Behaviour of bentazon as influenced by water and tillage management in rice growing conditions

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Abstract

BACKGROUND: Bentazon is a widely used herbicide in rice agroecosystems that has commonly been found in water resources. To assess how tillage and water regimes impact the sorption-desorption, dissipation, and leaching of bentazon in Mediterranean rice growing conditions, field experiments were carried out using tillage and flooding (TF), tillage and sprinkler irrigation (TS), no-tillage and sprinkler irrigation (NTS), and long-term no-tillage and sprinkler irrigation (NTS7).

RESULTS: After three years, the K_d values in TS were 2.3, 1.6, and 1.7 times lower than the values in NTS7, NTS, and TF, respectively. Greater sorption of bentazon was related to higher contents in total organic carbon (TOC) and, although to a lesser extent, in humic acids (HA) and dissolved organic carbon (DOC). The persistence of bentazon was significantly greater under anaerobic (half-life DT_{50} = 94.1–135 d) than aerobic (DT_{50} = 42.4–91.3 d) incubation conditions for all management regimes. Leaching losses of bentazon were reduced from 78% and 74% in TS and TF to 61% and 62% in NTS7 and NTS, respectively.

CONCLUSIONS: The mid- and long-term implementation of sprinkler irrigation in combination with no-tillage could be considered a management system that is effective at reducing water contamination by bentazon in Mediterranean rice-growing agroecosystems.

Keywords: Aerobic and anaerobic rice; Bentazon; Leaching; Persistence; Sorption; Tillage management.

1. Introduction

Traditionally, rice (*Oryza sativa* L.) is cultivated under agricultural practices that are intensive and costly, including tillage, flood irrigation, and high-intensity pesticide application. These practices negatively affect soil properties, consume large quantities of water, and can enhance the risk of surface and ground water contamination given that the fields are usually close to surface-water bodies.[1,2] Rice is widely cultivated in some areas in the south of Europe, especially in Mediterranean countries.[3] As is becoming especially evident in the Mediterranean region,[4] the sustainability of traditional rice production under flood irrigation is threatened by the growing lack of water resources due to the competition for them between humans and the environment. [5,6] Consequently, alternative management regimes need to be developed with which water can be used more efficiently to help cope with these problems.[7,8]

In this regard, aerobic (non-flooding) rice production is being proposed as an efficient management practice that can save water, hence making rice a more sustainable crop.[9] Also, techniques of conservation agriculture such as no-tillage with direct seeding are changing the traditional cultivation of this crop, since they are practices that conserve soil and water compared to tillage treatment, reduce the costs of production, and may be more environmentally sustainable.[10-12] Thus, aerobic rice growing using no-tillage and direct seeding has expanded greatly worldwide in the last few decades, including recently in areas with Mediterranean conditions, without there being any marked reduction in yield.[13-15] However, for this attractive alternative to be technically viable, greater amounts of herbicides may be required,[16,17] which could have negative consequences for the quality of surface and ground water.

In Europe, farmlands under rice cultivation in France, Spain, Italy, Portugal, and Greece are frequently near areas of high ecological interest or that are close to towns and villages.[18,19] Pesticide concentrations exceeding 0.1 μg L^{-1} in surface and ground water have been reported in several studies in rice-growing areas of Spain, Italy, Portugal, and Greece.[20,21] Indeed, the frequent detection of herbicides in surface and ground water systems neighbouring rice-growing basins is raising concerns in Europe about the potential impact on both the environment and human health, and pointing to the necessity to implement preventive measures to limit the risk of water contamination.[22]

While the implementation of aerobic rice growing using practices of conservation agriculture may enhance the sustainability of the crop in regions where the limiting factor is water, the techniques involved have a marked effect on soil properties, with which they may consequently also alter the environmental fate of pesticides applied to the soil,[23-25] affecting such processes as their sorption, leaching, and persistence. This last process is strongly dependent on microbial activity, with microbial degradation being considered the predominant pathway affecting pesticides' fate in soils, so that dehydrogenase activity is a measure of a soil's microbiological activity.[26] The trends found in studies of how modifications in soil properties can affect the behaviour of pesticides have frequently been contradictory, however.[25,27]Among rice pesticides, 3-isopropyl-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide, with the common name bentazon, is a thiadiazine herbicide used intensively across the world because of how effectively it controls broadleaf weeds and sedges.[28] This herbicide exhibits both acute and chronic toxicity, and presents a potential risk for non-target organisms, with the concern for both environmental and human health being very high because it is commonly found in the environment, especially in water resources.[29] Studies conducted in rice-growing areas worldwide have reported that bentazon is one of the most frequently found pesticides, with its levels in surface and ground water being high due to its extremely low sorption by soils and relatively high mobility. In the case of Mediterranean countries, concentrations of bentazon above the European threshold for drinking water (0.1 $\mu g\ L^{-1}$) have also frequently been detected.[30] Indeed, bentazon concentrations reaching 127 $\mu g\ L^{-1}$ have been found in one of the main rice-growing areas of Spain.[21] Similarly, studies monitoring selected locations near the main rice cropping areas of Italy[31] and France[32] revealed high levels of this herbicide in water, with peaks of 0.6 and 1.6 $\mu g\ L^{-1}$, respectively.

Although bentazon is used extensively and is an important cause of water pollution in

Mediterranean rice-growing ecosystems, we were unable to find any studies of how rice-farming practices impact its behaviour. Such information would be invaluable in planning its management and appropriate environmental protection. Therefore the goal of the present study was to assess in a field experiment the impacts of the water and tillage management in rice-growing on the sorption-desorption, dissipation, and leaching of the herbicide bentazon applied to a typical Mediterranean rice soil. Because the persistence of pesticides depends directly on microbial degradation, we also monitored soil dehydrogenase activity to assess how the management regimes affect the soil's microbial activity.

2. Materials and Methods

2.1. Chemicals and assays

Bentazon (98.0% purity) was supplied by Dr Ehrenstorfer, GmbH. It is a weak acid with a pKa of 3.28 at 24°C and water solubility at 20°C of 570 mg L^{-1}.[33] Bentazon concentration was determined by high-performance liquid chromatography (HPLC), using a device which was equipped with a UV diode-array detector and Nova-Pack column (150 × 3.9 mm, 4.5 µm particle size). The injection volume was 25 µL, flow rate 1 mL min^{-1}, and column temperature 35°C. The mobile phase was 40% water with 0.25% phosphoric acid and 60% acetonitrile (pH=2). The wavelength used for detection was 213 nm. The bentazon concentrations were quantified against a linear calibration relationship between chromatographic peak area and bentazon standards (0.05–50 µM).

