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Effect of Multiple Stresses, Organic Amendment and Compaction, on the Fate and Impact of Isoproturon in Soil

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Abstract: Organic matter decline and compaction are two major processes of soil degradation. Organic amendment is a current practice to compensate the loss of organic matter, which could in addition contribute to increase soil aggregate stability and limit compaction. Therefore, the objective of this work was to study the effect of multiple physico-chemical stresses, organic amendment (compost of sewage sludge and green waste) addition and soil compaction, on the fate and impact (measured through the urease enzyme activity) of isoproturon. Compost addition and compaction did not significantly affect the fate and impact of isoproturon. The lack of effect of compost can be due to the delay between soil sampling and soil amendment. Compaction had no effect probably because the porosity reduction does not affect the habitable pore space accessible to degrading microorganisms. Nevertheless, isoproturon significantly increased the urease enzyme activity in compacted and not compacted unamended soils contrary to the amended ones. It seems that the organic amendment could act as a buffer with regards to the impact of isoproturon. The results obtained in this work suggest that, in general, the fate and impact of isoproturon in soils will not change following compaction and/or organic amendment addition, neither the corresponding risks for the environment.

Keywords: pesticide; soil; compaction; organic amendment; fate; enzyme activity

1. Introduction

Soil is essentially a non-renewable resource which delivers services vital to human activities and ecosystems survival [1,2]. However, soils suffer from the increasing environmental pressure, driven or exacerbated by human activity, such as inappropriate agricultural practices, industrial activities or urban development. These activities are damaging the capacity of soil to continue to perform in full its broad variety of crucial functions. In addition, soil degradation has strong impacts on water resources, human health, climate change, nature and biodiversity protection and food safety. The European Commission thus identified the main eight threats to which soils are confronted: contamination, organic matter decline, compaction, erosion, salinisation, soil biodiversity loss, sealing, landslides and

flooding [2,3]. Among these threats, some of them directly result from agricultural practices such as contamination by pesticides, organic matter decline or compaction.

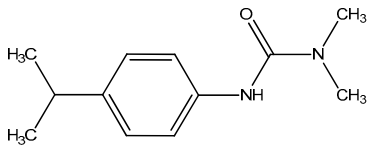
To compensate the loss of soil organic matter contents and/or to reduce the use of synthetic fertilizers, the addition of organic amendments is an increasingly used practice, which allows in parallel to add value to some organic wastes. However, the use of these organic wastes from various origins may have some impacts on the environment [4]. Organic amendment addition can change the physical (soil bulk density, aggregate stability, moisture retention and porosity), chemical (cationic exchange capacity, electric conductivity and addition of contaminants) and biological (stimulation of indigenous microflora and introduction of exogenous microorganisms) properties of soils. These changes can impact the fate of pesticides: in general, the mobility of pesticides decreases and their persistence increases in amended soils compared to unamended ones [4–14].

Compaction due to agricultural practices (farm traffic, soil tillage, short rotations...) leads to modifications in soil physical properties such as a decrease in porosity, air content or water infiltration that can modify the biological functioning of soil with, for example, creation of anaerobic media [15–18]. This can favour erosion by reducing water infiltration and lead to bad crop implantation by preventing root development which reduces crop yields [16,18,19]. The modification of soil properties due to compaction may change the fate and ecotoxicological effects of pesticides, but very few results have been published in the literature. Rahman et al. [19] observed higher phytotoxicity of two herbicides (atrazine and trifluralin) in heavy compacted soils compared to normally compacted ones. This was attributed to a combination of factors, including a decrease in leaching and an increase in persistence of herbicides in compacted soils. On the contrary, Mamy et al. [20] showed the degradation of isoproturon herbicide proceeded slightly faster in highly compacted soil probably because of higher soil degree of saturation. Finally, compaction did not modify significantly the impact of isoproturon on soil enzyme activities [20].

Both organic amendment addition and compaction are physico-chemical stresses that could affect the fate and impact of pesticides in soils but, to the best of our knowledge, this is not documented.

