No evidence for effect of soil compaction on the degradation and impact of isoproturon

Laure Mamy, Pauline Vrignaud, Nathalie Cheviron, Francois Perreau, Mehdi Belkacem, Agathe Brault, Sébastien Breuil, Ghislaine Delarue, Jean Pierre Petraud, Isabelle Touton, et al.

No evidence for effect of soil compaction on the degradation and impact of isoproturon

Laure Mamy * · Pauline Vrignaud · Nathalie Cheviron · François Perreau · Mehdi Belkacem · Agathe Brault · Sébastien Breuil · Ghislaine Delarue · Jean-Pierre Pétraud · Isabelle Touton · Christian Mougin · Véronique Chaplain

INRA, UR 251 PESSAC, Route de Saint Cyr 78026 Versailles, France

Abstract Soil is damaged by several threats and, among them, chemical contamination by pesticides and compaction. However, the effect of compaction on the fate of pesticides in soil and their impact on its biological functioning is unknown. Therefore, the aim of this work was to study the effect of soil compaction on (1) the degradation of one herbicide, isoproturon (2) the impact of this herbicide measured through one enzyme activity (β-glucosidase) involved in C cycle in soil. Undisturbed soil samples were prepared at different bulk densities, treated with isoproturon then incubated at 18°C in darkness for 63 days. Our results showed that soil compaction did not modify significantly the degradation of isoproturon, neither the formation rates nor the nature of its metabolites. Moreover, compaction did not modify the impact of isoproturon on β-glucosidase activity. To our knowledge, these are the first results concerning the interactions between soil compaction and the degradation and impact of a pesticide.

Keywords Bulk density · Undisturbed soil sample · β-glucosidase · Half-life · Metabolite

Introduction

Soil is a non-renewable resource which performs many functions and delivers services vital to human activities and ecosystems survival. However, the capacity of soil to keep on fully performing its broad variety of crucial functions is damaged by several threats, and among them chemical contamination by pesticides and compaction (Directive 2006/0086/EC). 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 (Dick, 1997;

Hamza and Anderson 2005). As a consequence, these modifications might change the fate and impact of pesticides in soils. But, to the best of our knowledge, no results have been yet published in the literature.

Isoproturon (N,N-dimethyl-N'-[4-(1-methylethyl)phenyl]-urea) is a selective herbicide used against annual grasses and broad-leaved weeds. It is one of the most used herbicide in European cereals production (Sørensen et al. 2003). In the soil, the degradation of isoproturon is well known: it is mainly biological (Sørensen et al. 2003) with laboratory half-life ranging from 6 to 223 days (Walker et al. 2002; Alletto et al. 2006). The main degradation pathway leads to the formation of three metabolites: monodesmethyl-isoproturon, didesmethyl-isoproturon and 4-isopropylaniline (Sørensen et al. 2003; Alletto et al. 2006). On the contrary, the effects of isoproturon on the biological functioning of soil and on soil organisms were less studied. Some results showed a low ecotoxicity of isoproturon (Agritox 2009).

Enzyme activities are relevant indicators of soil health that have been successfully used to discriminate a wide range of soil management practices including amendments, xenobiotics spreading, and landscapes disturbance (Dick 1997). Among these enzymes, β-glucosidase has a critical role in releasing low molecular weight sugars that are important sources of energy for micro-organisms, and catalyzes the hydrolysis of cellobiose thus playing a major role in the initial phases of the decomposition of organic C compounds (Eivazi and Tabatabai 1988; Dick 1997).

Therefore, the aim of this work was to study the effect of soil compaction on (1) the degradation of isoproturon, (2) the impact of this herbicide assessed through the β-glucosidase activity.

