditions (Table 3). Thus, while the $t_{1/2}$ values were greater by a factor of 1.2 in SNTW and STW than in the corresponding unamended soils (SNT and ST) under aerobic incubation conditions, and by factors of 1.4 and 1.1 in SNTW and STW, respectively, these values were lower by a factor of 3.2 in TFW than in the corresponding unamended soils under anaerobic incubation conditions (Table 3). Our findings could be explained by the unamended and amended SNT and ST soil microbial communities naturally not having any adaptation to anoxic conditions after years of non-flooding management implementation (Table 3). This finding is concordant with those of Tomco et al.[27] who report the importance of anaerobic bacteria degrading clomazone.

Our results are contrary in part to what we found in a previous work for BS[37] in rice soils after w addition in which BS persistence increased in the direct and residual years under aerobic conditions. However, under anaerobic conditions, while BS persistence decreased in the direct year, it increased in the residual year except for FT treatment.

3.2. Leaching studies

The cumulative clomazone breakthrough curves are shown in Figure 2. Table 4 presents the total clomazone leached and the percentage that was extracted from the soil columns at the end of the leaching study. For the unamended soils, the total clomazone leached ranged from 18.8 % to 29.9% of the initial amount applied (Table 4; Fig. 2). However, greater leaching losses of clomazone (59%) were reported by Gámiz et al.[50], although in an unamended soil whit a sorption valued on average 2.1 times lower tan those observed in our study. The total amount of clomazone leached was significantly influenced by the treatments, with their effects being different in the two years, as indicated by the significance ($p<0.05$) of the treatment × year interaction (Table 4). For the unamended soils, although no significant differences were found among treatments in the first year, FT leached 1.4 and 1.3 times more clomazone than SNT and ST, respectively. Three years after the implementation of the trials, the amount of leached clomazone was significantly lower in SNT (23.4%) than in ST (29.9%) and FT (29.7%), suggesting that the leaching of clomazone could be enhanced in soils with conventional tillage regardless of the water irrigation system implemented. The lower amount of clomazone in the leachates corresponding to the SNT treatment may be consistent with its higher TOC (Table 1) and H values (i.e., lower reversibility; Tables 2 and 4). However, these results contrast with those found in a previous study[37] in which the amount of BS leached was lower in FT than in SNT and NT. This effect was explained by the greater sorption and lesser persistence of BS in FT than in SNT and NT treatments.

The addition of W significantly reduced the quantity of clomazone that leached in the direct and residual years (Table 4). Thus, the amount of clomazone in the leachates was lessened by factors of 1.8, 2.4, and 2.8 in SNTW, STW, and FTW, respectively, than in the corresponding unamended soils (SNT, ST, and FT) in the direct year, and by factors of 1.3, 1.6 and 2.6 in SNTW, STW, and FTW than in their corresponding unamended soils in the residual year (Table 4). While in the direct year there were no significant differences among amended treatments, the amount of clomazone leached in the residual year was significantly lower in FTW (1.6 times less) than in SNTW and STW (Table 4). These results may be attributable to the greater values of K_d observed in amended compared to unamended soils (Tables 2 and 4). Indeed, the amount of clomazone leached had a significant negative correlation with K_d (r=-0.785, p <0.01). Furthermore, the lesser persistence may explain the greater fall in the quantity of clomazone in the FTW leachates in the residual year, compared to those observed in SNTW and STW (Tables 2, 3 and 4). These results suggest that using W as organic amendment in rice production strongly decreases clomazone leaching in the short-term, regardless of the irrigation and tillage management implemented. However, in the medium-term, this effect was much more evident under flooding irrigation with conventional tillage (Table 4), as was expected according to the above sorption-desorption and dissipation results. Similarly, Gómez et al.[37] also observed that the use of W reduced the amount of BS leached in the same treated soils, although only at the short-term (direct year), indicating that the effects of this organic amendment can be different for different types of pesticides.

At the termination of the trial, the total amount of clomazone recovered from the soil columns was significantly influenced by the treatments, with their effects differing between the two years, as shown by the significance (p<0.05) of the treatment × year interaction (Table 4). For all treatments and both years, clomazone was retained in the all four soil columns sections. In the unamended soils, while in the direct year no significant differences between treatments were observed, in the residual year the amounts of clomazone recovered were significantly lower in FT than in SNT and ST by factors of 1.9 and 2.1, respectively. For the direct year, the addition of W significantly decreased the amount of clomazone recovered in all treatments (Table 4). However, in the residual year the amounts of the herbicide extracted increased in all treatments, although this only was significant in FT. For both years, the results are consistent with those of the dissipation experiment described above, especially under anaerobic incubation conditions (Tables 3 and 4). Indeed, a significant positive correlation of the clomazone recovered was observed with the $t_{1/2}$ values under anaerobic conditions (r=0.705, p <0.01). The greater K_d value (Table 2) may explain the significant increase of clomazone retained in FTW in the residual year compared to that observed in FT (Table 4), despite its lower persistence (Table 3).