Isoproturon (3-(4-isopropylphenyl)-1, 1-dimethylurea) (Table 1) is a selective herbicide used against annual grasses and broad-leaved weeds. It was banned in Europe in 2016, but, for many years, it was one of the most used herbicides in European cereals production and, because of its high mobility in soils, it was frequently found in ground and surface waters and it is still today [8,21–25]. In the soil, the degradation of isoproturon is mainly biological [21] with laboratory half-life ranging from 7 to 223 days [22,24,26,27]. The main degradation pathway leads to the formation of three metabolites: monodemethyl-isoproturon, didemethyl-isoproturon and 4-isopropyl-aniline [21,22,26]. The effects of isoproturon on the biological functioning of soil and on soil organisms were less studied but, in general, the results show a low risk for soil micro- and macro-organisms [28,29].

Table 1. Main physico-chemical properties of isoproturon [24].

Structural Formula	Molecular Mass (g mol^{-1})	Water Solubility (mg L^{-1})	Log Kow	Vapor Pressure (mPa)
	206.28	70.2	2.5	5.5×10^{-3}

Soil enzyme activities are known to be relevant indicators of soil health, and they were successfully used to discriminate a wide range of agricultural practices such as organic amendments or pesticides addition, but also perturbation at the landscape scale [15]. They are considered as integrative bioindicators because they reflect the structure and functioning of microbial communities [30]. Isoproturon was shown to have some effects on various enzyme activities.

It decreases the dehydrogenase, acid and alkaline phosphomonoesterases, and leucine aminopeptidase activities [27,31,32]. By contrast, it increases the urease activity [33], but it had no effect on several other activities such as arylsulfatase, beta-glucosidase or esterase [20,34]. Mamy et al. [20] also showed that soil compaction did not modify the effect of isoproturon on the beta-glucosidase activity. Among the numerous soil enzymes, urease plays a key role because it catalyses the hydrolysis of urea into carbon dioxide and ammonia which can be assimilated by microbes and plants, and it is therefore a crucial component in the nitrogen cycle in soils [15,35].

Thus, the objective of this work was to study the effect of organic amendment addition and soil compaction multiple stresses on the fate and impact (measured through the urease enzyme activity) of isoproturon.

2. Materials and Methods

2.1. Soil and Organic Amendment

Undisturbed soil cores (5 cm diameter, 2 cm height) were sampled in two 450 m^2 plots located in a French long-term field experiment (QualiAgro, Feucherolles, Yvelines): one control plot (no amendment) and one plot receiving a co-compost of sewage sludge and green waste every two years. The QualiAgro field experiment was initiated in 1998 to investigate the long-term effects of repeated applications of urban waste composts on the soil, plant and water, as well as on the dynamics of various contaminants (pesticides, trace metals, pharmaceutical residues...). The plots were cropped with a winter wheat–maize rotation, where isoproturon was the main herbicide used on the wheat crop. The last compost addition (in the corresponding plot) and isoproturon application were carried out 12 months and 20 months before sampling, respectively.

Soil samples were randomly collected in the interfurrows of the two plots on 30 October 2008 and stored at 4 °C for 12 days before the beginning of the experiments. The interfurrows were selected as they concentrate the organic amendments (and crop residues) following ploughing [5]. The soil is a silt loam Glossic Luvisol containing 17% clay, 76% loam and 7% sand, with a pH in water of 6.75, in the ploughed layer. The organic carbon contents were 1.19% and 1.41% for control and compost amended soils, respectively.

Soil samples were prepared at two realistic levels of compaction (no and high). The increase in bulk density was 0.3 g cm^{-3} between no and high compaction, as observed following wheeling [36] (Table 2): from 1.16 ± 0.14 g cm^{-3} before compaction to 1.52 ± 0.14 g cm^{-3} after compaction for the control unamended soil and from 1.08 ± 0.13 to 1.41 ± 0.25 g cm^{-3} for the amended soil (Table 2). Compaction was achieved uniformly on the soil surface by applying a cylinder having a diameter equal to the inner diameter of the cylinders. High compaction resulted in a reduction in soil volume of 20%.

Table 2. Main soil physical properties of the control (unamended) and compost amended soils, not compacted and compacted.