Experimental

Soil samples

Undisturbed soil samples were taken, using plastic rings (5 cm diameter, 2 cm height), in the 2-4 cm layer of a plot located in a French experimental site (La Cage, INRA, Versailles, France). The samples were randomly collected in the totality of the plot in April 2008. The soil is a loam with 17.3% clay, 61.1% loam, 21.6% sand, 1.69% of organic matter and pH in water of 7.3. The field was cropped with oilseed rape under intensive agriculture practices and was previously treated with isoproturon in 2002, 2004 and 2006.

The soil samples were prepared at three realistic levels of compaction (no, moderate and high) so that the increase in bulk density was 0.3 g cm^{-3} between no and high compaction, as observed following wheeling (Défossez et al. 2003) (Table 1). Moderate and high compaction

resulted in a reduction in soil volume of 10% and 20%, respectively. Compaction was applied uniformly on the soil surface with a cylinder having a diameter equal to the inner diameter of the cylinders.

For each level of compaction, the physical properties of the soil samples were characterized (Table 1): the bulk densities (ρ) of three randomly selected samples were measured, then the porosity (P), the pore volume (Vp), the water (Vw) and air (Va) contents, and the degree of saturation (s) were calculated as follows:

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

where 2.65 is the particle density of soil solids;

Vp (cm^3) = Volume of soil sample – (Mass of dry soil / 2.65);

Vw (cm^3) = Initial soil water content + Volume of isoproturon added;

Va (cm^3) = Vp – Vw;

s (%) = (Vw / Vp) × 100.

The average dry weight of twelve soil samples was measured and was equal to 51.3 ± 4.8 g.

Chemicals

Isoproturon and two of its metabolites in soil (monodesmethyl-isoproturon, didesmethyl-isoproturon) were purchased from Dr Ehrenstorfer GmbH (Augsburg, Germany) with 99.0%, 99.5% and 99.0% purity for isoproturon, monodesmethyl-isoproturon and didesmethyl-isoproturon, respectively. A 20 mg L^{-1} aqueous solution of isoproturon was prepared in water and methanol (99.7/0.3; v/v). In addition, standard solutions of isoproturon and metabolites were prepared in acetonitrile at concentrations ranging from 5 to 50 mg L^{-1}.

Incubation procedure

The samples were placed in 500 mL hermetically stoppered jars. Two millilitres of isoproturon solution were added on the surface of soil to reach a final concentration of herbicide of 0.75 mg kg^{-1} dry soil, corresponding approximately to application rates of 1 kg ha^{-1}. Isoproturon-free not compacted soil samples were also incubated as control to study the effect of the herbicide on β-glucosidase activity (see below).

Each jar contained a vial with 10 mL of water to keep the relative humidity constant. Soil samples were incubated at 18 ± 1°C in the dark, for 63 days. Jars were opened weekly to maintained aerobic conditions, and soil water content was periodically adjusted by weighing each sample and adding the required amount of water. At 0, 2, 7, 14, 28, 63 days after the beginning of

the incubation, soil samples were analysed for isoproturon and its metabolites, and enzyme activity (see below). Six replicates were done for each compaction, and sampling dates (three replicates for chemical analyses and three replicates for enzyme activity analyses).

Chemical analysis

Soil samples were sequentially extracted three times (for 24, 5 and 24 hours) with 150 mL of methanol. They were mechanically shaken at 20 ± 2°C in the dark and then centrifuged for 20 minutes at 1800 × g. Average recovery of isoproturon was 93.3 ± 6.0%.

The three successive methanol extracts of each soil sample were pooled. They were concentrated using a rotary evaporator (Büchi, Flawil, Switzerland) under vacuum, and filtered through a syringe-PTFE filter (0.45 µm, Millipore, Molsheim, France).

HPLC-UV analyses were performed with a Dionex (Voisins le Bretonneux, France) system, including an ASI100 autosampler, a P580 Pump, a STH585 column oven and a UVD340S UV-diode-array detector. Separation of isoproturon was conducted on an Nucleodur C8 column (250 × 4.6 mm i.d., 5 µm granulometry; Macherey-Nagel, Hoerdt, France), with a gradient of water/acetonitrile as mobile phase at 1 mL min^{-1}. Samples were kept at 4°C in the autosampler before being injected (30 µL). Quantification was done at 250 nm.

