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Environmental Fate of Trifluralin, Procymidone, and Chlorpyrifos in Small Horticultural Production Units in Argentina

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Abstract Pesticide biodegradation was studied in soil samples of a representative small periurban production unit (Moreno District, Argentina). The mean periods required for the 50 % dissipation of chlorpyrifos (16 days±1 day), procymidone (3.7 days±0.6 day), and trifluralin (3.6 days±0.6 day) were significantly lower than those measured for reference soil samples of a close location, using doses similar to the manufacturer's recommendation. A preliminary screening scheme for pesticide-degrading bacteria on horticultural soil allowed the isolation of nine culturable bacterial strains, eight of which belonged to *Pseudomonas* genus. In order to consider the influence of the variability of soil properties on the biodegradation results, humidity, organic matter, conductivity, pH, water retention volume, density, respiration, and total phosphorous content were studied for different soil samples, finding no significant differences in the performed analysis. Overall, although the horticultural activity alters the natural soil, pesticide contamination effects could be reversed by the autochthonous microbial community.

Keywords Horticulture · Soil · Biodegradation · Chlorpyrifos · Procymidone · Trifluralin

1 Introduction

It has been pointed out that the main impacts of agriculture on soil are erosion, salinization, compaction, reduction of organic matter, and diffuse pesticide pollution (Zalidis et al. 2002). At the same time, there is a general perception that soil quality is a critical value for a sustainable agriculture practice. In particular, horticulture is an important agricultural activity that plays a strategic role providing fresh fruit and vegetables to big cities (UNDP 1997). Frequently, their production units are located in "green belts" close to urban concentrations (Paquette and Domon 1999). This is the case of Buenos Aires City, where small farm clusters (less than 5 ha each) are mostly located in the western districts (Fig. 1a), sharing the territory with floriculture enterprises and experiencing the consequences of the city's expansion (Craig et al. 2002; Hughes et al. 2008). In this area, horticulture has been developed with a high crop rotation and an intensive use of pesticides.

In this context, one important environmental issue is to assess if the use of pesticides could affect nontarget

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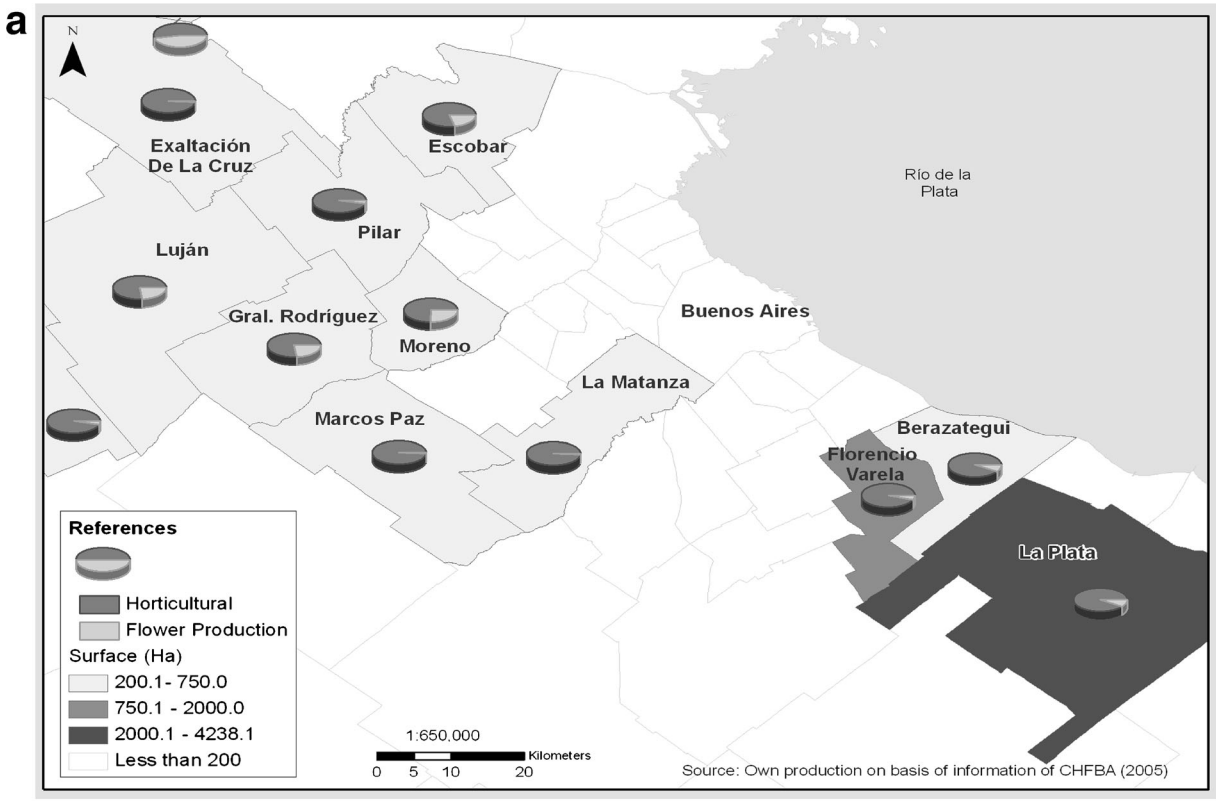