Soil	Bulk Density ρ (g cm^{-3})	Porosity P (-)	Pore Volume Vp (cm^3)	Water Content Vw (cm^3)	Degree of Saturation s (%)
Control, No compaction	1.16 ± 0.14	0.56 ± 0.05	22.0 ± 2.0	8.5 ± 0.9	39.3 ± 7.5
Control, Compaction	1.52 ± 0.14	0.43 ± 0.05	13.4 ± 1.7	8.8 ± 0.8	67.3 ± 13.2
Compost, No compaction	1.08 ± 0.13	0.59 ± 0.05	23.2 ± 1.9	8.2 ± 0.9	35.9 ± 7.3
Compost, Compaction	1.41 ± 0.25	0.47 ± 0.09	14.7 ± 2.9	8.5 ± 1.4	62.3 ± 23.9

For each level of compaction, the physical properties of the soil samples were characterised (Table 2): the bulk densities (ρ) of all samples were measured; then, the porosity (P), the pore volume (Vp), the water content (Vw) and the degree of saturation (s) were calculated as follows:

$$P = 1 - (\rho/2.65) \quad (1)$$

where 2.65 is the particle density of soil solids;

$$\text{Vp } (\text{cm}^3) = \text{Volume of soil sample} - (\text{Mass of dry soil}/2.65) \quad (2)$$

$$\text{Vw } (\text{cm}^3) = \text{Initial soil water content} + \text{Volume of added isoproturon} \quad (3)$$

$$\text{s } (\%) = (\text{Vw}/\text{Vp}) \times 100. \quad (4)$$

2.2. Incubation Procedures

2.2.1. Fate of Isoproturon

^{14}C-ring-labelled isoproturon (475 MBq mmol^{-1}, 95% purity) was purchased from Izotop (Budapest, Hungary). Soil samples (control unamended and compost amended, not compacted and compacted) were treated with an aqueous solution of ^{14}C-isoproturon (0.06 MBq per soil sample) to reach a final concentration of 0.29 mg g^{-1} dry soil (equivalent to the agronomic dose of 1.2 kg ha^{-1}). Soil water content was adjusted to reach 80% of the humidity at pF 2.5. Then, soil samples were placed in 500 mL jars containing vials with 10 mL of NaOH to trap the $^{14}CO_2$ and with 10 mL of water to maintain the ambient humidity. The jars were incubated at 28 °C in darkness for 49 days. They were opened at least weekly to preserve aerobic conditions, and soil water content was periodically adjusted by weighing each sample and adding the required amount of water. The NaOH traps were periodically sampled (after 1, 3, 7, 14, 21, 28, 35, 43 and 49 days) and replaced to determine the mineralisation kinetics of ^{14}C-isoproturon. At 0, 7 and 49 days, four sequential extractions of soil samples were done: one with $CaCl_2$ 0.01 M for 24 h and three with CH_3OH, each for 18 h. Samples were mechanically shaken at 20 °C in the dark and then centrifuged for 15 min at 9000 g. Non-extractable (bound) residues corresponded to the radioactivity remaining in the soil pellet after the four extractions. Five replicates were done for each incubation condition (control and amended soils, compacted and not compacted soils) and date of measurement.

Total radioactivity contents of the NaOH traps and of the $CaCl_2$ and CH_3OH extracts were measured by liquid scintillation counting using a Tri-Carb 2100 TR counter (Packard Instruments, Meriden, CT, USA) with external standardisation and Ultima Gold XR (Packard Instruments) as liquid scintillation cocktail. One-millilitre aliquots of NaOH trap and of each extract were mixed with 10 mL of liquid scintillation cocktail. Radioactivity in the solid samples containing the non-extractable residues was measured by liquid scintillation counting of the $^{14}CO_2$ evolved after combustion in triplicate of 150 mg of ground dry soils using a Sample Oxidizer 307 (Packard Instruments).

HPLC analyses were carried out on combined $CaCl_2$ and CH_3OH extracts because of too low ^{14}C contents of the $CaCl_2$ extracts. The $CaCl_2$ extracts were filtered through Whatman 90 filter discs, passed through Isolute® Env+ cartridges (IST, Mid Glamorgan, UK) and then eluted with 5 mL CH_3OH before the HPLC analysis. Methanol extracts were concentrated by evaporation near to dryness under vacuum at 60 °C using a Rotavapor R-200 (Büchi, Champigny, France). The residues were filtered through regenerated cellulose disc filters (0.45 mm, Alltech France, Templemars). Samples were analysed using a Waters HPLC appliance (600 E Multisolvent Delivery System, 717 Autosampler, and Nova-Pak C18 column) equipped with a 996 Photodiode Array Detector (Waters) and a radioactive flow detector (Packard-Radiomatic Flo-One A500). The mobile phase was 45/55 (*v*/*v*) methanol/water with a flowrate of 1 mL min^{-1}. In these conditions, the retention times of isoproturon and of three of its metabolites, monodemethyl-isoproturon (3-(4-isopropylphenyl)-1-methylurea), didemethyl-isoproturon (4-isopropylphenyl) urea) and 4-isopropyl-aniline, were 27, 25.8, 22 and 28 min, respectively [5].