4. Conclusions

Different tillage management and irrigation regimes alone or in combination with W application induce major changes in the properties of the soil, e.g., in the quantity and nature of soil organic matter and the DA, and these in turn can modify the behaviour of the herbicide clomazone used in rice production. While in the short-term, the implementation of sprinkler irrigation under tillage and non-tillage management reduced clomazone leaching, in the medium-term this effect was present only under non-tillage management. For both the residual and the direct years, the W field application significantly influenced clomazone's sorption and persistence, reducing its leaching, especially under conventional tillage and flooding management. Therefore, although there may be a greater risk of water being contaminated by clomazone in rice production under conventional tillage regardless of the irrigation system (sprinkler or flooding), using W as organic amendment might greatly reduce it for the short- and medium-terms, especially under flooding irrigation. However, on the basis of our previous studies for BS[37], amending soils with W may have different effects on the environmental fate of pesticides depending of their characteristics. Therefore, future studies are needed evaluating how the behaviour of each pesticide used in rice production is affected by amendment with W under different tillage and water management practices.

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Table 1. Effect of different treatments on the soil's physicochemical properties

	TOC ($g\ kg^{-1}$)	WSOC ($mg\ kg^{-1}$)	HA ($g\ kg^{-1}$)	FA ($g\ kg^{-1}$)	pH (H_2O)	EC ($dS\ m^{-1}$)
2015						
SNT	10.2±0.070aA	210±0.624cA	1.62±0.103bA	1.10±0.009cB	5.77±0.029cA	2.14±0.109aA
SNTW	21.3± 0.097dB	448±21.8dA	2.35±0.049dA	1.29±0.002dA	6.58±0.003eA	3.69±0.101bA
ST	10.7±0.085aA	137±2.60bA	1.34±0.027aA	0.97±0.008bA	5.64±0.012bA	3.74±0.072bA
STW	15.7±0.522bA	497±18.7eB	2.02±0.062cA	1.29±0.054dA	5.99±0.006dA	4.19±0.231cA
FT	10.9±0.186aA	72.8±4.58aA	1.50±0.053abA	0.85±0.012aA	4.93±0.026aA	3.60±0.032bA
FTW	20.3±0.058cB	489±6.23eB	1.60±0.007bA	1.02±0.002bA	6.06±0.052dB	4.46±0.035cB
2017						
SNT	15.1±0.205cB	316±0.443bB	1.44±0.086aA	0.95±0.002aA	6.08±0.017cB	3.96±0.188cB
SNTW	19.1±0.262eA	498±44.9dA	2.24±0.073cA	1.24±0.031dA	6.61±0.020eA	3.44±0.035abA
ST	13.1±0.284aB	336±1.33bcB	1.55±0.054aB	1.48±0.045eB	5.61±0.043aA	4.62±0.038dB
STW	18.2±0.023dB	395±17.5cA	2.40±0.026cB	1.13±0.010cA	6.35±0.075dB	3.73±0.064bcA
FT	13.9±0.034bB	220±2.87aB	1.48±0.020aA	1.03±0.019bB	5.65±0.012aB	3.37±0.170aA
FTW	19.1±0.171eA	325±7.68bA	1.88±0.030bB	1.19±0.020cdB	5.80±0.023bA	3.29±0.012aA
Y	***	**	**	***	***	NS
M	***	***	***	***	***	***
Y x M	***	***	***	***	***	***

TOC: Total organic carbon; WSOC: Water soluble organic carbon; HA: Humic acids; FA; Fulvic acids, EC: Electrical conductivity. The data of TOC, WSOC, HA, FA, pH, and EC are presented as mean values ± standard error. ANOVA factors are Y: year; M: management regime; Y x M: interaction year x management regime; *, **, and *** significant at a levels of 0.05, 0.01, and 0.001, respectively; NS: not significant. Different letters indicate significant differences ($p < 0.05$) between management regimes in the same year (lower case letters) and between years within the same management regime (upper case letters).