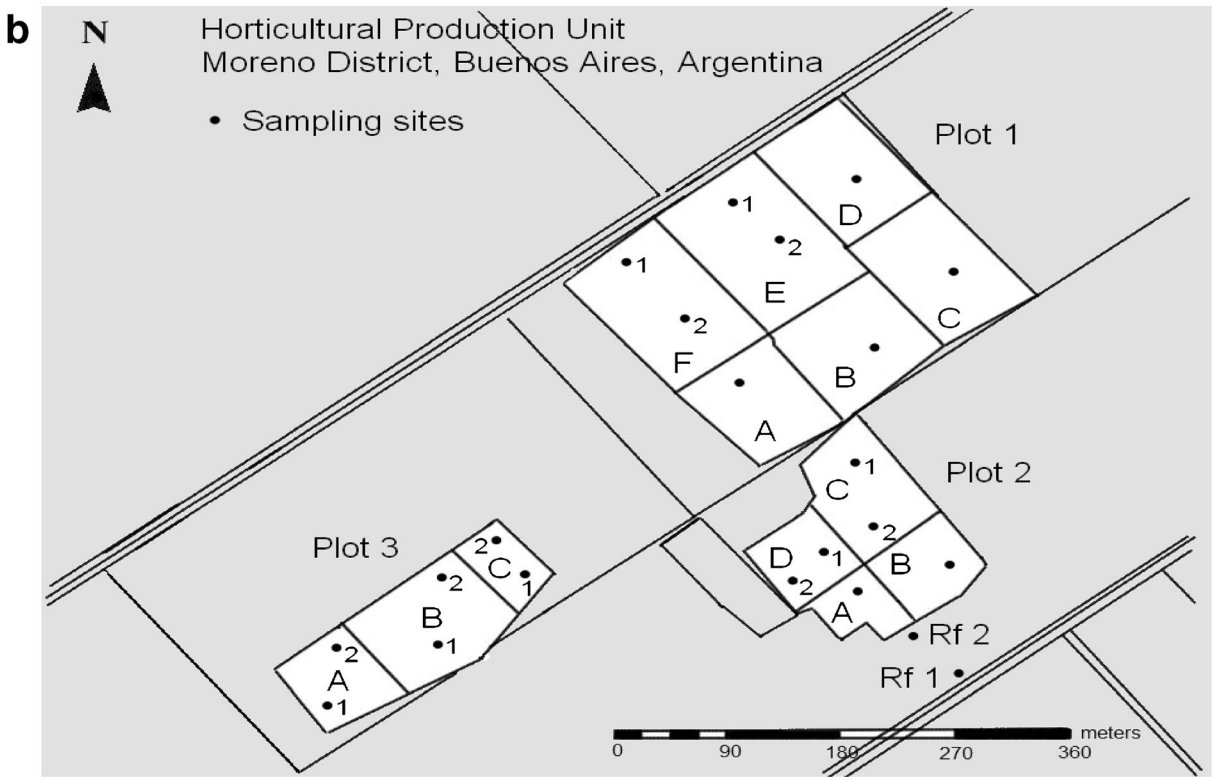

Fig. 1 **a** Location of horticultural and floricultural production units close to Buenos Aires City. **b** Location of farm sampling points for the spatial study

systems, like water (Licciardello et al. 2011; Oliver et al. 2012) and soil (González et al. 2010). Regarding the latter matrix, we have recently reported that during pesticide application in horticultural and floricultural production units, the soil was the most exposed nontarget subsystem (Querejeta et al. 2012). These results are in accordance with persistent pesticide contamination in areas under intensive horticultural activity (Goncalves et al. 2006; Rosendahl et al. 2009). In this sense, the study of the residence and degradation times of a pesticide in agricultural soils is an issue of great importance in terms of its potential accumulation and the consequent environmental damage.

Taking into account that the soil is a reservoir of microorganisms, microbial diversity is an indicator of its health (Brussaard et al. 2007); therefore, the impact of pesticide application on the microbial community is a relevant matter. It is generally accepted that pesticide degradation in soil is mainly microbiological in nature (Fomsgaard 1995) and that xenobiotic degradation is a narrow niche function performed by a small subset of soil microbiota (Girvan et al. 2005). It has also been noted that in chemically perturbed soils, microbial community adaptation is a well-known phenomenon (Spain and Van Veld 1983). For example, after one chlorotalonil application on soil, the microbial community changed, experiencing both enhancements and inhibitions of microorganisms (Siegler and Turco 2002). It has also been reported that the degradation of the fungicide carbendazin by soil microcosms was accelerated by increasing its application frequency, indicating a certain microbial adaptation (Yunlong et al. 2009). In the same trend, the repeated application of *N*,*N*′-dibutylurea, a benomyl (fungicide) breakdown product, accelerated its degradation in soil (Bischoff et al. 2005). Other reports accounted for different impacts of pesticide application on soil. For example, Hua et al. (2009) reported a small but significant inhibition of soil microbial communities after chlorpyrifos application, observing microorganism rehabilitation or recolonization a long time after exposure.

On the other hand, the microbial community's adaptation usually enhances product degradation. For example, it has been shown that chlorpyrifos can be either degraded by axenic cultures, as described for *Pseudomonas* genus (Singh et al. 2003) and *Bacillus pumilus* (Anwar et al. 2009), or co-metabolized by bacterial consortia (Sasika et al. 2012). In the case of trifluralin biodegradation, while bacterial metabolization seems to play a minor role (Jolley et al. 1990), fungal herbicide reduction was observed (Zayed et al. 1983). It has also been reported that consecutive procymidone applications on soil increased its degradation rates (García-Cazorla and Xirau-Vayreda 1998), though, as far as we know, no characterized strains were related to its biodegradation.

It has been pointed out that the variability in pesticide degradation rates between different soils could be caused mainly by the heterogeneity of soil properties (Walker et al. 2001). Thus, the selection of a set of experimental soil properties such as pH, organic C content, and bulk density was considered important for establishing soil variability. Taking into account these ideas, the aim of our work was focused on (1) a case study of pesticide degradation rates in horticultural and control soils, looking for preliminary differences on microbial population behavior between them, and (2) a case study of spatial and temporal soil properties variability (respiration, humidity, organic matter, conductivity, pH, retention volume, density, and total phosphorous) of a horticultural production unit, in order to evaluate their effect on pesticide degradation.

2 Experimental Methods

2.1 Soils

All soil samples were taken from the same farm, where horticultural soil (HS) has been under continuous production for at least 20 years. A grassland of the same soil order as HS, close to the productive plots but fallow for at least 20 years, was sampled as reference soil (RF).