2.2. Rice cultivation, and sampling and analysis of soils

The field experiment location and site description are given in López-Piñeiro *et al.*[34] Four management regimes were considered: (TF) tillage to 25 cm and flooding; (TS) tillage and sprinkler irrigation; (NTS) no-tillage and seeding by direct drilling with sprinkler irrigation; and (NTS7) no-tillage and seeding by direct drilling with sprinkler irrigation in fields where this regime had been in use for 7 years so as to be able to detect possible long-term effects. Soil samples were collected from the surface 0-20 cm after the harvest of the rice in November of the years 2011, 2012, and 2013 (for the sorption-desorption study), 2011 and 2013 (for the leaching study), and 2013 (for the dissipation study). We measured in the sieved (<2 mm) soils the total organic carbon (TOC), dissolved organic carbon (DOC), humic and fulvic acids (HA and FA, respectively), humic index (HI), electrical conductivity (EC), and pH as described in the study by Sánchez-Llerena *et al.*[14]

2.3. Sorption-desorption experiments

All the bentazon sorption-desorption experiments were done in triplicate. For the sorption, we used a batch equilibration technique[35] as detailed in Cañero *et al.*[36] The initial concentrations of the bentazon solutions used were from 10 to 100 μM in 0.01 M $CaCl_2$. After the sorption experiment, we studied the desorption using the tubes to which the highest initial concentration of bentazon (100 μM) had been added, replacing the 5 mL of the supernatant which had been taken for the HPLC assay of the remaining bentazon with 5 mL of 0.01 M $CaCl_2$ as extractant, and then following the same operating procedure as in the sorption experiment. This step was repeated twice more for a total of three desorption measurements for each aliquot. The sorption-desorption results were fitted to the Freundlich equation, $C_s = K_f C_e^{1/nf}$, where C_s is the concentration (μM kg^{-1}) of bentazon sorbed to the solid phase at equilibrium, C_e is the bentazon concentration (μM L^{-1}) in the aqueous phase at equilibrium, and K_f and n_f are respectively the Freundlich coefficient and linearity parameter. Partition coefficients, K_d (L kg^{-1}), where $K_d = C_s/C_e$, and the percentage of bentazon desorbed with respect to that previously sorbed, D, were also calculated. The Freundlich equation was preferred to other sorption models because it is appropriate for heterogeneous surfaces, such as soils, and gave good descriptions of experimental results. [37] Additional details for sorption-desorption experiments are given in Text S1 of the Supplementary Material.

2.4. Dissipation of bentazon

A full description of the method followed for the dissipation study can be found in López-Piñeiro *et al.*[34] Aliquots (5 g) of soil from each treatment were weighed into glass tubes, and supplemented with sterile distilled water to obtain non-flooded (80% field capacity) and flooded (1:1.25 w/v soil/water) moisture conditions. Bentazon was applied to correspond to a field dose rate of 2 kg ha^{-1}. At periodic intervals until 49 days from preparation, soil samples in triplicate were removed for analysis. Herbicide was extracted from these samples by adding 10 mL of a 40:60 (v/v) mixture of acetonitrile/distilled water containing 0.25% phosphoric acid. After centrifuging, the supernatants were analysed by HPLC. Bentazon dissipation curves were fitted to a first-order kinetics equation, and the half-life DT_{50} (time required for 50% of the initial dose of pesticide to be dissipated) was calculated in each treatment. Additional details for dissipation study are given in Text S2 of the Supplementary Material.

2.5. Dehydrogenase activity

Dehydrogenase activity (DHA) was determined in another three replicate tubes (from the dissipation studies) from each treatment following the method described in García *et al.*[38] (for additional details see Supplementary Material, Text S3).

2.6. Leaching studies

We studied leaching as described by Peña *et al.*[39] using PVC columns of 30-cm length × 5-cm internal diameter. Each determination was done in triplicate. Bentazon corresponding to an application rate of 2 kg ha^{-1} dissolved in water was applied dropwise to the top of the columns so as to cover the entire column surface. Each day we leached the columns with 50 mL of 0.01M $CaCl_2$ until herbicide was no longer detected in the leachates (18-20 d). Bentazon in the leachates was assayed by HPLC. The columns of soil were sectioned into 5-cm deep portions after the leaching experiment to determine the residual amount of bentazon at different depths, following the same extraction procedure as described in Sec. 2.4. Additional details for leaching studies are given in Text S4 of the Supplementary Material.

2.7. Statistical analyses

We carried out statistical analyses using the IBM SPSS Statistics 22.0 program package. We subjected the data to a two-way ANOVA with repeated measures on the factor "year". The Duncan test was applied for multiple comparisons, and Pearson's correlation coefficient was employed to find possible correlations between the various parameters. Variations at a $p<0.05$ level of probability between the results were considered statistically significant.

3. Results and Discussion

3.1. Soil properties

Table S1 of the supporting information lists the values of the soils' physicochemical parameters at a depth of 0-20 cm for the years 2011, 2012, and 2013. These data have been extensively discussed in a previous work[14] (briefly discussed in Text S5 of the Supplementary Material).

3.2. Sorption-desorption studies

In all cases, the regression coefficients (R^2) were 0.915 or greater (Table 1), which is indicative of the acceptability of the assumption of the Freundlich equation. The K_d values went from 0.073 to 0.272 (Table 1 and Fig. S1 of the Supplementary Material), and the TOC content from 7.03 to 16.2 g kg^{-1} (Table S1). Although these values indicate very weak sorption, they are still far greater than the value found by Cañero *et al.*[36] of 0.03 for a soil with 14.6 g kg^{-1} TOC content, but with a pH of 7.95. In general, the K_d values of the present study are of the same order as those of the data reported by Li *et al.*[40] with K_d values ranging from 0.140 to 0.321 for a soil with 3.71–24.9 g kg^{-1} TOC content and pH of 4.15–7.60. Bergstrom and Jarvis[41] reported a K_d value of 2.9, but for a soil with much higher TOC content (34.2 g kg^{-1}) and pH of 6.2. The K_d values were significantly influenced by the management regimes (Table 1). The significant management × year interaction (p<0.01; Table 1) showed that effects of the management regimes on K_d and n_f were different in the three years. NTS7 had the highest K_d values for all three years (Table 1), while the differences between the other three treatments were smaller as well as being without significance in 2011 and 2012. Under all management regimes, the sorption K_d reduced over the 3 years. The reduction was greatest in the tilled/sprinkler (TS) soil, which indicates that it is this combination of factors that leads to the greatest reduction in sorption.This contrasts with the results given by Gaston *et al.*[42] who did not find any differences in this sense between tillage and no-tillage.