Table 2. Effect of different treatments on Clomazone sorption–desorption parameters

	n_f	R^2sorp	K_d (L kg^{-1})	H[a]	R^2des
2015					
SNT	0.893bB	0.999	1.85bB	301cA	0.968
SNTW	0.837aA	0.999	3.05eB	221aA	0.970
ST	0.903bA	0.999	1.54aB	318cA	0.983
STW	0.879bB	0.998	2.27cB	261bA	0.996
FT	0.953cB	0.998	1.81bA	247bA	0.998
FTW	0.906bB	0.999	2.82dA	218aA	0.996
2017					
SNT	0.775aA	0.994	1.24aA	420cB	0.999
SNTW	0.806aA	0.988	2.06dA	290aB	0.999
ST	0.797aA	0.956	1.42bA	383bcB	0.999
STW	0.778aA	0.995	2.06dA	318abA	0.975
FT	0.794aA	0.994	1.69cA	341abA	0.988
FTW	0.852aA	0.994	2.95eA	263aA	0.998
Y	***	-	***	***	-
M	*	-	***	***	-
Y x M	NS	-	***	NS	-

[a] Hysteresis values. The data of n_f, K_d, and H are presented as mean values. ANOVA factors are Y: year; M: management regime; Y x M: interaction year x management regime; *, **, and *** significant at a levels of 0.05, 0.01, and 0.001, respectively; NS: not significant. Different letters indicate significant differences ($p < 0.05$) between management regimes in the same year (lower case letters) and between years within the same management regime (upper case letters).

Table 3. Effect of different treatments on dehydrogenase activity and clomazone dissipation parameters

	$t_{1/2\ 80\%}$ (days)	$R^2_{\ 80\%}$	$t_{1/2\ 1:1.25}$ (days)	$R^2_{\ 1:1.25}$	$DA_{B80\%}$	$DA_{A80\%}$	$DA_{T80\%}$	$DA_{B1:1.25}$	$DA_{A1:1.25}$	$DA_{T1:1.25}$
					(μg INTF g^{-1} h^{-1})					
2015										
SNT	36.3cA	0.976	5.40bA	0.934	0.344aA	0.535aA	4.39aA	2.42aA	4.81bB	23.7bB
SNTW	21.1abA	0.953	3.56aA	0.957	0.718bA	1.79cB	13.8cB	5.85bA	7.60dB	52.0dB
ST	33.5cA	0.949	10.8cA	0.858	0.337aA	0.585aB	5.36aB	1.62aA	3.43aB	13.5aB
STW	29.7bA	0.976	5.44bA	0.956	0.650bA	1.06bA	9.22bA	4.83bA	6.06cA	29.6cB
FT	33.1cA	0.904	3.43aA	0.980	0.269aA	0.573aA	4.53aA	2.30aA	2.54aA	11.8aA
FTW	18.1aA	0.917	3.06aA	0.986	0.710bA	2.05cB	17.1dB	4.38bA	7.33dA	50.2dA
2017										
SNT	54.6abB	0.888	14.7cB	0.961	0.793bcB	0.901cA	6.41bB	4.53bcB	2.04aA	10.5bA
SNTW	64.1cB	0.873	21.3dB	0.857	1.04eB	0.999cA	9.31dA	5.43cA	4.59bA	19.4cA
ST	52.7aB	0.959	20.5dB	0.868	0.472aB	0.444aA	4.33aA	1.34aA	1.18aA	6.04aA
STW	63.4cB	0.888	23.2eB	0.879	0.838cB	0.792abA	8.61dA	3.79bA	5.38bA	20.9cA
FT	62.6bcB	0.885	8.23bB	0.824	0.762bB	0.984cA	7.58cB	8.19dB	8.50cB	36.8dB
FTW	60.3bcB	0.895	2.53aA	0.984	0.962dB	0.761abA	7.51cA	9.76eB	7.66cA	43.2eA
Y	***	-	***	-	***	**	***	***	*	***
M	*	-	***	-	***	***	***	***	***	***
Y x M	**	-	***	-	**	***	***	***	***	***

Half-lives: $t_{1/2\ 80\%}$ in soils at 80% field water capacity; $t_{1/2\ 1:1.25}$ in soils with 1:1.25 (w/v) (soil/water) moisture content. DA: dehydrogenase activity two hours before (B) and after (A) the application of the herbicide to soils conditioned to 80% field capacity and 1:1.25 (w/v) (soil/water) moisture content. DA_T: total dehydrogenase activity considering all the incubation times in soils conditioned to 80% field capacity and 1:1.25 (w/v) (soil/water) moisture content. The data of $t_{1/2\ 80\%}$, $t_{1/2\ 1:1.25}$, $DA_{B80\%}$, $DA_{A80\%}$, $DA_{T80\%}$, $DA_{B1:1.25}$, $DA_{A1:1.25}$, $DA_{T1:1.25}$ are presented as mean values. ANOVA factors are Y: year; M: management regime; Y x M: interaction year x management regime; *, **, and *** significant at a levels of 0.05, 0.01, and 0.001, respectively; NS: not significant. Different letters indicate significant differences ($p < 0.05$) between management regimes in the same year (lower case letters) and between years within the same management regime (upper case letters).