The sampled soils were Argiudols, and their compositions were the following: HS: 9 % sand, 21 % clay, 70 % silt, 0.15 % N total, 7.5 meq Ca^{2+}/100 g soil, 4.2 meq Mg^{2+}/100 g soil, 2.0 meq K^{+}/100 g soil, and 0.6 meq Na^{+}/100 g soil; RF: 12 % sand, 18 % clay, 70 % silt, 0.30 % N total, 9.5 meq Ca^{2+}/100 g soil, 5.5 meq Mg^{2+}/100 g soil, 1.6 meq K^{+}/100 g soil, and 0.2 meq Na^{+}/100 g soil.

2.2 Reagents and Materials

The used pesticides were the following commercial formulations: procymidone (3-(3,5-dichlorophenyl)-1,5-dimethyl-3-azabicyclo[3.1.0]hexane-2,4-dione, CASRN [32809-16-8]), Sumilex® (CS, 50 %*w*/*v*, Summit Agro

Argentina); chlorpyrifos (*O*,*O*-diethyl-*O*-(3,5,6-trichloro-2-pyridinyl)-phosphorothioate, CASRN [2921-88-2]), Lorsban® (EC, 48 %*w*/*v*, Dow AgroSciences); and trifluralin (α,α,α trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine), CASRN [1582-09-8], Trigermin® (CS, 48 %*w*/*v*, CHEMINOVA). Reference materials were prepared by extraction/recrystallization (>95 % pure by GC-FID) and confirmed by ^{1}H- and ^{13}C-NMR. Primary stock solutions (1,500–2,900 μg/L) were prepared in cyclohexane and diluted as needed. Cyclohexane (Aberkon p.a. grade), distilled prior to use and chromatographically checked as suitable for use under GC-electron capture detector (ECD) conditions, was used for every solution and extract preparation.

2.3 Pesticide Extraction and Chromatographic Quantification

For pesticide extraction, 50 g of soil was treated with 100 mL of a mixture of cyclohexane and acetone (50:50). The suspension was sonicated for 20 min, and 10 mL was filtered through a 1 g silica column. The filtrate was transferred to 2 mL vials and analyzed by gas chromatography.

All chromatographic analyses were performed on a PerkinElmer (Norwalk, CT, USA) AutoSystem XL gas chromatograph with an Autosampler automatic injector, equipped with an ECD and a fused silica capillary column (PE-5, 5 % diphenylpolysiloxane–95 % dimethylpolysiloxane stationary phase, 30 m length, 0.25 mm i.d., and 0.25 μm film thickness). The GC-ECD operating conditions were injector temperature 280 °C; ECD temperature 375 °C; oven temperature 190 °C for 1.5 min, 45 °C/min to 300 °C then 10 °C/min to 320 °C, and hold 2 min; injection volume 1 μL, splitless; carrier gas N_2, 30 psi; and ECD auxiliary flow 30 mL/min.

Methodology validation data are given in the Supplementary Information Section. For all studies, the initial content in soil of trifluralin, procymidone, and chlorpyrifos was always checked, being in all cases below the limit of detection of the method.

2.4 Pesticide Degradation Assays

In order to obtain representative soil samples for the pesticide degradation assays, HS samples collected from different sections of the horticultural field (Fig. 1b) were thoroughly mixed; the same was done with the reference soils. Each composite soil sample (HS and RF) was placed in individual pots (in triplicate) where an initial dose of an aqueous solution of the three pesticides (Sumilex, Lorsban, Trigermin) was applied. An additional pot of each soil without pesticide was used as a supplementary control.

Samples were taken from each pot at different times (from 0 to 768 h), and humidity, microbial respiration, total viable heterotrophic bacteria count, and pesticide quantitations were done as described in Sections 2.3, 2.6.1 and 2.6.2.

2.5 Isolation and Preliminary Characterization of Degrading Bacteria

HS and RF soil samples were suspended in sterile physiological solution (1:10), and 2.5 mL of the suspension was transferred into a 125-mL Erlenmeyer flask charged with 25 mL M9 medium (g/L: K_2PO_4H, 6.0; KPO_4H_2, 3.0; NaCl, 0.5; NH_4Cl, 1.0; $MgSO_4 \cdot 7H_2O$ 1 M, 0.8; $CaCl_2 \cdot 2H_2O$, 1.47), supplemented with 1 % for each of the commercial formulations of Lorsban, Sumilex, and Trigermin. Cultures were kept for 15 days at 25 °C in a shaker. Three replicates of each soil were processed.

After observing microbial development, cultures were streaked on Petri dishes containing the same medium (M9-pesticides-15 g/L agar). Colonies were purified, and a preliminary characterization was done on the basis of their morphology, Gram staining, and biochemical assays (API 20 E-Biomérieux).

2.6 Soil Properties

2.6.1 Physicochemical Characterization

Organic matter content was determined using the ignition method, destroying organic matter by heating in an oven at 400 °C for 18 h (Nelson and Sommers 1996). Conductivity and pH were measured using a multiparameter probe (Horiba, Water Quality Checker U10), suspending 10 g of soil in 10 mL of distilled water.

Water retention volume of the soil was determined filling a glass column with 30 g of soil and pouring 50 mL of distilled water on it. The drained water was collected at the bottom of the column until no more dripping was observed. The retention volume was calculated as the difference between initial and final water volumes. Humidity was measured by weighing soil

samples before and after drying at 105 °C. Bulk density was obtained by weighing a soil cylinder of a known volume (Wilke 2005).

Total phosphorous content was determined spectrophotometrically using the phosphomolybdate method (Kuo 1996). Briefly, 5.0 g of soil was extracted using 0.5 M HCl and 0.5 M H_2SO_4. Then, 5 mL of the supernatant was mixed with 5 mL of ammonium molybdate 12.5 %*w*/*v* and 3 mL of ascorbic acid solution 1 %*w*/*v*, and the absorbance at 660 nm was measured. A calibration curve (1 μg/L to 10 μg/L) was prepared using K_3PO_4(s).