2.2.2. Soil Enzyme Activity

A 20 mg L^{-1} isoproturon aqueous solution (Dr Ehrenstorfer GmbH, Augsburg, Germany, 99% purity) was prepared using water and methanol (99/1; *v*/*v*). The incubation conditions were

similar as those of the isoproturon fate study (0.29 mg isoproturon g^{-1} dry soil, 28 °C, 80% of pF 2.5, darkness) (Section 2.2.1), except that the soil samples were treated with non-labelled isoproturon solution, and that no NaOH trap was needed. Some soil samples (amended or not, compacted or not) were also incubated without isoproturon treatment as controls. The activity of urease was determined by incubating 1 g of soil with urea 0.4 M (3 h at 25 °C). The absorbance of the reaction product (NH_4^+ released) was determined with HACH reagents (Loveland, CO, USA) by using a microplate luminometer (Xenius SAFAS, Monaco) at 610 nm [37]. Four measures of enzyme activity were achieved by the Biochem-Env platform for each soil sample, and four replicates were done for each treatment (no isoproturon/isoproturon, unamended/amended, no compaction/compaction) and sampling date.

2.3. Statistical Analyses

The effects of organic amendment and/or compaction on the mineralisation kinetics and on the overall balances of isoproturon were determined with an analysis of variance (ANOVA) after the Shapiro–Wilk and Bartlett tests were done. Then, the Tuckey test allowed pairwise comparisons. When no ANOVA was possible, significant differences between treatments were determined with the test of Kruskal–Wallis followed by the test of Holm–Sidak for pairwise comparisons.

The effect of isoproturon, organic amendment and compaction on the urease activity were determined with an ANOVA. The homogeneity of variances was checked with the test of Levene, and pairwise comparisons were done with the test of Wilcoxon.

All statistical analyses were performed at the significance level $p < 0.05$ with the R software [38].

3. Results and Discussion

3.1. Fate of Isoproturon in Soil under Different Stress Conditions: Organic Amendment Addition and Compaction

The balances between the mineralised, $CaCl_2$ and CH_3OH extractable and non-extractable fractions ranged from 85.8% to 134.7% of the initial ^{14}C.

Both organic amendment and compaction did not affect the fate of isoproturon in soil ($p > 0.05$) (Figures 1 and 2). In any case, the main dissipation pathway was the formation of non-extractable residues: they reached more than 65% of the recovered ^{14}C 49 days after treatment (Figure 2). This was in agreement with the findings of Vieublé-Gonod et al. [5], Sørensen et al. [21] and Rodríguez-Cruz et al. [27]. There was no significant difference among treatments ($p > 0.05$) though the amounts of bound residues generally increase in the presence of organic amendment due to an increase in soil organic carbon content [39]. However, for low sorbed pesticides such as 2,4-D and carbetamide, which have similar mobility properties as isoproturon, no effect of compost addition on non-extractable residues was found [24,39,40]. The formation of non-extractable residues leads to a decrease in the toxicity and bioavailability of pesticides in the short term, but it could lead to further environmental contamination in the long term [39].

At the end of the incubation, the mineralisation of isoproturon ranged from 19.0% to 22.4% (in per cent of initial ^{14}C) which was consistent with previous observations [5,14,21,27]. There was no significant difference among the various soil treatments (control unamended and compost amended, compacted and not compacted) ($p > 0.05$) (Figure 1).

From 0 to 49 days, a decrease in the extractable ^{14}C was observed with a concomitant increase in the mineralisation and in the amounts of non-extractable residues (Figure 2). The amounts of extractable ^{14}C were very high at Days 0 and 7 (more than 90% and 45% of recovered ^{14}C, respectively), but low at Day 49 (<15%). At Day 0, most of the ^{14}C was extracted with $CaCl_2$ while, at Day 49, it was mainly extracted by methanol (Figure 2). The $CaCl_2$-extractable ^{14}C provides an estimate of the availability of pesticide residues, which could be directly related to the risk of pesticide leaching and consequently to the risk of groundwater contamination by the herbicide and/or its metabolites. Therefore, the results indicate that these risks are very high in the first days following the application of isoproturon as an