Table 4. Effect of different treatments on clomazone leaching parameters

	Initial pore volume	Max. Concentration leached (μM)	Total leached (%)	Total extracted (%)
2015				
SNT	1.40bA	0.856cA	18.8bA	18.3bA
SNTW	2.33dA	0.444abA	10.4aA	13.8aA
ST	1.68cB	0.740bcA	19.3bA	13.6bA
STW	1.88cA	0.256aA	7.96aA	5.52aA
FT	0.666aA	1.47dB	25.6bA	18.7bA
FTW	2.34dB	0.662bcA	8.92aA	10.3aA
2017				
SNT	1.98cB	0.656abA	23.4cA	26.8bB
SNTW	2.61dB	0.410aA	18.0bB	32.4bB
ST	1.36bA	1.07cB	29.9dB	30.2bB
STW	2.47dB	0.535aB	18.5bB	33.4bB
FT	0.659aA	0.890bcA	29.7dA	14.4aA
FTW	0.611aA	0.515aA	11.4aA	21.2bB
Y	***	*	***	**
M	***	***	***	*
Y x M	***	*	*	*

The data of initial pore volume, Max. Concentration leached, Total leached, and Total extracted are presented as mean values. ANOVA factors are Y: year; M: management regime; Y x M: interaction year x management regime; *, **, and *** significant at a levels of 0.05, 0.01, and 0.001, respectively; NS: not significant. Different letters indicate significant differences ($p < 0.05$) between management regimes in the same year (lower case letters) and between years within the same management regime (upper case letters).