β-glucosidase activity

The activity of *β*-glucosidase (EC 3.2.1.21) was measured according to the method of Eivazi and Tabatabai (1988) at the soil pH. Each soil sample was homogenized, then 1 g of soil was dissolved in 4 mL water (MilliQ, Millipore) followed by the addition of 1 mL of 0.05 M *p*-nitrophenyl-*β*-D-glucopyranoside. Samples were vortexed and incubated for 2 hours at 25°C. One millilitre of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH were then added and the mixture was agitated. Finally, 1 mL of the suspension was centrifuged 2 min at 12000 × g and the absorbance of the supernatant was determined by UV spectrophotometer at 410 nm. Three replicates were done for each soil sample.

Data analysis

Herbicide degradation was described using single first-order kinetics:

$C(t) = C_0 \exp(-kt)$

where $C(t)$ is amount of remaining extractable herbicide (% of initial applied dose) at time t, C_0 is initial extractable percentage of herbicide, and k the first-order rate constant of degradation (day^{-1}).

The values of k were determined by non-linear regression (Marquardt-Levenberg algorithm, SigmaPlot, SPSS Inc., Chicago, IL, USA) and were used to calculate the herbicide degradation half-lives ($T_{1/2}$) with the following equation:

$T_{1/2}$ (days) = ln(2) / k.

Statistical analyses were done with XLstat (AddinSoft, Brooklyn, NY, USA). The impact of isoproturon on β-glucosidase activity was tested by the Mann-Whitney test, and the effects of soil compaction on isoproturon degradation and β-glucosidase activity were tested by the Kruskal-Wallis test. In addition, the Dunn test was done with the correction of Bonferroni for pairwise comparison between compaction conditions. The effects were considered significant when $P < 0.05$.

Results and discussion

Effect of soil compaction on the degradation of isoproturon

In any case, the degradation of isoproturon fitted well a first-order kinetic ($r^2 > 0.89$) (Table 2, Fig. 1a). However, the model deviated slightly from observed values at the end of incubation, probably because of lowest availability of isoproturon with time due to long-term sorption phenomenon, including non-extractable residues formation (Alletto et al. 2006). Several other models (like bi-exponential) were tested to fit the data but they did not allow a better description of the observations (data not shown).

Degradation did not evidence a lag phase indicating that no adaptation of microorganisms was necessary, and that isoproturon was co-metabolized. The half-lives of isoproturon, according to the adjustment with the first-order kinetic model, were 11.9, 17.3 and 10.5 days in the not, moderately and highly compacted soils, respectively (Table 2). They are in agreement with the lowest values found in the literature probably because of accelerated degradation due to several previous applications of isoproturon in the field in combination with a soil pH higher than 7 (Cox et al. 1996; Walker et al. 2002).

No significant modification in the degradation of isoproturon with compaction was observed (Fig 1a), except a difference between the moderately and not compacted soils after 63 days. Though compaction can facilitate the contact between isoproturon and soil constituents therefore decrease its bioavailability for microorganisms (by adsorption phenomena) (Sørensen et al. 2003), there was no increase in the persistence of isoproturon. On the contrary, the degradation of isoproturon proceeded slightly faster in the highly compacted soil (Table 2), probably because the water content (or degree of saturation) was higher than in the moderately and not compacted soils (Table 1) (Alletto et al. 2006).

The lack of significant modification in isoproturon degradation with soil compaction can be related to the results of Shestak and Busse (2005) and Gregory et al. (2007) showing that compaction generally did not affect microbial communities and processes (in laboratory compacted soils and in durably compacted soils in the field) because of reduction in porosity favouring smaller, habitable pore volume accessible primarily to bacteria and fungi. Soil respiration is also known to be unaffected if more than 10% of the pore volume is air-filled (Table 1) (Shestak and Busse 2005). As the degradation of isoproturon is mainly biological (Sørensen et al. 2003), it is not modified by soil compaction. In addition, because applied on soil surface (as often in crop treatment), 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.