2.6.2 Biological Determinations

Microbial respiration was determined by capture and titration of the carbon dioxide generated by biological activity. In short, 20.0 g of soil was placed in a small airtight vessel which also contained a small beaker with 25 mL of NaOH(aq) 0.1 M to absorb the CO_2. Then, 1.0 mL of a glucose solution (20 %*w*/*v*) was added to the previously homogenized soil, and the vessel was closed. After 24 h at 32 °C, the alkaline solution was titrated with HCl(aq) 0.1 M to obtain the CO_2 content. Microbial respiration was expressed as milligrams of CO_2 per gram of dry soil.

Total viable aerobic heterotrophs were evaluated by counting colony forming units (CFU) per gram soil: 1 g of soil was suspended in 10 mL of NaCl 150 mM, and serial dilutions were plated in plate count agar (PCA, g/L: casein peptone 5.0, yeast extract 2.5, D(+)-glucose 1.0; agar-agar 14.0) and incubated for 48 h at 32 °C.

2.6.3 Spatial Sampling Scheme

The horticultural production unit was constituted by three separate plots (plots 1, 2, and 3, Fig. 1b). Each plot was divided by small paths separating different crops (A–F for plot 1, A–D for plot 2, A–C for plot 3, Fig. 1b). A set of sampling points were defined within each subplot and located with the help of a GPS; for the biggest subplots, two sampling points were considered (for example, E and F, plot 1, Fig. 1b). At each sampling point, a small pit (approximately 20 cm in diameter, 10 cm in depth) was excavated, and these samples were kept in labelled polyethylene bags until analysis. At the laboratory, they were individually sieved by hand to separate stones and plant residues. If the analysis was not performed immediately, samples were stored at 4 °C.

Reference soil samples were taken from the neighboring fallow grassland (RF, Fig. 1b). In this case, three independent samples separated by 3 m were collected and mixed to obtain a composite sample. The results for each sample site are shown in the Supplementary Information (Table 1).

2.6.4 Seasonal Sampling Scheme

In order to analyze if the physical and biological properties of soil varied with the seasons, a set of sampling sites was defined for each plot (Fig. 1c, Supplementary Information). These were different from the set defined in Fig. 1b, in order to reduce the number of analysis, but equally distributed in the three plots in order to be representative of the HS. Soil was extracted and processed in the same way as in Section 2.6.3. The results for each point are shown in Table 2 of the Supplementary Information. Sampling times were as follows: t_1, October 2010, spring; t_2, May 2011, autumn; and t_3, August 2011, winter.

2.7 Statistical Analysis

2.7.1 Pesticide Degradation DT50

All temporal points of chlorpyrifos, trifluraline, and procymidone were the results of three independent experiments. For each time, the mean and the standard deviation were calculated. To obtain the DT50, the natural logarithmic integrated version of the first-order kinetic equation ($\ln[A]=\ln[A_0]-k.t.$) was used for procymidone and trifluraline (Origin software). For chlorpyrifos, a direct linear regression of the mass of chlorpyrifos per gram of soil versus time was done (Origin software), and the DT50 was calculated.

2.7.2 Horticultural and Reference Physical and Biological Soil Properties

The microbial respiration, humidity, organic matter, conductivity, pH, water retention volume, density, and total phosphorous values for the horticultural soil (Fig. 4) were calculated as the mean (plus the standard deviation) over all the sampling points defined in Fig. 1 (Supplementary Table 1B). The RF properties indicated

in Supplementary Table 1 correspond to the mean of three sampling points (plus the standard deviation).

A HS or RF soil property was considered statistically different when the range of the property defined by the property mean value±SD of the HS was not part of the range defined by the property mean value±SD for the same property in the RF soil. The same criterion was applied to consider a statistically significant difference for the soil properties in the different plots (Fig. 5).

3 Results

3.1 Chlorpyrifos, Procymidone, and Trifluralin Degradation in Soil

In order to evaluate chlorpyrifos, procymidone, and trifluralin degradation in soil, consolidated samples were made with equal amounts of soil from each sampling point for HS and RF. Soil influence on pesticide degradation was studied applying a single pulse of a mixture of pesticides (chlorpyrifos, procymidone, and trifluralin as their commercial formulations) to the composite samples of HS and RF (see Section 2.4). We decided to study the simultaneous degradation of a group of pesticides because simultaneous application of different active ingredients was a common practice amongst the horticultural workers. The three selected pesticides were chosen as representative of the herbicide, insecticide, and fungicide groups. A single dose of 0.015–0.035 mg of each pesticide per gram of dried soil, similar to the manufacturer's recommended dose, was applied. Soil pesticide content was determined by solvent extraction and quantification by GC-ECD at different exposure times. Figure 2 shows the chlorpyrifos, procymidone, and trifluralin degradation profiles as a function of time for both soils. All pesticides experienced faster degradation in HS than in RF, showing practically first-order exponential kinetics for procymidone and trifluralin in HS. In order to evaluate if the pesticide application affected the microbiota, microbial respiration was measured in parallel experiments at the same time intervals, using composite samples with and without pesticides. Figure 3 shows that there were no statistically important differences in microbial respiration versus time for both experiments.

3.2 Isolation of Pesticide-Degrading Bacteria

In an attempt to explain the differences between the degradation performances of the horticultural and the reference soils, a screening of pesticide-degrading bacteria was carried out with HS and RF samples. Seven strains were isolated from HS and two from RF; all nine responded to Gram-negative rod-shaped bacteria. A preliminary biochemical characterization of the isolated strains was performed with API 20E (Table 3, Supplementary Information). Eight of the nine strains belonged to *Pseudomonas* genus, with an acceptable approximation, while the ninth could be included in the *Bordetella*, *Alcaligenes*, *Moraxella*, or *Ochrobactum* genera; the result was obtained with low discrimination.