aqueous solution (Figure 2). The amounts of extractable ^{14}C (with $CaCl_2$ and methanol) were not significantly different ($p > 0.05$) among the four incubation conditions, suggesting a lack of effect of compost addition and/or compaction. Nevertheless, though not significant, the proportions of $CaCl_2$-extractable ^{14}C were lower in the amended soils (<50% of recovered ^{14}C) than in the not amended ones (>58% of recovered ^{14}C) at Day 0. Indeed, organic amendments are known to decrease the mobility of isoproturon because of an increase in its sorption due to an increase in soil organic matter content [8,41]. The effects of organic amendment addition on the fate of isoproturon also depend on the nature of the organic matter: Vieublé-Gonod et al. [5] evidenced that it was more affected by the presence of a municipal solid waste compost, mainly composed of labile organic matter which is directly available for microorganisms, than of a co-compost of sewage sludge and green waste with stabilised organic matter.

Chromatographic analyses showed that the remaining amounts of isoproturon after 49 days ranged from 28.9 ± 7.3% of extractable ^{14}C in the amended not compacted soil to 47.0 ± 10.4% in the unamended not compacted soil (Figure 3), but there was no significant difference among the various treatments ($p > 0.05$). The degradation of isoproturon mainly led to the formation of the monodemethyl-isoproturon metabolite, from 37.7 ± 22.7% in the control not compacted soil to 65.9 ± 5.3% in the amended not compacted soil ($p > 0.05$) (Figure 3). These results are in agreement with those of many authors who observed the formation of monodemethyl-isoproturon as the major metabolite of isoproturon [20–22]. The didemethyl-isoproturon and 4-isopropyl-aniline metabolites were not observed in this work while some minor polar metabolites were detected, but they did not exceed a total of 15% (Figure 3). In general, the degradation of isoproturon is initiated by successive N-demethylations of the N,N-dimethylurea side chain, leading first to the metabolite monodemethyl-isoproturon then to the didemethyl-isoproturon, before cleavage of the urea side chain resulting in the 4-isopropyl-aniline metabolite, but a metabolic pathway involving cleavage of the methylurea group of monodemethyl-isoproturon directly to 4-isopropyl-aniline was also observed and may have occurred in our experiment [21]. As a result, organic amendment and/or compaction did not modify the formation rates nor the nature of the metabolites of isoproturon. This was consistent with the findings of Vieublé-Gonod et al. [5] and Perrin-Ganier et al. [42] for compost addition, and with those of Mamy et al. [20] for compaction.

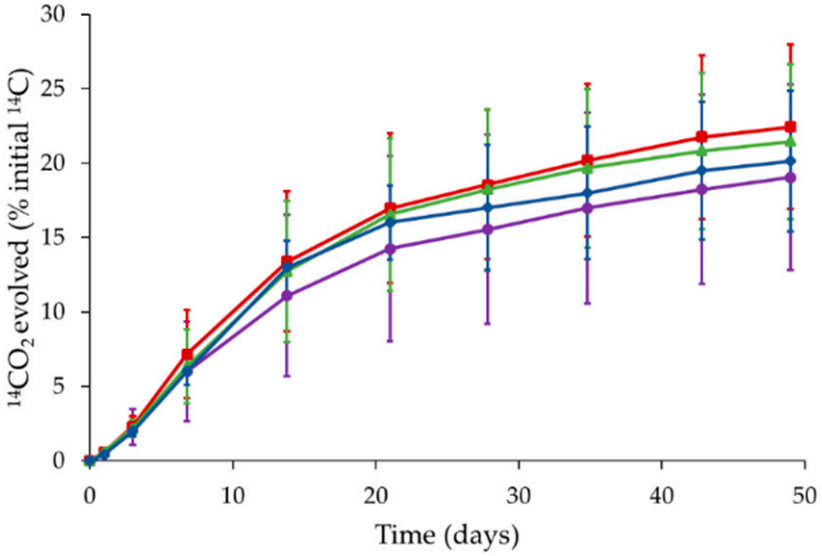


Figure 1. Mineralisation kinetics of isoproturon in control and compost amended soils, not compacted and compacted: ■ Control (unamended), Compaction; ▲ Compost amended, No compaction; ◆ Compost amended, Compaction; ● Control (unamended), No compaction.