The sorption findings indicated an increasing affinity for bentazon both with increasing quality (HA) and especially quantity of the soil's organic matter content (Table S1 and Table 1). Indeed, K_d was strongly correlated with TOC (r = 0.740**) and, to a lesser extent, with dissolved organic carbon (DOC) ($r = 0.693^{**}$) and HA ($r = 0.506^{*}$). We found no significant correlation between K_d and pH, however, which is not in agreement with Boivin *et al.*[43], although the soils in their study had a wider range of pH values than those of the present study. The present results are consistent with those of Li *et al.*[40] and Cañero *et al.*[36] who reported that bentazon sorption was a function of TOC and of DOC, respectively. Other studies have also reported that sorption coefficients for bentazon[27] and other pesticides[44,45] are greater in no-tillage soils than in conventional tillage soils due to the former's greater organic matter content.

The data of desorption were described adequately by the Freundlich model, with R^2>0.915 (Table 1). In all treatments and years, $n_{f\ sorption}$ was greater than $n_{f\ desorption}$ (Table 1), a finding which is indicative of hysteresis phenomena.[46] In all three years, the percentage desorption (D) was significantly influenced by the management regimes (Table 1), with the greatest values being for TF in all three years (Table 1), while there were no significant differences between the other three treatments. There were increases in D in 2013 relative to 2011 for all the treatments (Table 1). These increases were by factors of 1.3, 1.1, 1.4, and 1.4 for NTS7, NTS, TS, and TF, respectively, indicating that sorbed bentazon may be more weakly retained in tillage rice soils, regardless of what kind of irrigation is used. This could be attributable to the significant declines in TOC content observed in the TS and TF treatments (by 23.9% and 13.4%, respectively, Table S1) for 2013 compared to 2011, corroborating the positive influence of the organic matter of the soil on the bentazon sorption-desorption process.

3.3. Dissipation studies

Figure 1 shows the bentazon dissipation and the dehydrogenase activity (DHA) for each treatment. All the treatments' experimental data fit first-order kinetics, with R^2>0.953 and R^2>0.806 for aerobic and anaerobic conditions, respectively (Table 2). In aerobic conditions, the DT_{50} values were between 42 d for NTS7 and 91 d for NTS. These DT_{50} values lie within the range of 8–133 d reported by Ghafoor *et al.*[47] for sixteen different soils under aerobic conditions. However, they are much greater than those reported by Cañero *et al.*[48] of 21 d and 37 d for soils with similar (10.9

g kg^{-1}) or much lower (2.5 g kg^{-1}) TOC contents, respectively, although the moisture content was adjusted to 40% field capacity in that study instead of the 80% field capacity of the present study. They are also much greater than those reported by Larsbo *et al.*[27] which ranged from 3 d to 15 d in soils under conventional and reduced tillage, respectively, but with higher TOC (18–30 g kg^{-1}) and the moisture content adjusted to 60% field capacity.

The DT_{50} values were significantly influenced by the management regime (Table 2). The trend in bentazon dissipation rates was found to be NTS = TS < TF < NTS7. Thus, while by the end of the experiment 55% of the applied herbicide had been dissipated in NTS7, in the rest of the treatments (NTS, TS, and TF) dissipation was less than 33% (Fig. 1), reflecting the long-term effects of the no-till management system.There is conflicting evidence in the literature on the effect of tillage on degradation rates. Some studies show that tillage can suppress degradation rates[27] and some show that tillage enhances degradation rates.[42,45] Our results agree with the first proposition, that tillage reduces degradation. Our simple correlation analysis found a significant correlation (r = 0.784**) of TOC with DHA_A (the value two hours after the herbicide application; Table 2) and an even stronger correlation with DHA considering all incubation times (Fig. 1) (r = 0.936**), which agrees with various authors who report finding that a soil's enzymatic activity is strongly connected to its organic matter content (see, for example, the work of Romero *et al.*[49]). The order of bentazon dissipation noted above reflects an inverse relationship with the soil's organic matter content (Table S1 and Table 2). Indeed, DT_{50} had a negative and highly significant correlation with TOC (r = -0.976**) and HA (r = -0.926**), indicative that bentazon dissipation also depends upon the degree of polymerization of the soil's organic matter. Furthermore, DT_{50} was negatively correlated with DHA_A (Table 2) (r = -0.756**) and even more strongly with DHA as determined by considering all incubation times (Fig. 1) (r = -0.908**). These results indicate that, for the soils of the rice-growing ecosystems in the present study, the availability of organic carbon favoured microbial growth, thus increasing the bentazon dissipation rates as a consequence of the greater biological activity.[50]

The DHA values before bentazon application were higher in all treatments under anaerobic than under aerobic conditions (Table 2). This agrees with previous studies, and indicates that DHA reaches greater values when the soil's oxygen diffusion rate, water potential, and redox potential are lower, all characteristics typical of anaerobic systems.[51-53] Under aerobic conditions, the DHA values rose in all management regimes after applying the herbicide at the rate of 2 kg ha^{-1}, which suggests that the application of bentazon was non-toxic, and may even have been stimulating for the microorganisms, although the impact on soil-enzyme activities depended upon the management system (Table 2). Under anaerobic conditions, however, the DHA values fell significantly ($p<0.05$) in the herbicide-treated soils, suggesting a negative effect of bentazon on anaerobic microorganisms, at least in the first stages after its application (2h – 3d) (Table 2; Fig. 1). This agrees with Allievi *et al.*[54] who noted that, after bentazon application, only the number of anaerobic N_2-fixing bacteria decreased significantly. The observed trend (Fig. 1) is logical and it should be expected if one takes into account that the contribution of nutrients and easily mineralizable carbon also tends to decline with time, reason why in turn the microbial population decreases and, consequently, also DHA decreases (in our case after approximately 10 d).

In all treatments, the herbicide was less persistent under aerobic (DT_{50} = 42–91 d) than under anaerobic conditions (DT_{50} = 94–136 d) (Table 2). Thus, by the end of the study, on average there remained 62% of the bentazon under aerobic conditions compared to 74% under anaerobic conditions (Fig. 1). This is consistent with studies reporting that degradation potentials for organic pollutants are higher under aerobic conditions since anaerobic metabolic rates are slower than aerobic rates (see, for example, the works of Bondarenko and Gan[55] and Charnay *et al.*[56]). It is worth noting that, after 28 days of incubation under anaerobic conditions, less than 4% of the applied herbicide had been dissipated in the soils of all the management regimes. This is compatible with the results of Knauber *et al.*[57] who, in an experiment in which the samples were also incubated at 20°C for 28 days, found that under anaerobic conditions bentazon mineralization was less than 5% of that under aerobic conditions. The present DT_{50} values are greater than the value noted by

Leistra *et al.*[58] who found a DT_{50} of 38 days in a water-saturated subsoil material but with a greater redox potential than in our study. The results of our study contrast with those of Levi *et al.*[59] who found no degradation of bentazon in aquifer sediment material under anaerobic conditions, although in that study the incubation temperature was 10°C, whereas in the present study the samples were incubated at 20°C.