3.3 Horticultural Soil Properties Variability

Taking into account that the horticultural soils have been under intensive use and exposed to both mechanical and chemical stresses for a long time, it would be interesting to determine if the history of the land management could have affected the pesticide fate (Johnsen et al. 2001).

With this purpose, a set of soil properties: pH, retention volume, conductivity, density, microbial respiration, humidity, total phosphorous, and organic matter content were selected as indicators. The studied farm was divided into three different plots (P1, P2, P3, Fig. 1b). Each plot has internal subdivisions, usually associated with different crops (lettuce, broccoli, Swiss chard, strawberry, etc.). Figure 1b shows the production unit scale and the defined sampling scheme. In the first place, a comparison of these properties between the HS and an RF from a neighboring Arguidol grassland, 50 m distant from the production unit, was done. No agricultural activity was recorded in the reference soil for at least 20 years. For the horticultural-reference soil comparison, averaged values of soil properties and the standard deviation for HS and RF were determined (Fig. 4). A statistically significant difference could be observed for humidity, total phosphorous and organic matter content, and retention volume, while microbial respiration, pH, conductivity, and density have similar values.

An interesting issue is the degree of temporal and spatial heterogeneities of the HS. To evaluate this, Fig. 5 shows the mean value of each soil property for plots P1 and P2 (the soil properties obtained for each sampling point are given in the Supplementary Information, Table 1). Except for microbial respiration for

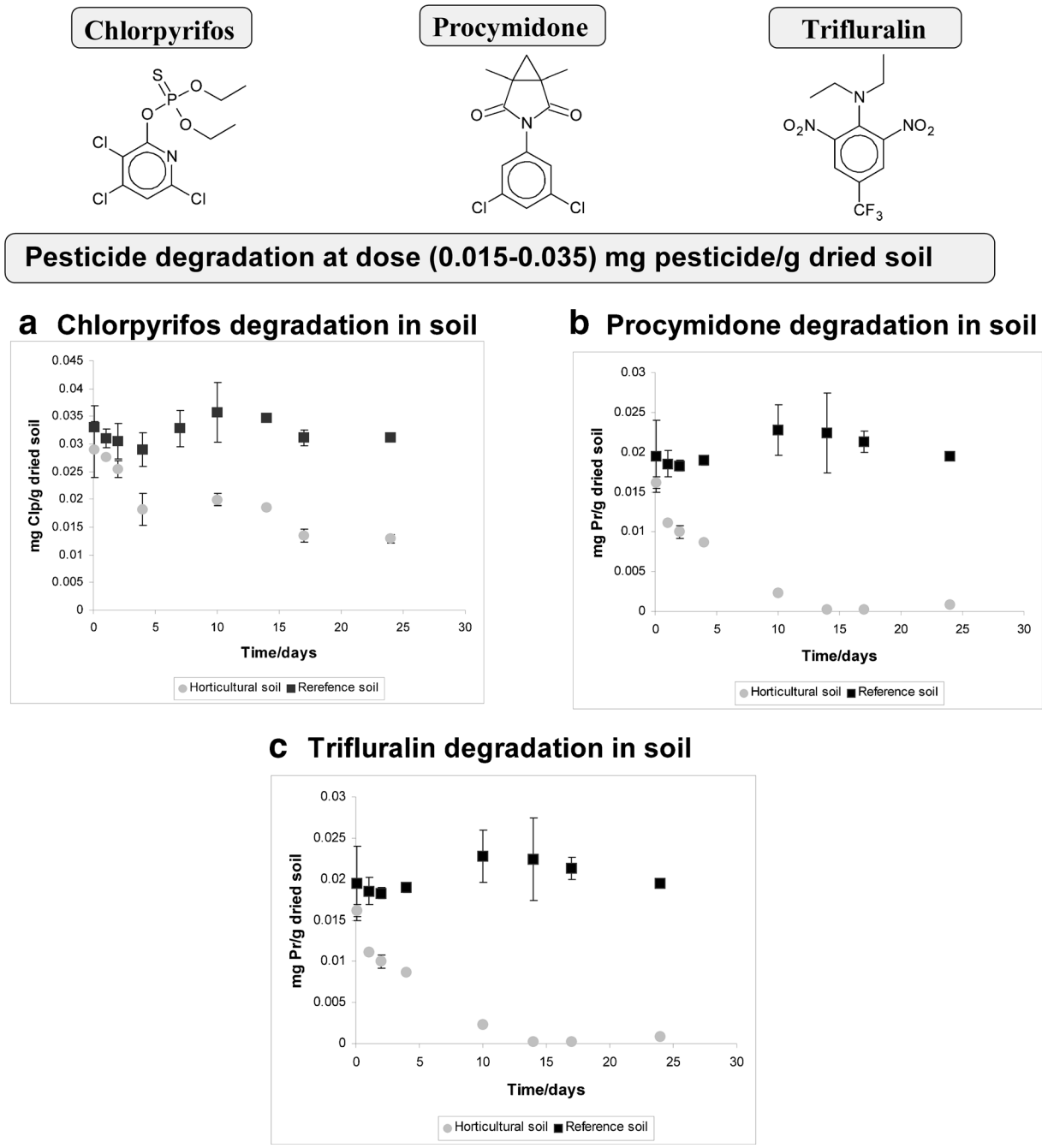

Fig. 2 Degradation of chlorpyrifos (**a**), procymidone (**b**), trifluralin (**c**) in horticultural and reference soils at dose of 0.015–0.035 mgpesticide/g dried soil

P2 and retention volume for P1, the remaining properties showed similar values whatever the observed plot was.

In order to estimate temporal variations of a selected subset of soil properties, a consolidated sample of nine representative points of plots 1, 2, and 3 (Fig. 1c, Supplementary Information) was prepared, and each set of analysis (microbial respiration, humidity, organic matter, and total phosphorous content) was repeated at three different seasons during 2010 and 2011 (see Section 2). Figure 6 shows that for the selected properties (as averaged values), no statistical significant differences with time could be found.