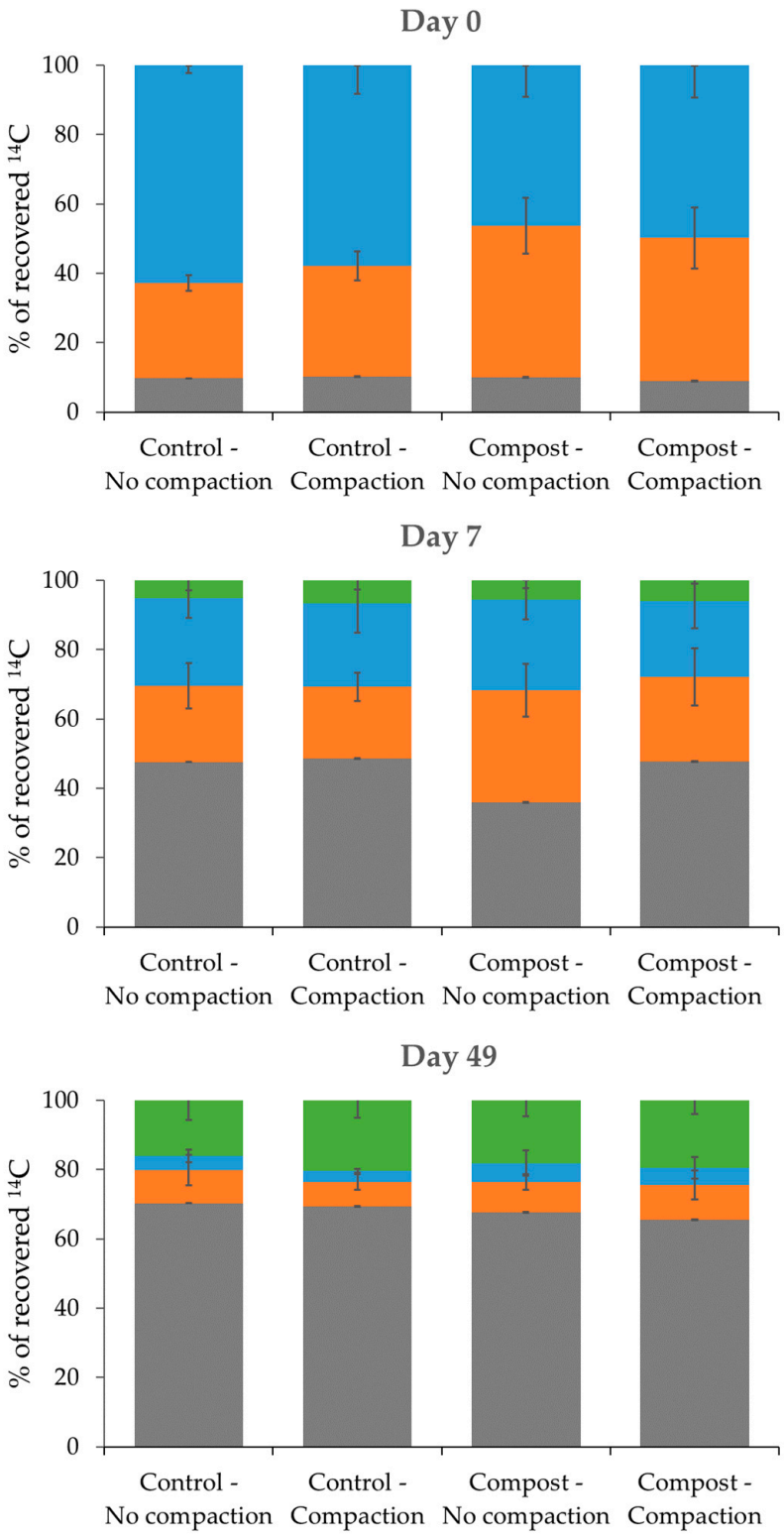


Figure 2. Overall balance of the fate of isoproturon after 0, 7 and 49 days in control (unamended) and compost amended soils, not compacted and compacted: Mineralised; $CaCl_2$ extractable; CH_3OH extractable; Non-extractable (bound) residues.