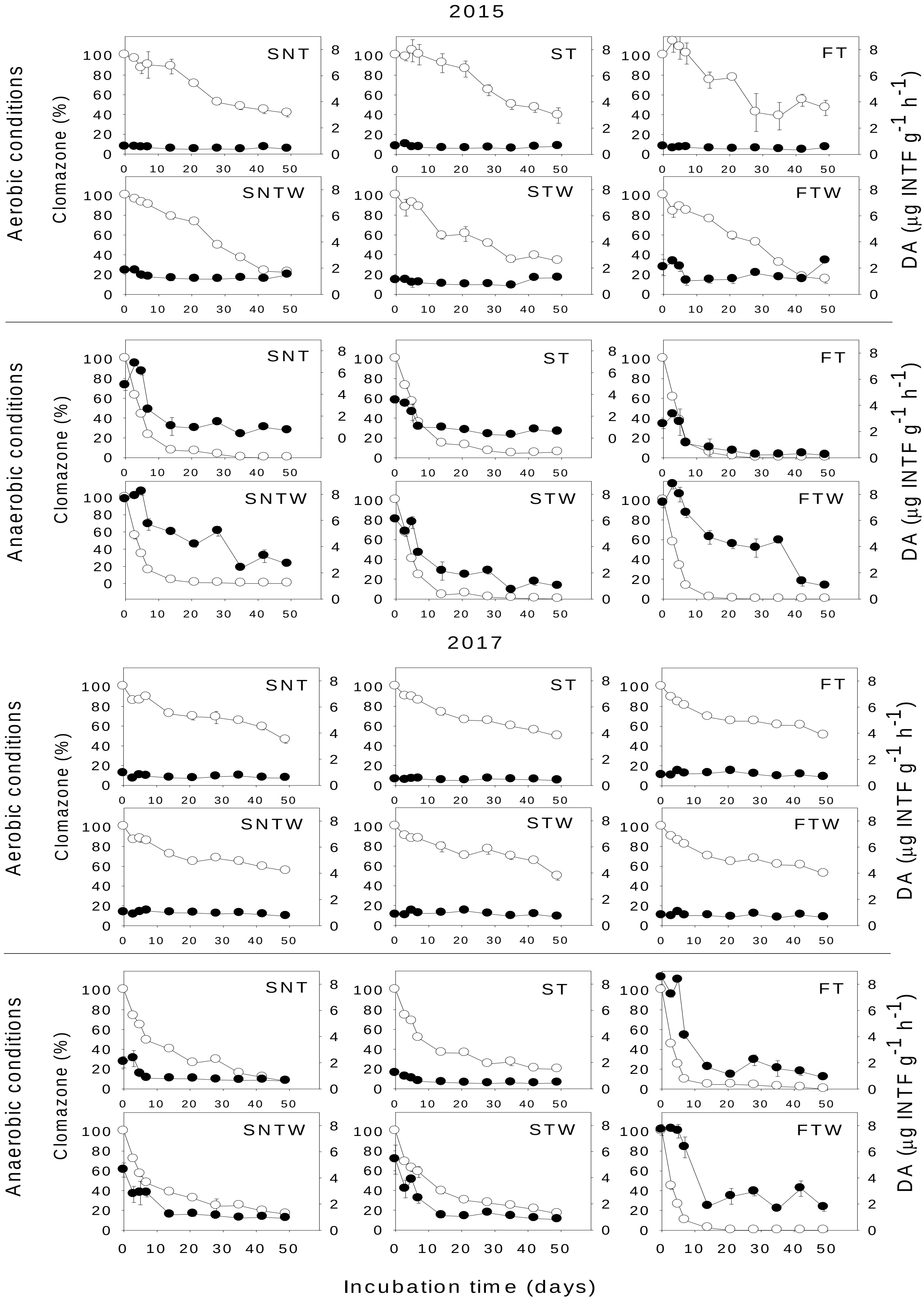

Figure 1. Effects of treatments on the clomazone dissipation (○) and DA (dehydrogenase activity, ●) under aerobic and anaerobic incubation conditions. Vertical bars representing one standard error of the mean were smaller than the symbols in some cases. Treatments are: No-tillage and sprinkler irrigation without (SNT) or with compost application (SNTW); conventional tillage and sprinkler irrigation without (ST) or with compost application (STW), continuos flooding irrigation and tillage without (FT) or with compost application (FTW).

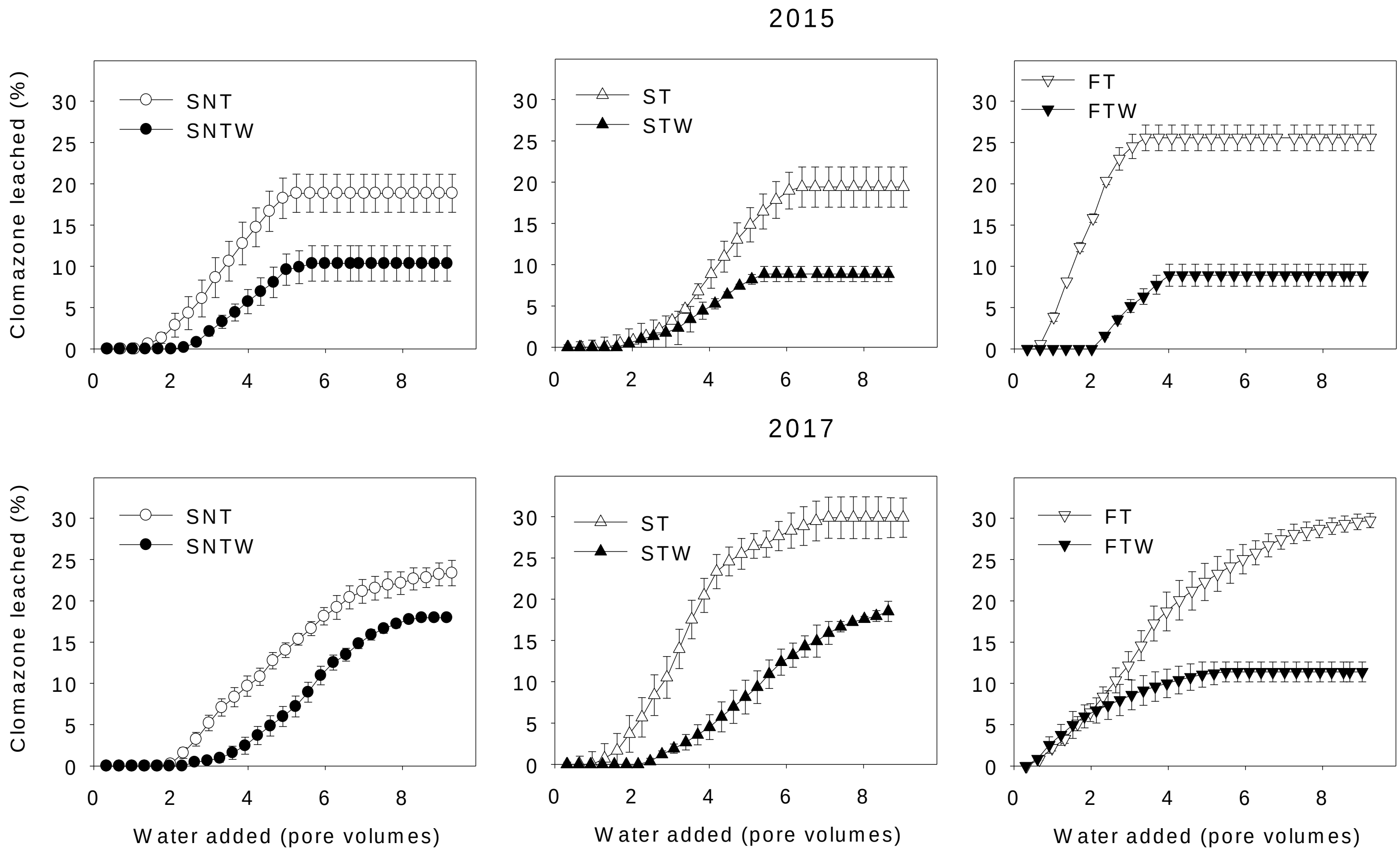

Figure 2. Effects of treatments on the cumulative breakthrough curves of clomazone. Vertical bars represent one standard error of the mean. Treatments are: No-tillage and sprinkler irrigation without (SNT) or with compost application (SNTW); conventional tillage and sprinkler irrigation without (ST) or with compost application (STW), continuous flooding irrigation and tillage without (FT) or with compost application (FTW).