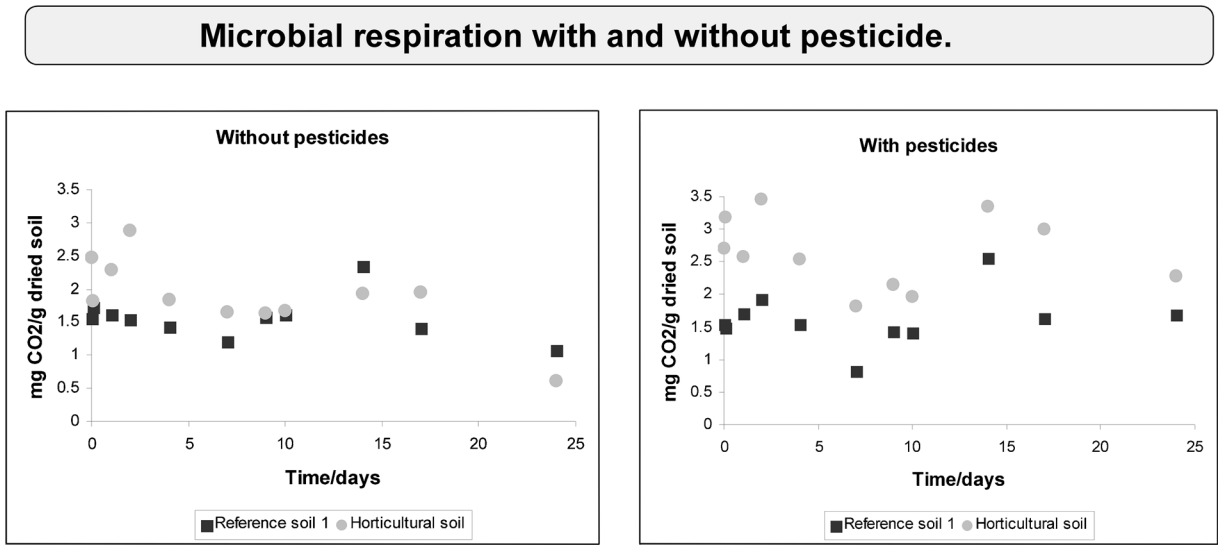

Fig. 3 Microbial respiration at the degradation experiment in horticultural and reference soils

4 Discussion

4.1 Chlorpyrifos, Procymidone, and Trifluralin Degradation in Soil

When simultaneous degradation of chlorpyrifos, procymidone, and trifluralin was studied in HS at doses of 0.015–0.035 mg of pesticide, a clear first-order exponential decrease for procymidone and trifluralin was observed (Fig. 2), but not for chlorpyrifos. When degradation results were analyzed in the RF, a practically negligible degradation of all tested products was observed. This behavior is particularly evident considering the differences with the HS at 15 and 24 days. The period required for the 50 % dissipation (DT50) for the three pesticides in the HS was calculated by logarithmic linearization of procymidone and trifluralin curves, while in the case of chlorpyrifos, the direct linear mean square of the data was used. The mean values were 16±1, 3.7±0.6, and 3.6±0.6 days for chlorpyrifos, procymidone, and trifluralin, respectively, while the concentrations in RF remained practically constant (Fig. 2). The DT50 values found in HS are also significantly lower than the half-lives in soil reported in literature. For example, the DT50 values of 76, 48–189, and 181 days were reported for chlorpyrifos, procymidone, and trifluralin, respectively, in laboratory degradation experiments in soil at 20 °C (Pesticide Properties Database 2013). These results could be indicative of a remarkable soil microbial adaptation in the HS as a consequence of the repeated use of different pesticides.

Microbial respiration was measured during a degradation assay (Fig. 3, right) together with a control experiment (Fig. 3, left), in order to check if the degradation rate is related to microbial biomass. No significant discrepancy between both experiments was observed, indicating that at least the total number of microorganisms showed no appreciable changes when the pesticide was applied.

4.2 Isolation of Pesticide-Degrading Bacteria

There was a clear difference in the number of bacterial isolations achieved with the HS and the RF under the selection pressure of chlorpyrifos, procymidone, and trifluralin as unique carbon sources in liquid media. This result could be explained by an adaptation response to the pesticide's continuous presence, affecting the bacterial community composition in the HS, as a consequence of intensive applications. It is not surprising that the majority of the isolates were preliminarily identified as belonging to *Pseudomonas* genus, one of the most versatile microorganisms in terms of metabolic diversity, culturability, and survival strategies in nature. However, studies will be started in our laboratory for a deeper analysis of the microbial behavior.