Independently of compaction, the formation of the monodesmethyl-isoproturon metabolite was observed (Fig. 1b) but not that of didesmethyl-isoproturon. This is in agreement with other results concerning the degradation of isoproturon in the soil (Sørensen et al. 2003; Alletto et al. 2006). The maximum amounts of monodesmethyl-isoproturon were 5.1 ± 0.8% in the not compacted soil after 7 days, 5.0 ± 0.3% in the moderately compacted soil at the end of incubation and 3.8 ± 0.6% in the highly compacted soil 14 days after treatment. In addition, two unidentified polar metabolites were detected (maximum amounts of 6.2 ± 0.4% after 14 days and 10.5 ± 4.1% after 28 days) that can be 4-isopropylaniline and/or hydroxylated metabolites (Sørensen et al. 2003; Alletto et al. 2006). The formation kinetics of these three metabolites were not significantly different among the three levels of compaction with the exception of the amounts of monodesmethyl-isoproturon in the moderately compacted soil that are different from those of the not and highly compacted soils after 63 days. This can be related to the different behaviour of isoproturon in this soil 63 days after treatment (see above).

In the conditions of this experiment, our results showed that soil compaction did not modify the degradation of isoproturon, neither the formation rates nor the nature of its metabolites. Therefore, the persistence of pesticides in the field is not expected to increase under zones that are compacted because of traffic, tillage or short rotations.

Effect of soil compaction on β-glucosidase activity

The kinetics of β-glucosidase activity showed the same trends in all experiments (Fig. 2a, b). There was an increase in the activity till 28 days followed by a decrease till the end of incubation. Similar trends were observed in soils treated with fungicides like mefenoxam (Demanou et al. 2006) but not

with the herbicide 2,4-D (Bécaert et al. 2006), however, the applied dose was considerably higher than in our study. The increase may reflect the degradation of dead cell walls after isoproturon application as β-glucosidase is involved in the degradation steps of complex sugars which are important constituents of plant and fungal cell walls (Demanou et al. 2006). The following decrease may result from a decrease in microbial biomass (Waldrop et al. 2000) rather than exhaustion of C-substrates and nutrients (Monkiedje et al. 2002) or air-drying of soil (Bandick and Dick 1999) as the soil moisture was constant all along the experiment.

No significant impact of isoproturon on the β-glucosidase activity was observed compared to the control (Fig. 2a). Unlike what was evidenced for other pesticides (Monkiedje et al. 2002; Bécaert et al. 2006), there was no decrease in β-glucosidase activity after application of isoproturon. The lack of effect of isoproturon on the activity can be due to the adaptation of microorganisms following the previous applications of the herbicide in the field (Cox et al. 1996).

In general, compaction did not modify significantly the impact of isoproturon on β-glucosidase (Fig. 2b). However, the activity in the moderately compacted soil was different from those of the not and highly compacted soils at the beginning of incubation (day 0), and after 14 and 63 days. The initial difference in the activity might explain the differences in isoproturon degradation and monodesmethyl-isoproturon formation in this soil compared to the other soils (see above), as soil enzymes are the mediators of detoxification of xenobiotics (Dick 1997). Nevertheless, the general trend showed no modification in the β-glucosidase activity following compaction. This result is in agreement with previous studies showing that compaction was without effect on enzyme activities (dehydrogenase, protease, phosphatase), and is of little consequence on the microbial communities (Shestak and Busse 2005; Gregory et al. 2007; Tan et al. 2008).

As a result, the impact of isoproturon on β-glucosidase activity was not affected by soil compaction, suggesting that in the field, the biological functioning of soil might not be different under the compacted zones and the less compacted ones.