Although the management regimes significantly influenced the bentazon half-lives (DT_{50}) under both aerobic and anaerobic incubation conditions (Table 2), there was a significant management × incubation condition interaction ($p<0.01$). This was particularly notable for the case of the NTS7 soil for which the aerobic half-life was shorter than that of the other three soils (by factors of 2.1, 2.1, and 1.9 for NTS, TS, and TF, respectively), but the anaerobic half-life was longer (by factors of 1.4, 1.2, and 1.1). The differences in TOC content between treatments may well be the explanation for the bentazon dissipation rates observed under anaerobic conditions. Thus, contrary to the aerobic case, DT_{50} was positively correlated with TOC for the anaerobic incubation conditions (r = 0.709**), suggesting that the anaerobic microorganisms in the soils may have preferentially used the soil organic matter as a carbon source instead of bentazon, at least in the first part of the incubation process (Fig. 1). The soil with the greatest TOC content, NTS7, would therefore show the slowest bentazon dissipation rate, as indeed was observed (Table 2).

3.4. Leaching studies

Relative and cumulative bentazon breakthrough curves are shown in Fig. 2. Table 3 presents the total bentazon leached as well as the remaining percentage extracted from the soil columns. In all management regimes, the position of the maximum of all the breakthrough curves was close to one pore volume in 2011, or even lower in 2013 (TS) (Fig. 2), as is typical of highly mobile compounds.[60] The total bentazon leached went from 60.7% to 82.5% of the amount initially applied (Table 3; Fig. 2). The results confirm this compound's high mobility and low retention, reflected in its common detection in surface and ground water around the world. The present amounts of bentazon leached are greater than the 17%–45% reported by Larsbo *et al.*[27] for three different soils under conventional and reduced tillage, although the sorption values for those soils were on average 2.7 times greater than for the soils of the present study. However, much higher bentazon leaching losses were detected by Cañero *et al.*[48] who stated having recovered 100% of the herbicide initially applied herbicide from leachates of two soils with sorption values similar to those of the soils of the present study, although those two soils were very alkaline (pH≥8.4). The bentazon leaching was significantly influenced by the management regimes in both years (Table 3). The effects on the breakthrough and percentage of herbicide leached differed in the two years as determined by the significant treatment × year interaction (p<0.001; Table 3). Thus, in 2011, the bentazon breakthrough took place after 0.72 pore volumes of water in NTS7, but after 0.38, 0.34, and 0.36 pore volumes in NTS, TS, and TF, respectively (Table 3). But in 2013, these breakthrough thresholds were 0.70 pore volumes for both NTS7 and NTS, and 0.42 and 0.36 pore volumes for TS and TF, respectively (Table 3) despite the NTS7 and NTS soils having on average some 2.3 times greater DOC content than the TS and TF soils (Table S1). This suggests that increased DOC content did not necessarily imply more rapid movement of the bentazon. The mass balance calculation showed that in 2011 the significantly (p<0.05) smallest amount of bentazon leached corresponded to the NTS7 soil (Table 3). Two years later in 2013 after three years of the trial, NTS7 was joined by the other no-till soil, NTS, in having significantly (p<0.05) less bentazon leached than the two tillage soils (TS and TF). In particular, while 60.7% and 62.3% of the bentazon initially applied was recovered from the NTS7 and NTS leachates, respectively, these amounts were 77.5% and 74.1% for the TS and TF soils, respectively (Fig. 2; Table 3). Furthermore, the average maximum concentration of bentazon in the tillage treatment leachates (20.9 µM) was 1.6 times greater than that in the no-till treatment leachates (13.4 µM) (Table 3). The rank order of these herbicide mass losses mainly reflects differences in the TOC and, above all, HA contents (Table S1 and Table 3). In particular, the percentage of bentazon leached had negative correlations with TOC (r = -0.586**) and HA (r = -0.711**), which indicates that bentazon leaching is dependent not only on the quantity of the soil's organic matter but also on its degree of polymerization.

All the columns were sectioned into four at the end of the leaching experiment, and the bentazon that remained in each section was extracted and quantified. We did not find any significant differences ($p>0.05$) between years or between management regimes in the amount of herbicide remaining in the soils (Table 3). Nonetheless, while in the year 2011 no unleached bentazon was recovered from any of the soils, in 2013 small amounts of the herbicide were recovered from the NTS7 and NTS soil columns, although in both cases the total amounts were less than 5% of the initially applied herbicide (Table 3). This is coherent with the smaller amount of bentazon leached from these two no-tillage soils than from the two tilled soils. Considering the total amounts recovered (leached + soil extract), in 2013 after the three years of the trial, these were on average 12% less for the no-till soils (NTS7 and NTS) than for the tilled soils (TS and TF) (Table 3).

4. Conclusions

Irrigation and tillage management significantly affected soil properties, and consequently also affected the behaviour of the herbicide bentazon. It was found that the soil organic matter and its transformation into humic substances are major factors influencing the behaviour of bentazon in rice agroecosystems under different water and tillage managements. Conventional tillage practices promote the loss of bentazon by leaching regardless of which irrigation method is applied (flooding or sprinkler irrigation). Furthermore, for all management regimes tested in our study, bentazon was significantly more persistent under anaerobic than aerobic incubation conditions, which might be a factor that could contribute to faster disappearance of the herbicide from rice-growing environments under sprinkler irrigation than traditional flooding. Therefore, under conventional tillage, both aerobic (non-flooding) and anaerobic (flooding) rice production may make water contamination by bentazon a greater risk in Mediterranean rice agroecosystems. However, under no-tillage practices, aerobic (*i.e.*, sprinkler irrigated) rice production may reduce that risk, even in just the mid-term once this management system has been implemented.

Acknowledgements

Support for the present work was provided by Spain's Ministries of Science and Innovation (AGL

2010-21421-CO2-02) and of Economics and Competitiveness (AGL2013-48446-C3-2-R).

References

[1] Machado SLO, Marchezan E, Righes AA, Carlesso R, Villa SCC, Camargo ER, Water use and nutrients and sediments losses on the initial water drainage on flooded rice. *Cienc Rural* **36**:65-71 (2006).