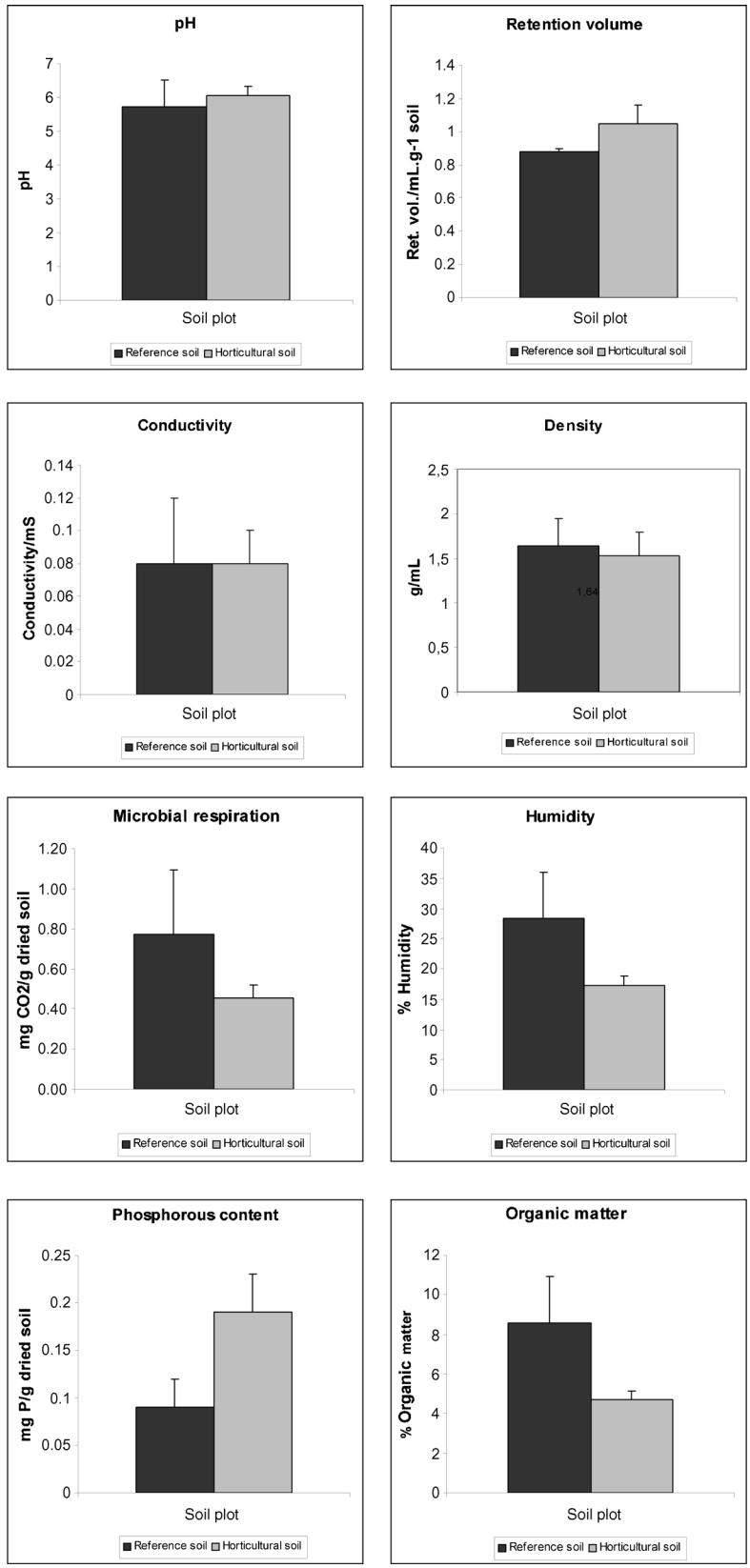

Fig. 4 Microbial respiration, humidity, organic matter, conductivity, pH, retention volume, density, and phosphorous content for reference and horticultural soil

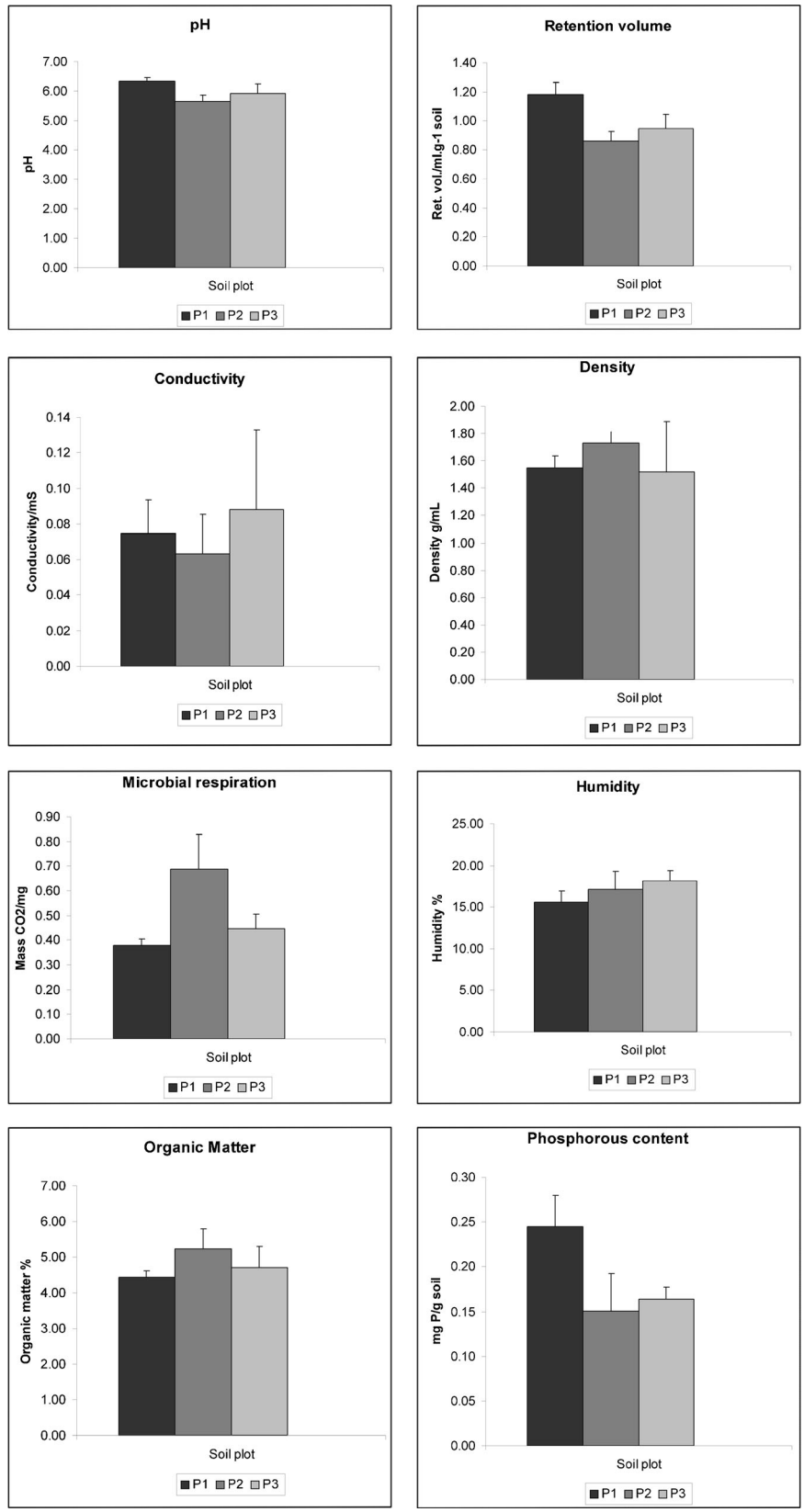

Fig. 5 Microbial respiration, humidity, organic matter, conductivity, pH, retention volume, density, and phosphorous content of the soil in farm plots 1, 2, and 3