Conclusion

The effects of soil compaction on the degradation and impact of isoproturon were studied in laboratory. Our experimental conditions were closed to the field ones: undisturbed soil samples, application of pesticide on soil surface, same water contents in all samples. To our knowledge, these are the first results concerning the interactions between soil compaction and the degradation and impact of a pesticide. In the conditions of this experiment, compaction did not modify the degradation of isoproturon, neither the formation rates nor the nature of its metabolites. In addition, compaction did not modify the impact of isoproturon on β-glucosidase activity. Therefore, our

results suggest that under soils compacted zones (due to traffic, tillage or short rotations), the persistence of pesticides should not increase and their impact should not be modified. However, further studies measuring additional biological indicators (phospholipids fatty acids, microbial biomass…), studying the relationship between compaction and the degree of soil saturation, and studying the overall balance of the fate of pesticides (mineralization, extractability, formation of non-extractable residues) should be performed.

Acknowledgement The authors are grateful to Dr Jérémie D. Lebrun for constructive comments and to Dr Florent Allais, Rachid Benabdallah and Cédric Repinçay of INRA for complementary chemical analyses.

References

Agritox (2009) http://www.dive.afssa.fr/agritox/ Accessed 9 December 2009

Alletto L, Coquet Y, Benoit P, Bergheaud V (2006) Effects of temperature and water content on degradation of isoproturon in three soil profiles. Chemosphere 64:1053-1061

Bandick AK, Dick RP (1999) Field management effects on soil enzyme activities. Soil Biol. Biochem. 31:1471-1479

Bécaert V, Samson R, Deschênes L (2006) Effect of 2,4-D contamination on soil functional stability evaluated using the relative soil stability index (RSSI). Chemosphere 64:1713-1721

Cox L, Walker A, Welch SJ (1996) Evidence for the accelerated degradation of isoproturon in soils. Pestic. Sci. 48:253-260

Défossez P, Richard G, Boizard H, O'Sullivan MF (2003) Modeling change in soil compaction due to agricultural traffic as a function of soil water content. Geoderma 116:89-105.

Demanou J, Sharma S, Dörfler U, Schroll R, Pritsch K, Njine T, Bausenwein U, Monkiedje A, Munch JC, Schloter M (2006) Structural and functional diversity of soil microbial communities as a result of combined applications of copper and mefonoxam. Soil Biol. Biochem. 38:2381-2389

Dick RP (1997) Soil enzyme activities as integrative indicators of soil health. In: Pankhurst CE, Doube BM, Gupta VVSR (eds) Biological indicators of soil health, CAB international, Wallingford, pp 121-156

Directive 2006/0086/EC (2006) Directive of European Parliament and of the Council establishing a framework for the protection of soil and amending Directive 2004/35/EC, Brussels 22.9.2006, COM 232 final

Eivazi F, Tabatabai MA (1988) Glucosidases and galactosidases in soils. Soil Biol. Biochem. 20:601-606

Gregory AS, Watts CW, Whalley WR, Kuan HL, Griffiths BS, Hallett PD, Whitmore AP (2007) Physical resilience of soil to field compaction and the interactions with plant growth and microbial community structure. Eur. J. Soil Sci. 58:1221-1232

Hamza MA, Anderson WK (2005) Soil compaction in cropping systems. A review of the nature, causes and possible solutions. Soil Till. Res. 82:121-145

Monkiedje A, Olusoji Ilori M, Spiteller M (2002) Soil quality changes resulting from the application of the fungicides mefenoxam and metalaxyl to a sandy loam soil. Soil Biol. Biochem. 34:1939-1948

Shestak CJ, Busse MD (2005) Compaction alters physical but not biological indices of soil health. Soil Sci. Soc. Am. J. 69:236-246