Supplementary Material: "How the environmental fate of clomazone in rice fields is influenced by amendment with olive-mill waste under different regimes of irrigation and tillage"

Text S1. The herbicide was assayed by HPLC, using a chromatograph (Waters 600E) coupled to a diode-array detector (Waters 996). The conditions used were: Nova-Pack column (150 × 3.9 mm, 4.5 μm particle size), acetonitrile:water (70:30) mobile phase at a flow rate of 1 mL min^{-1}, 25 μL injection volume, and UV detection at 214 nm. The limits of detection and quantification, calculated as the herbicide concentrations resulting in signal-to-noise ratios of 3:1 and 10:1 respectively, were 0.015 μM and 0.047 μM, respectively.

Text S2. Total organic carbon content (TOC) was determined by dichromate oxidation. Water-soluble organic carbon (WSOC) was extracted with de-ionized water at a 3:1 (water to soil) ratio. Humic and fulvic acids (HA and FA, respectively) were extracted by a solution of 0.1 M $Na_4P_2O_7$ + NaOH using a ratio of extractant to sample of 10:1, and to precipitate humic acid the supernatant was acidified to pH 2 with H_2SO_4. The WSOC and the total organic carbon associated with each fraction of HA and FA were determined by dichromate oxidation and absorbance at 590 nm to detect Cr^{3+} formation. The pH was measured in a 1:1 (w/v) soil/water using a combination electrode. Electrical conductivity (EC) was measured in a saturation extract.

Text S3. Soil samples (5 g) were treated by mechanical shaking at 20±1 °C for 24 h with 10 mL of solutions of clomazone in 0.01M $CaCl_2$ at initial concentrations (C_i) of 5, 10, 20, 40, and 50μM. Equilibrium concentrations in the supernatants were determined by high-performance liquid chromatography (HPLC). The amount of clomazone sorbed (C_s) was calculated from the difference between the initial (C_i) and the equilibrium (C_e) solution concentrations. The values of K_d were calculated from the fit of the experimental sorption isotherms ($K_d = C_s/C_e$) at a selected C_e (20 μM). The measurements with control samples containing only clomazone but no soil showed that there were no losses of the herbicide due to microbial activity, volatilization, or sorption onto the surface of the tubes. Hysteresis coefficients (H) were calculated: $H = (n_{fa}/n_{fd})\times 100$, where n_{fa} and n_{fd} are the Freundlich n_f constants obtained from the sorption and desorption isotherms, respectively.

Text S4. For each treatment, triplicate soil samples (5 g) were weighed into 50 mL glass tubes. Soils were supplemented with distilled water to obtain non-flooded (80% field capacity) and flooded (soil-to-water ratio 1:1.25, w/v) moisture conditions. Prior to the clomazone addition, the soils were pre-incubated for 7 days in the dark at 20±1 °C to allow the soil microorganisms to adapt to the non-flooded (aerobic) and flooded (anaerobic) incubation conditions, and also to allow the development of reducing conditions in the flooded soils. Then clomazone dissolved in distilled water was applied at a rate equivalent to 1 kg ha^{-1}, and the tubes were incubated in the dark at 20±1 °C for 49 days. Moisture was maintained at a constant level throughout the experiment by adding distilled water as necessary. Three replicate tubes were removed (at 2 h and at 2 days after herbicide application, and then at 7-day intervals for 49 days) from each treatment to measure the herbicide concentrations. The soils (5 g) were extracted with methanol (10 mL) by shaking mechanically on an end-over-end shaker at 20±1°C for 24 h followed by centrifugation, and the residues of the herbicide in the extracts were determined by HPLC. Recoveries were greater than 95% of the herbicide applied to the soil. Clomazone residues from water samples were also determined by HPLC. The clomazone dissipation data in soils and water were fitted to a first-order kinetics equation, $C = Co\ e^{-kt}$, where C is the clomazone concentration at time t (days), Co is the initial herbicide concentration, and k (day^{-1}) is the degradation constant, and the half-lives ($t_{1/2}$) were calculated.

To measure the dehydrogenase activity (DA), another three replicate soil samples of each treatment were weighed out into glass tubes, and supplemented with sterile distilled water to obtain aerobic (80% field capacity) and anaerobic (1:1.25 w/v soil/water) moisture conditions. From each treatment, the tubes were removed before clomazone application and at the same times as for the dissipation experiment. The tubes were incubated for 20 h at 20±1 °C in the dark with 1 mL of 0.4% 2-p-iodophenyl-3p-nitrophenyl-5 tetrazolium chloride (INT) as substrate. At the end of the incubation, the iodonitrotetrazolium formazan (INTF) produced was extracted with methanol, and the absorbance was measured at 490 nm.