[2] La N, Lammers M, Nguyen VV, Streck T, Modelling the fate of pesticides in paddy rice-fish pond farming systems in northern Vietnam. *Pest Manag Sci* **70(1)**:70-79 (2014).

[3] FAO, Agricultural data. Food and Agriculture Organization of the United Nations, Rome. FAOSTAT on-line database (2009), http://faostat.fao.org/ [accessed 4 July 2016].

[4] Chenini F, Effects of hydrologic alterations on the ecological quality of river ecosystems, in: *Water scarcity in the Mediterranean perspectives under global change,* ed. by Sabater S and Barceló D, The Handbook of Environmental Chemistry 8, pp. 162-172 (2010).

[5] Richter BD, Mathews R, Harrison DL, Wigington R, Ecologically sustainable water management: managing river flows for ecological integrity. *Ecol Appl* **13**:206-24 (2003).

[6] Ison R, Röling N, Drennan W, Challenges to science and society in the sustainable management and use of water: investigating the role of social learning. *Environ Sci Policy* **10(6)**:499–511 (2007).

[7] Feng LP, Bouman BAM, Tuong TP, Cabangon RJ, Li, YL, Lu GA, Feng YH, Exploring options to grow rice under water-short conditions in northern China using a modeling approach. I: field experiments and model evaluation. *Agr Water Manage* **88**:1-13 (2007).

[8] Qu Y, Ping M, Hongliang Z, Chen Y, Gao Y, Tian Y, Wen F, Li Z, Mapping QTLs of root morphological traits at different growth stages in rice. *Genetica* **133**:187-200 (2008).

[9] Bhushan L, Ladha JK, Gupta RK, Singh S, Padre AT, Saharawat YS, Gathala M, Pathak H, Saving of water and labour in a rice–wheat system with no tillage and direct seeding technologies. *Agron J* **99**:1288-96 (2007).

[10] Farooq M, Siddique KHM, Rehman H, Aziz T, Lee DJ, Wahid A, Rice direct seeding: experiences, challenges and opportunities. *Soil Till Res* **111(2)**:87-98 (2011).

[11] Kumar V, Ladha JK, Direct seeding of rice: recent developments and future research needs. *Adv Agron* **111**:297-396 (2011).

[12] Gianessi LP, The increasing importance of herbicides in worldwide crop production. *Pest Manag Sci* **69(10)**:1099-1105 (2013).

[13] Moreno-Jiménez E, Meharg A, Smolders E, Manzano R, Becerra D, Sánchez-Llerena J, Albarrán A, López-Piñeiro A, Sprinkler irrigation of rice fields reduces grain arsenic but enhaces cadmium. *Sci Total Environ* **485-486**:468-473 (2014).

[14] Sánchez-Llerena J, López-Piñeiro A, Albarrán A, Peña D, Becerra D, Rato-Nunes JM, Short and long-term effects of different irrigation and tillage systems on soil properties and rice productivity under Mediterranean conditions. *Eur J Agron* **77**:101-110 (2016).

[15] Nascente AS, Crusciol CAC, Cobucci T, The no-tillage system and cover crops-Alternatives to increase upland rice yields. *Eur J Agron* **45**:124-131 (2013).

[16] Mortimer A M, Riches CR, Mazid M, Pandey S, Johnson, DE, Issues related to direct seeding of rice in rainfed cropping systems in northwest Bangladesh, in: Direct seeding of rice and weed management in the irrigated rice-wheat cropping system of the Gangetic plains, ed. by Singh Y, Singh VP, Chauhan B, Orr A, Mortimer AM, Johnson DE and Hardy B, International Rice Research Institute, and Pantnagar (India): Directorate of Experiment Station, G.B. Pant University of Agriculture and Technology, Philippines, pp. 23-30 (2008).

[17] Galhano V, Peixoto F, Gomes-Laranjo J, Fernández-Valiente E, Comparative toxicity of bentazon and molinate on growth, photosynthetic pigments, photosynthesis, and respiration of the portuguese ricefield cyanobacterium Nostoc muscorum. *Environ Toxicol* **25(2)**:147-156 (2010).

[18] Ramos C, Carbonell G, García Baudín JM, Tarazona JV, Ecological risk assessment of pesticides in the Mediterranean region. The need for crop-specific scenarios. *Sci Total Environ* **247(2-3)**:269-278 (2000).

[19] Miao Z, Padovani L, Riparbelli C, Ritter AM, Trevisan M, Capri E, Prediction of the environmental concentration of pesticide in paddy field and surrounding surface water bodies. *Paddy Water Environ* **1(3)**:121-132 (2003).

[20] Papadopoulou-Mourkidou E, Karpouzas DG, Patsias J, Kotopoulou A, Milothridou A, Kintzikoglou K, Vlachou P, The potential of pesticides to contaminate the groundwater resources of the Axios river basin in Macedonia, Northern Greece. Part I. Monitoring study in the north part of the basin. *Sci Total Environ* **321(1-3)**:127-146 (2004).

[21] Kuster M, López de Alda MJ, Barata C, Raldúa D, Barceló, D, Analysis of 17 polar to semi-polar pesticides in the Ebro river delta during the main growing season of rice by automated on-line solid-phase extraction-liquid chromatography-tandem mass spectrometry. *Talanta* **75(2)**:390-401 (2008).

[22] Barceló D, Hennion MC, Trace determination of pesticides and their degradation products in water. Techniques and Instrumentation in Analytical Chemistry. Elsevier, Amsterdam. 1997.

[23] Vasquez ME, Holstege DM, Tjeerdema RS, Aerobic versus anaerobic microbial degradation of etofenprox in a California rice field soil. *J Agr Food Chem* **59**:2486-2492 (2011).

[24] Álvaro-Fuentes J, Easter M, Paustian K, Climate change effects on organic carbon storage in agricultural soils of northeastern Spain. *Agr Ecosyst Environ* **155**: 87-94 (2012).

[25] Alletto L, Benoit P, Bolognési B, Couffignal M, Bergheaud V, Dumény V, Longueval C, Barriuso E, Sorption and mineralisation of S-metolachlor in soils from fields cultivated with different conservation tillage systems. *Soil Till Res* **128**:97-103 (2013).

[26] Moreno B, Vivas A, Nogales R, MacCi C, Masciandaro G, Benitez E, Restoring biochemical activity and bacterial diversity in a trichloroethylenecontaminated soil: the reclamation effect of vermicomposted olive wastes. *Environ Sci Pollut Res* **16**, 253–264 (2009).