Sørensen SR, Bending GD, Jacobsen CS, Walker A, Aamand J (2003) Microbial degradation of isoproturon and related phenylurea herbicides in and below agricultural fields. FEMS Microbiol. Ecol. 45:1-11

Tan X, Chang SX, Kabzems R (2008) Soil compaction and forest floor removal reduced microbial biomass and enzyme activities in a boreal aspen forest soil. Biol. Fertil. Soils 44:471-479

Walker A, Bromilow RH, Nicholls PH, Evans AA, Smith VJR (2002) Spatial variability in the degradation rates of isoproturon and chlorotoluron in a clay soil. Weed Res. 42:39-44

Waldrop MP, Balser TC, Firestone MK (2000) Linking microbial community composition to function in a tropical soil. Soil Biol. Biochem. 32:1837-1846

Table 1. Soil physical properties as a function of compaction

Compaction	Bulk density ($g\ cm^{-3}$)	Porosity	Pore volume (cm^3)	Water content (cm^3)	Air content (cm^3)	Degree of saturation (%)
No	1.33 ± 0.16	0.50 ± 0.06	19.6 ± 2.4	8.6 ± 1.1	10.9 ± 3.3	45.3 ± 11.4
Moderate	1.45 ± 0.02	0.45 ± 0.01	15.9 ± 0.3	8.2 ± 0.3	7.7 ± 0.5	51.8 ± 2.6
High	1.57 ± 0.13	0.41 ± 0.05	12.8 ± 1.6	8.0 ± 1.5	4.7 ± 3.1	64.6 ± 18.5

Table 2. Isoproturon degradation rates, initial concentrations, halflives and correlation coefficients estimated according to single firstorder kinetic

Compaction	Degradation rates (day^{-1})	Initial concentrations (% of isoproturon applied)	Half-life (days)	r^2
No	0.058 ± 0.008	87.6 ± 4.9	11.9	0.91
Moderate	0.040 ± 0.006	87.5 ± 4.4	17.3	0.89
High	0.066 ± 0.008	93.7 ± 4.6	10.5	0.92

Fig. 1 Degradation kinetics of isoproturon (a) and formation kinetics of monodesmethyl-isoproturon (b) in (—●—) not compacted, (---■---) moderately compacted and (– –▲– –) highly compacted soil. For isoproturon, lines are adjustment with first-order kinetics. The degradation of isoproturon and the formation rates of its metabolite were not significantly modified by compaction.

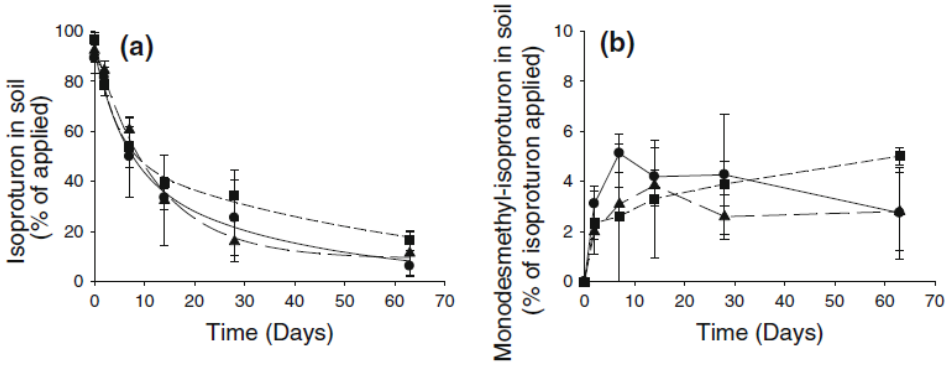

Fig. 2 *β*-glucosidase activity in (a) not compacted soil treated (—●—) or not (···○···) with isoproturon; and (b) (—●—) not compacted, (---■---) moderately compacted, (– –▲– –) highly compacted soil treated with isoproturon. The *β*-glucosidase activity was not significantly affected by isoproturon and by compaction.