Text S5. For the leaching studies, PVC was used to prepare disturbed soil columns (5-cm i.d. × 30-cm length). To minimize losses of soil during the experiment, the top 5 cm of the columns was filled with sea sand and the bottom 5 cm with sea sand plus glass wool. The remaining 20 cm was hand-packed with air-dried soil. The soil columns were saturated with 0.01 M $CaCl_2$ and allowed to drain for 24 h. Then, clomazone was applied to the top of the soil columns at a rate equivalent to 1 kg ha^{-1}. Leachates containing the clomazone were collected daily, filtered, and assayed by HPLC. After the leaching experiments, the soil columns were left to drain for 24 h and then sectioned into the follow depths: 0-5 cm, 5-10 cm, 10-15 cm, and 15-20 cm. In order to determine the residual amounts of clomazone, the soils (5 g) from different depths were extracted with methanol (10 mL) by shaking for 24 h at 20±1°C, and assayed by HPLC.

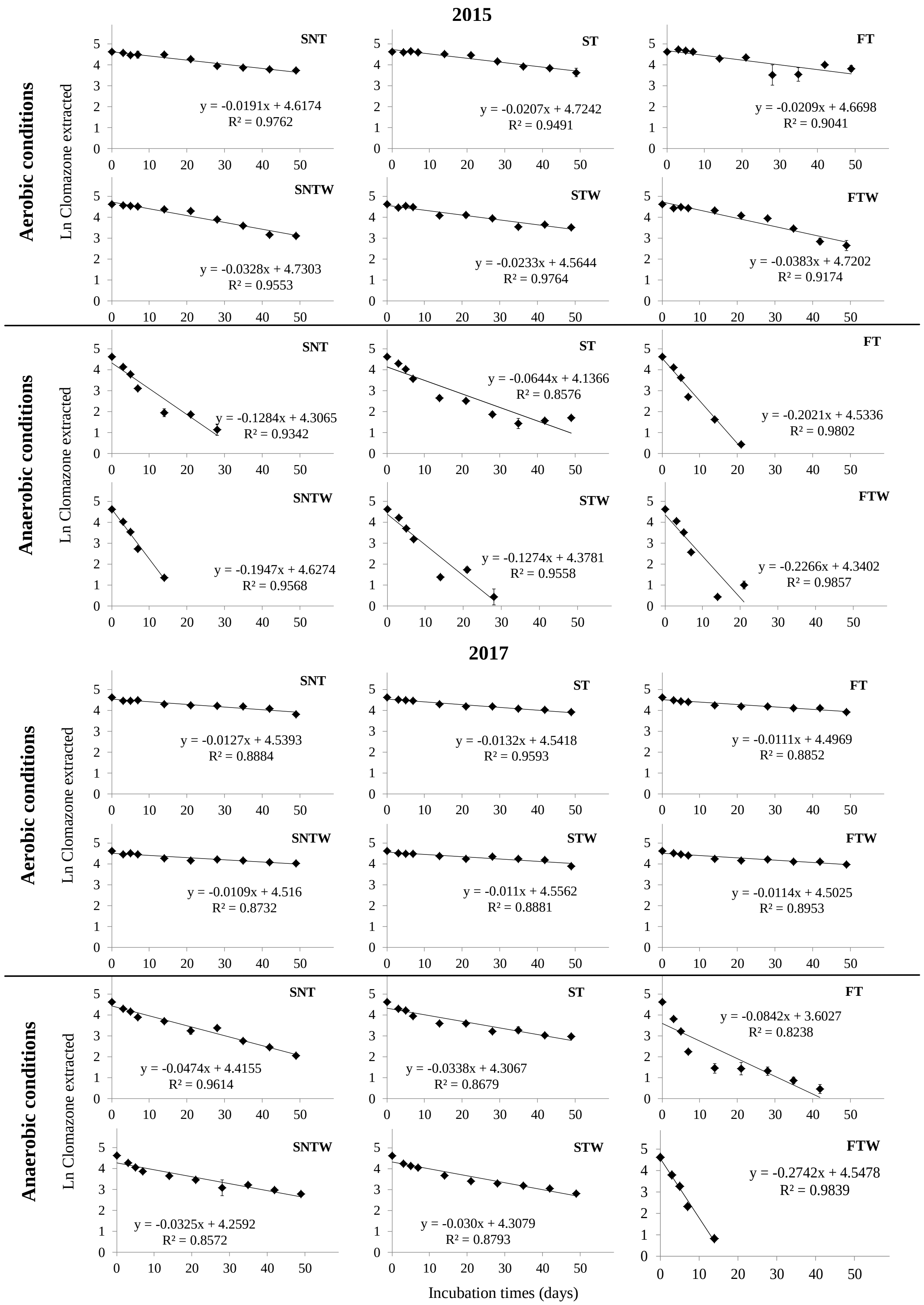

Figure S1. The symbols indicate the experimental data points of clomazone dissipation studies whereas the lines correspond to the fits to first-order dissipation kinetics for those experimental data. Error bars denote standard errors of triplicate measurements. Treatments are: No-tillage and sprinkler irrigation without (SNT) or with compost application (SNTW); conventional tillage and sprinkler irrigation without (ST) or with compost application (STW), continuous flooding irrigation and tillage without (FT) or with compost application (FTW).