[27] Larsbo M, Stenström J, Etana A, Börjesson E, Jarvis NJ, Herbicide sorption, degradation, and leaching in three Swedish soils under long-term conventional and reduced tillage. *Soil Till Res* **105**:200-208 (2009).

[28] Hua R, Spliid NH, Laursenb B, Influence of surfactants on the leaching of bentazone in a sandy loam soil. *Pest Manag Sci* **65(8)**: 857-861 (2009).

[29] Abudayyak M, Ozden S, Alpertunga B, Özhan G, Effects of bentazone on lipid peroxidation and antioxidant systems in human erythrocytes in vitro. *Drug Chem Toxicol* **37(4)**:410-414 (2014).

[30] Loos R, Locoro G, Comero S, Contini S, Schwesig D, Werres F, Balsaa P, Gans O, Weiss S, Blaha L, Bolchi M, Gawlik BM, Pan-European survey on the occurrence of selected polar organic persistent pollutants in ground water. *Water Res* **44(14)**:4115-4126 (2010).

[31] Salvati S, Alessi R, Policicchio R, Spinello O, Sollazzo C, Carere M, Fasanelli F, Muto L, Iannuzzi P, Prima ricognizione sulla presenza di sostanze pericolose nei corpi idrici in Italia. Report 34/2003. Agenzia per la protezione dell'ambiente e per i servizi tecnici (APAT), Roma, Italy. 2003

[32] Comoretto L, Arfib B, Chiron S, Pesticides in the Rhône river delta (France): Basic data for a field-based exposure assessment. *Sci Total Environ* **380(1-3)**:124-132 (2007).

[33] Styczen M, Petersen S, Christensen M, Jessen OZ, Rasmussen D, Andersen MB, et al. Calibration of models describing pesticide fate and transport in Lillebaek and Odder Baek Catchment. Ministry of the Environment, Danish Environmental Protection Agency, Pesticides Research N^{o} 62, 2002.

[34] López-Piñeiro A, Sánchez-Llerena J, Peña D, Albarrán A, Ramírez M, Transition from flooding to sprinkler irrigation in Mediterranean rice growing ecosystems: Effect on behaviour of bispyribac sodium. *Agric Ecosyst Environ* **223**:99-107 (2016).

[35] OECD guideline for testing of chemicals 106. Adsorption-desorption using batch equilibrium method. OECD, adopted 21 January (2000).

[36] Cañero AI, Becerra D, Cornejo J, Hermosín MC, Albarrán Á, López-Piñeiro A, Cox L, Transformation of organic wastes in soil: Effect on bentazone behaviour. *Sci Total Environ* **433**:198-205 (2012).

[37] Calvet R, Adsorption of organic chemicals in soils. *Environ Health Perspect* **83**:145-177 (1989).

[38] García C, Hernández T, Costa C, Ceccanti B, Masciandaro G, Ciardi C, A study of biochemical parameters of composted and fresh municipal wastes. *Bioresource Technol* **44**:17-23 (1993).

[39] Peña D, López-Piñeiro A, Albarrán A, Rato-Nunes, JM, Sánchez-Llerena J, Becerra D, Ramírez M, De-oiled two-phase olive mill waste may reduce water contamination by metribuzin. *Sci Total Environ* **541**:638-645 (2016).

[40] Li K, Liu W, Xu D, Lee S, Influence of organic matter and pH on bentazone sorption in soils. *J Agr Food Chem* **51(18)**:5362-5366 (2003).

[41] Bergstrom L F, Jarvis NJ, Leaching of dichlorprop, bentazon and Cl in undisturbed field lysimeter of different agricultural soils. *Weed Sci* **41**:251-261 (1993).

[42] Gaston LA, Locke MA, Zablotowicz RM, Sorption and degradation of bentazon in conventional- and no-till Dundee soil. *J Environ Qual* **25**:120-126 (1996).

[43] Boivin A, Cherrier R, Schiavon, M, Bentazone adsorption and desorption on agricultural soils. *Agron Sustain Dev* **25**:309-15 (2005).

[44] Reddy KN, Locke MA, Sulfentrazone sorption, desorption, and mineralization in soils from two tillage systems. *Weed Sci* **46**:494-500 (1998).

[45] Zablotowicz RM, Locke MA, Gaston LA, Bryson CT, Interactions of tillage and soil depth on fluometuron degradation in a Dundee silt loam soil. *Soil Till Res* **57**,61-68 (2000).

[46] Seybold CA, Mersie W, Adsorption and desorption of atrazine, deethylatrazine, deisopropylatrazine, hydroxyatrazine, and metolachlor in two soils from Virginia. *J Environ Qual* **25(6)**:1179-1185 (1996).

[47] Ghafoor A, Moeys J, Stenström J, Tranter G, Jarvis NJ, Modeling spatial variation in microbial degradation of pesticides in soil. *Environ Sci Technol* **45(15)**:6411-6419 (2011).

[48] Cañero AI, Cox L, Cabrera A, Cornejo J, Different Effects of a By-product From Olive Oil Extraction on S-Metolachlor and Bentazone Soil Fate. *Clean - Soil Air Water* **43(6)**:948-957 (2015).

[49] Romero E, Fernández-Bayo J, Díaz JMC, Nogales R, Enzyme activities and diuron persistence in soil amended with vermicompost derived from spent grape marc and treated with urea. *Appl Soil Ecol* **44(3)**:198-204 (2010).

[50] Thorstensen CW, Lode O, Organic compounds in the environment: Laboratory degradation studies of bentazone, dichlorprop, MCPA, and propiconazole in Norwegian soils. *J Environ Qual* **30(3)**:947-953 (2001).

[51] Brzezińska M, Stępniewski W, Stępniewska Z, Przywara G, Wlodarczyk T, Effect of oxygen deficiency on soil dehydrogenase activity in a pot experiment with triticale cv. Jago Vegetation. *Int Agrophys* **15(3)**:145-149 (2001).

[52] Wolińska A, Bennicelli RP, Dehydrogenase activity response to soil reoxidation process described as varied conditions of water potential, air porosity and oxygen availability. *Pol J Environ Stud* **19**:651-657 (2010).

[53] Weaver MA, Zablotowicz RM, Krutz LJ, Bryson CT, Locke MA, Microbial and vegetative changes associated with development of a constructed wetland. *Ecol Indic* **13(1)**:37-45 (2012).

[54] Allievi L, Gigliotti C, Salardi C, Valsecchi G, Brusa T, Ferrari A, Influence of the herbicide bentazon on soil microbial community. *Microbiol Res* **151(1)**:105-111 (1996).