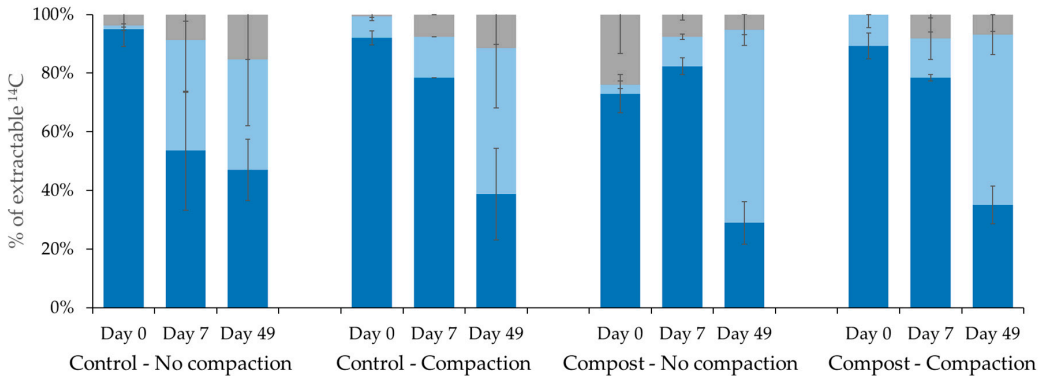


Figure 3. Chromatographic analysis of combined $CaCl_2$ and CH_3OH extracts after 0, 7 and 49 days in control (unamended) and compost amended soils, not compacted and compacted: Isoproturon; Monodemethyl-isoproturon; Other metabolites.

Overall, the combination of organic amendment addition and compaction stresses did not modify the fate of isoproturon in soil. The unexpected lack of effect of compost can be due to the physico-chemical characteristics of this amendment [5], to the lack of significant change in the soil physical properties after compost addition (Table 2), or because the effect of organic amendment on microbial activity strongly varies according with the time of sampling and the delay with the last amendment which was one year long in the present work [4,43]. Despite compaction changes the physical properties of the soil (Table 2), it had no effect on the fate of isoproturon (Figures 1 and 2) probably because the reduction in porosity did not affect the habitable pore space accessible to degrading microbial communities [44,45]. Vieublé-Gonod et al. [14] showed that the spatial distribution of isoproturon in soil cores containing compost with different localisations had a significant effect on the mineralisation and fate of isoproturon. In our experiment, isoproturon was applied on the soil surface (as often in crop treatment), thus the herbicide might have been not uniformly distributed in the soil samples and mainly been located on the surface. Its fate would have been therefore less influenced by compaction. However, this is representative of field conditions, particularly if no rainfall occurs to facilitate the infiltration of pesticides after their application [20].

3.2. Soil Enzyme Activity under Different Stress Conditions: Herbicide and Organic Amendment Additions and Compaction

The urease activity showed the same trends in all incubation conditions, with an increase from 0 to 60 days (Figure 4), which is consistent with the findings of many authors [46–50].

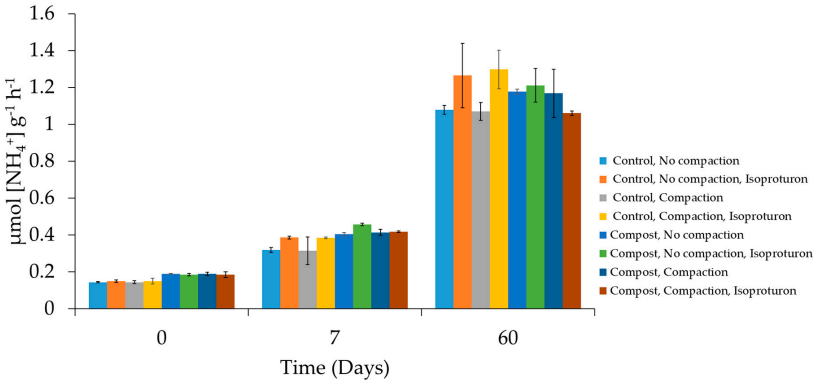


Figure 4. Urease enzyme activity in control and compost amended soils, not compacted and compacted, not treated and treated with isoproturon after 0, 7 and 60 days.

In both compacted and not compacted soils without isoproturon, the addition of compost increased the urease activity ($p < 0.05$), as observed in several works [49–52] (Figure 4 and Table 3). Organic amendments are known to stimulate the microbial activity and to have a high level of substrates capable of activating enzyme synthesis [4,51], and positive correlations between soil organic matter content and urease activity were frequently observed [53].

Table 3. Effect of compost amendment and compaction on the impact of isoproturon measured through the urease enzyme activity. s: significant effect (Wilcoxon test, $p < 0.05$); ns: no significant effect ($p > 0.05$).