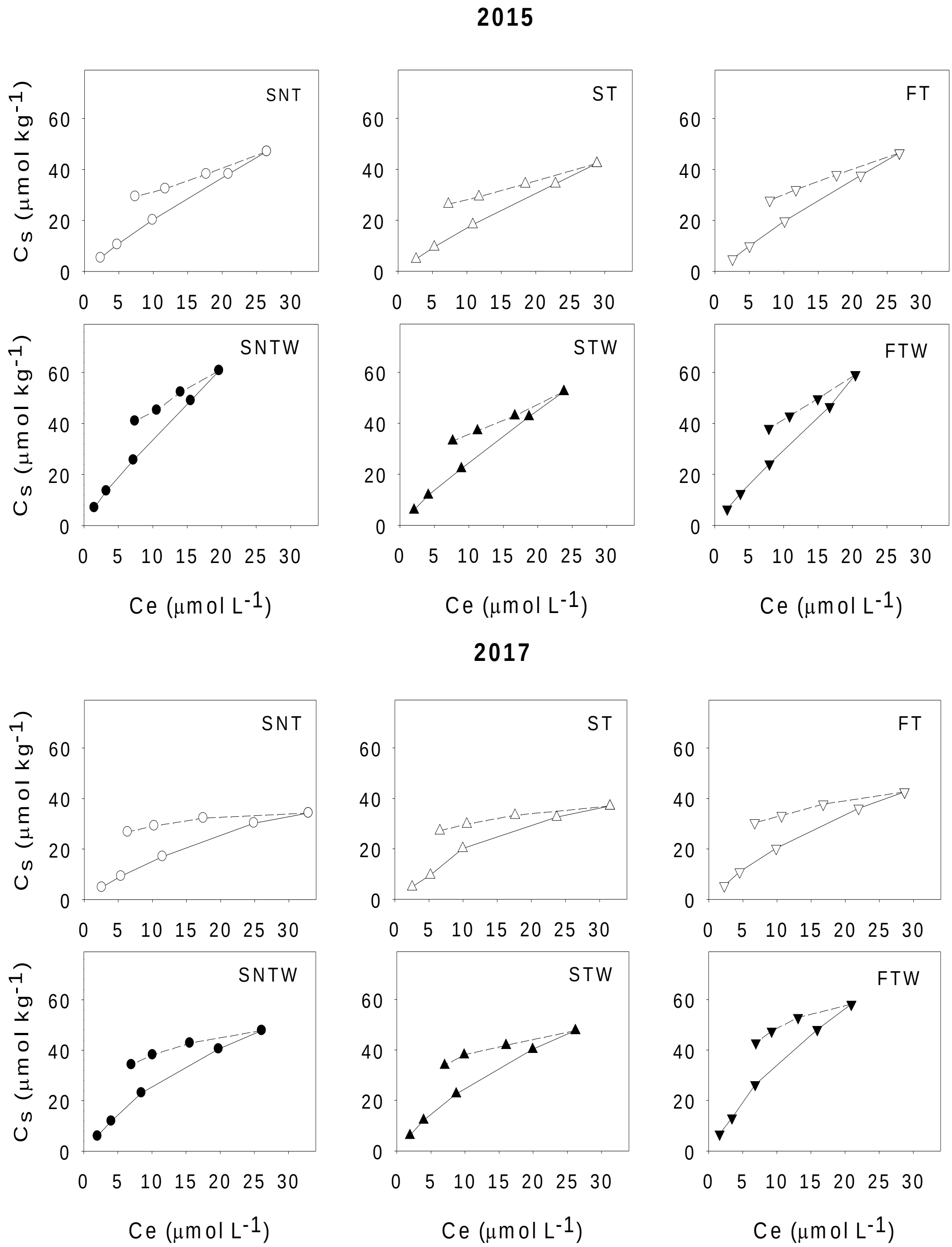

Figure S2. Effects of treatments on the clomazone sorption and desorption isotherms. Vertical bars representing one standard error of the mean were smaller than the symbols in all cases. Ce: equilibrium clomazone concentration; Cs: amount of clomazone sorbed. Treatments are: No-tillage and sprinkler irrigation without (SNT) or with compost application (SNTW); conventional tillage and sprinkler irrigation without (ST) or with compost application (STW), continuous flooding irrigation and tillage without (FT) or with compost application (FTW).