[55] Bondarenko S, Gan J, Degradation and sorption of selected organophosphate and carbamate insecticides in urban stream sediments. *Environ Toxicol Chem* **23(8)**:1809-1814 (2004).

[56] Charnay MP, Tuis S, Coquet Y, Barriuso E, Spatial variability in 14C-herbicide degradation in surface and subsurface soils. *Pest Manag Sci* **61(9)**:845-855 (2005).

[57] Knauber WR, Krotzky AJ, Schink B, Microbial metabolism and further fate of bentazon in soil. *Environ Sci Technol* **34(4)**:598-603 (2000).

[58] Leistra M, Smelt JH, Matser AM, Boyte JJ, Van der Pas LJT, Rate of bentazone transformation in four layers of a humic sandy soil profile with fluctuating water table. *Pest Manag Sci* **57**:1023-32 (2001).

[59] Levi S, Hybel AM, Bjerg PL, Albrechtsen HJ, Stimulation of aerobic degradation of bentazone, mecoprop and dichlorprop by oxygen addition to aquifer sediment. *Sci Total Environ* **73-474**:667-675 (2014).

[60] Beck, AJ, Johnston AEJ, Jones KC, Movement of nonionic organic chemicals in agricultural soils. *Cr Rev Environ Sci Technol* **23**:219-248 (1993).

Table 1. Bentazon sorption–desorption parameters for each management regime and year.

	$n_{f\ sorption}$	K_d	$R^2_{\ sorption}$	$n_{f\ desorption}$	D (%)	$R^2_{\ desorption}$
			2011			
NTS7	0.466 aA	0.272 bC	0.950	0.086 aA	14.9 aA	0.988
NTS	0.853 bA	0.164 aB	0.952	0.099 aB	18.8 aA	0.918
TS	0.599 abA	0.160 aC	0.936	0.071 aA	15.7 aA	0.992
TF	0.702 abA	0.178 aC	0.939	0.109 aA	25.1 aA	0.964
			2012			
NTS7	0.562 aA	0.199 bB	0.915	0.024 aA	6.32 aA	0.998
NTS	0.563 aA	0.100 aA	0.983	0.095 aB	16.3 aA	0.983
TS	0.566 aA	0.113 aB	0.939	0.039 aA	5.84 aA	0.953
TF	0.720 aA	0.138 aA	0.988	0.068 aA	20.2 aA	0.959
			2013			
NTS7	0.755 aB	0.164 dA	0.996	0.045 aA	19.8 aA	0.915
NTS	0.765 aA	0.114 bA	0.986	0.044 aA	20.1 aA	0.998
TS	0.845 aB	0.073 aA	0.984	0.066 aA	22.7 aA	0.999
TF	1.123 bB	0.125 cA	0.995	0.071 aA	35.5 bA	0.943
Y	**	***	-	NS	NS	-
M	***	***	-	NS	**	-
Y x M	*	**	-	NS	NS	-

Table 2. Bentazon dissipation parameters and dehydrogenase activity for each management regime.

	$DT_{50\ 80\%}$ (days)	$R^2_{80\%}$	$DT_{50\ 1:1.25}$ (days)	$R^2_{1:1.25}$	$DHA_{B80\%}$ (µg INTF g^{-1} h^{-1})	$DHA_{A80\%}$ (µg INTF g^{-1} h^{-1})	$DHA_{B1:1.25}$ (µg INTF g^{-1} h^{-1})	$DHA_{A1:1.25}$ (µg INTF g^{-1} h^{-1})
NTS7	42.4 a	0.984	135.6 b	0.824	0.456 b	0.816 a	1.14 c	0.752 c
NTS	91.3 c	0.972	94.1 a	0.857	0.441 b	0.699 a	0.721 b	0.334 a
TS	88.9 b c	0.981	116.1 a b	0.905	0.531 c	1.01 b	0.583 a	0.393 a b
TF	82.0 b	0.954	121.9 b	0.807	0.382 a	0.715 a	0.581 a	0.442 b
M	***	-	***	-	***	***	***	***

Half-lives: $DT_{50\ 80\%}$ in soils at 80% field water capacity; $DT_{50\ 1:1.25}$ in soils with 1:1.25 (w/v) (soil/water) moisture content. DHA: 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. Significant differences ($p<0.05$) between management regimes are indicated by different lower case letters. M is the ANOVA factor: management regime. ***: significant at an α level of 0.001.

Table 3. Bentazon leaching for each management regime.

	Initial pore volume[a]	Max. concentration leached (µM)	Total leached (%)	Total extracted (%)
		2011		
NTS7	0.721 bA	16.2 aA	61.4 aA	0 aA
NTS	0.383 aA	29.6 bA	82.1cB	0 aA
TS	0.344 aA	29.8 bB	70.5 bA	0 aA
TF	0.364 aA	26.4 bA	82.5cB	0 aA
		2013		
NTS7	0.688 bA	13.6 aA	60.7 aA	3.56 aA
NTS	0.707 bB	13.2 aA	62.3 aA	4.62 aA
TS	0.417 aB	16.1 aA	77.5 bA	0 aA
TF	0.359 aA	25.7 bA	74.1 bA	0 aA
Y	**	**	***	NS
M	***	**	***	NS
Y x M	***	NS	***	NS