	Conditions of Soil Incubation	Urease Activity
Effect of compost (no isoproturon)	No compaction (compost/no compost)	s
	Compaction (compost/no compost)	s
Effect of compaction (no isoproturon)	No compost (compaction/no compaction)	ns
	Compost (compaction/no compaction)	ns
Effect of isoproturon	No compost, no compaction (isoproturon/no isoproturon)	s
	No compost, compaction (isoproturon/no isoproturon)	s
	Compost, no compaction (isoproturon/no isoproturon)	ns
	Compost, compaction (isoproturon/no isoproturon)	ns
Effect of compaction on the impact of isoproturon	No compost (compaction/no compaction)	ns
	Compost (compaction/no compaction)	ns
Effect of compost on the impact of isoproturon	No compaction (compost/no compost)	ns
	Compaction (compost/no compost)	ns

Although soil compaction is associated with changes in physical properties such as a decrease in porosity and air content and an increase in water content (Table 2), it had no effect on the urease activity ($p > 0.05$) (Figure 4 and Table 3). This agrees with numerous works showing that compaction was without effect on various enzyme activities in soil surface [15,20,54] because it has little consequences on the microbial communities [44,45,54].

The application of isoproturon at recommended dose significantly increased the urease activity in unamended soils, either compacted or not, compared to the untreated ones ($p < 0.05$) (Figure 4 and Table 3). This positive effect of isoproturon on urease activity was also observed by Nowak et al. [33]. Similarly, some other urea herbicides, diuron and nicosulfuron, were shown to increase the urease activity although in a transitory manner [35,55–57]. The increase in urease following soil treatment with isoproturon may have several explanations: (1) isoproturon (and its metabolites) may act as an organic carbon source to some microorganism species which increased microbial biomass and in turn increased the activity of urease [35,58]; (2) isoproturon presents an urea structure, and it could be used as a substrate for the urease enzyme as observed for diuron and nicosulfuron [55–57]; and (3) the adsorption of isoproturon in soils is moderate [22,24,27] but there may be some competition between immobilised enzymes and isoproturon, as urease is bonded to the organic matter and mineral particles of soil [59], with the subsequent release of free enzymatic molecules from matrices [52]. Finally, the increase in urease activity from 0 to 7 then from 7 to 60 days may show that isoproturon metabolites would have similar effect on urease than isoproturon (Figures 2 and 3). Karas et al. [34] evidenced that monodemethyl-isoproturon and didemethyl-isoproturon inhibited various enzyme activities while isoproturon did not, but the urease activity was not considered. In amended soils, on the contrary, isoproturon and its metabolites had no effect on the urease activity either in compacted and not compacted soils ($p > 0.05$) (Figure 4 and Table 3). It seems that the compost acts as a buffer with regard to the effects of isoproturon and metabolites on urease.

Compost addition and compaction together did not modify the effect of isoproturon on urease activity ($p > 0.05$) (Figure 4 and Table 3). In addition to the lack of effect of compaction on microbial

communities and processes (see above), this can be explained by incubation conditions not limiting for the biological activity and/or by the delay between the last application of organic amendment and the soil sampling (one year) [4,5,43,60].

4. Conclusions

The effect of multiple stresses, organic amendment addition and compaction, on the fate and impact of isoproturon in soil was studied under laboratory conditions. The fate of isoproturon did not change compared to unamended and not compacted soils as the mineralisation and the amounts of extractable and non-extractable residues were not significantly different, neither the nature of metabolites nor their formation rates. In general, the effect of isoproturon on the urease activity was not modified by organic amendment and/or by compaction. However, isoproturon was shown to significantly increase the urease activity in the unamended (compacted and not compacted) soils, while it had no effect in the amended ones. It seems that the organic amendment could act as a buffer with regards to the effects of isoproturon on urease activity, either in compacted and not compacted soils. Overall, the results obtained in this work suggest that the fate and impact of isoproturon in soils will not change following compaction and/or organic amendment addition, neither the risks for the environment (groundwater contamination, biological activity...).

Nevertheless, additional research is needed to better understand the role of organic amendment addition and compaction in the fate and effects of pesticides. In particular, further studies should consider various organic amendments, pesticides, and biological indicators (e.g., other enzyme activities, microbial diversity, fatty acids...), as well as other localisations in soils, such as plough pan, clods, etc., as interfurrows constituted a special local environment with high level of microbial biomass and respiration levels [5].

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