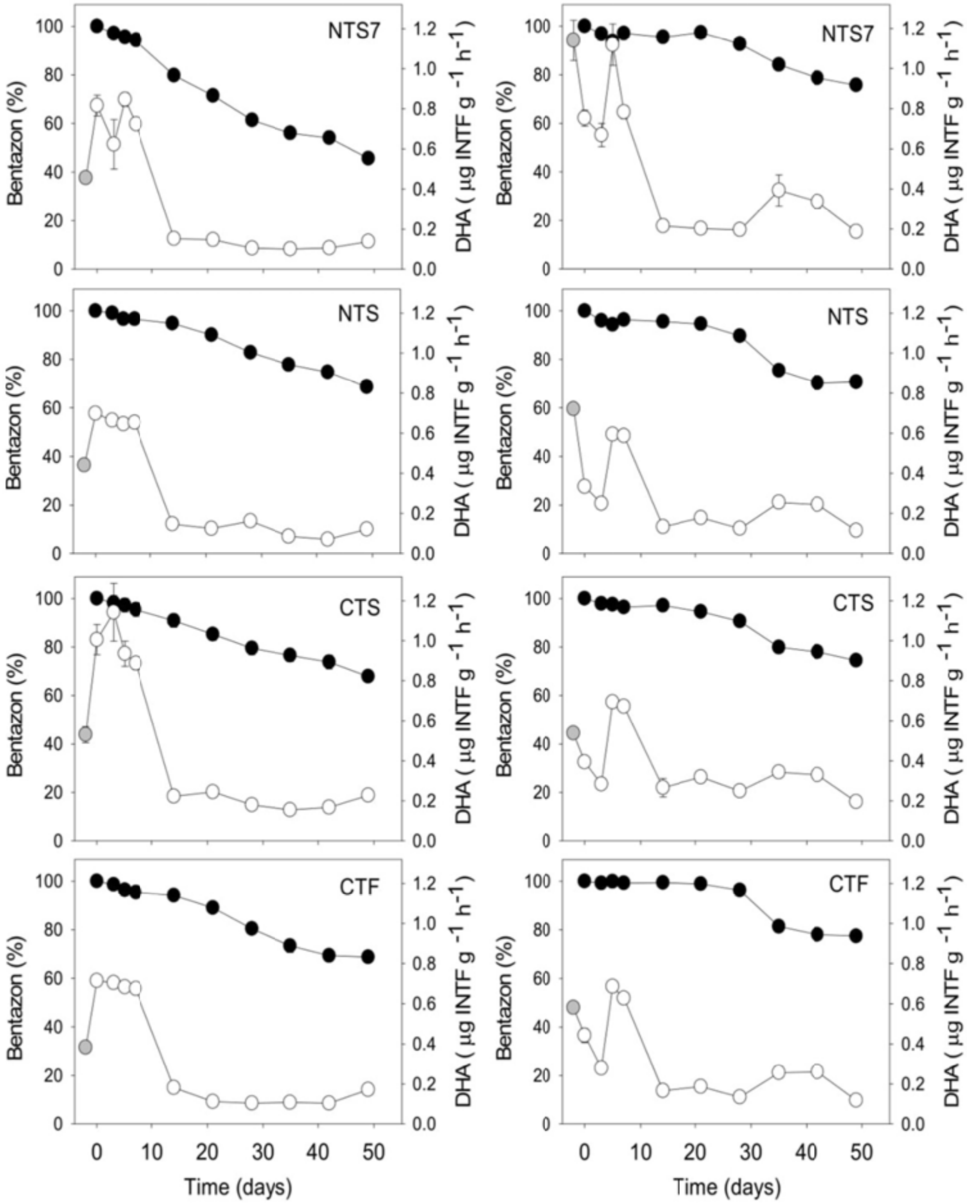
[a] values of pore volume when bentazon was for the first time found in the leachate. Significant differences ($p<0.05$) between management regimes in the same year are indicated by different lower case letters, and between years in the same management regime by different upper case letters. The meaning of the ANOVA factors is: Y, year; M, management regime; Y × M, interaction between year and management regime; *, **, and *** significant at α levels of 0.05, 0.01, and 0.001, respectively; NS, not significant.

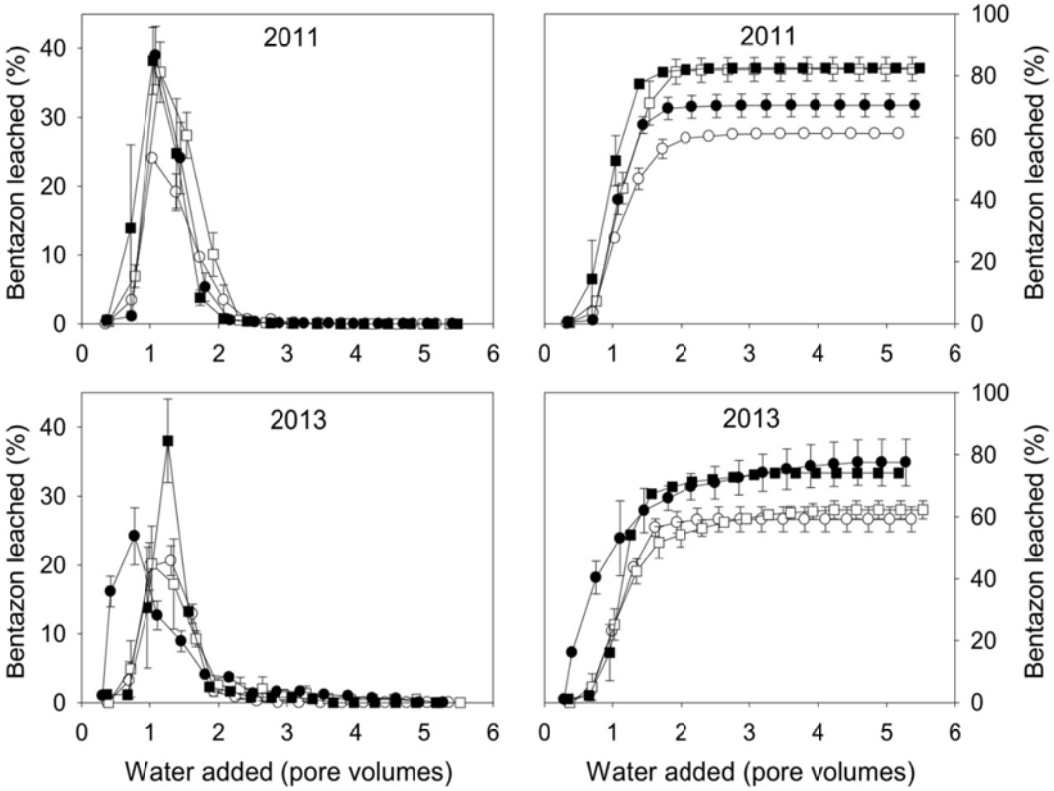
Figure Captions

Figure 1. Bentazon dissipation (●) and dehydrogenase activity (○) in soils under aerobic (left) and anaerobic (right) conditions for each management regime. Dehydrogenase activity two hours before the application of the herbicide to soils (●). Error bars represent one standard error of the mean.

Figure 2. Relative (left) and cumulative (right) breakthrough curves of bentazon for each management regime. NTS7 (○), NTS (□), TS (●) and TF (■). Error bars represent one standard error of the mean.

Figure S1. Bentazon sorption isotherms. NTS7 (○), NTS (□), TS (●) and TF (■). Error bars represent one standard error of the mean.


NTS7
NTS7
NTS
NTS
CTS
CTS
CTF
CTF
Bentazon (%)
DHA (µg INTF g -1 h-1)
0
20
40
60
80
100
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0
10
20
30
40
50
Time (days)


2011
2011
2013
2013
Bentazon leached (%)
Bentazon leached (%)
Bentazon leached (%)
Bentazon leached (%)
0
10
20
30
40
0
20
40
60
80
100
0
1
2
3
4
5
6
Water added (pore volumes)
Water added (pore volumes)