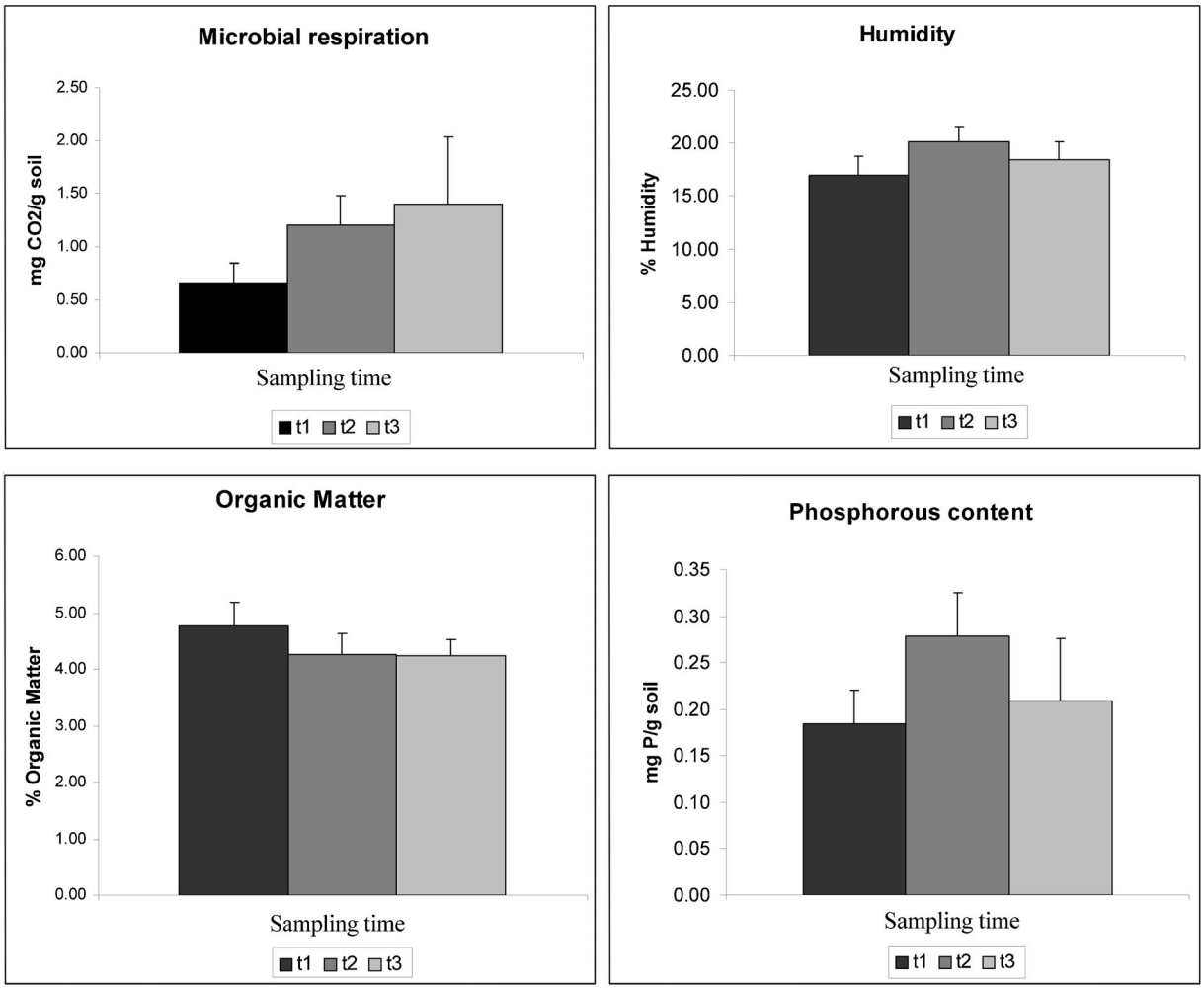

Fig. 6 Temporal evolution of the microbial respiration, humidity, organic matter, and phosphorous content in horticultural soil

4.3 The Effect of Horticultural Soil Variability on Pesticide Degradation

Figure 4 compares the values of the selected properties of the HS (microbial respiration, humidity, organic matter, conductivity, pH, and total phosphorous content) with those of the RF. Although the soils are similar in composition and texture (see Section 2), organic content was higher in RF (8.6 %±2.3 %, Fig. 4) than in HS (4.7 %±0.4 %, Fig. 4). The low organic matter content found in the HS is consistent with the lower total nitrogen content (see Section 2) and reflects the stresses resulting from the intensive production practices. Another important difference was found in the total phosphorous content, where the levels measured in the HS (0.19 mg±0.04 mg P/g dried soil) doubled the amounts found in RF (0.09 mg±0.03 mg P/g dried soil). This fact is in agreement with the producer's use of poultry litter, as well as high doses of synthetic phosphorous additives.

The results shown in Fig. 5 indicate that the HS did not present a relevant heterogeneity within the production unit, according to the selected properties (microbial respiration, humidity, organic matter, conductivity, pH, retention volume, density, and total phosphorous content). No statistically meaningful differences were observed between the subplots P1, P2, and P3 (Fig. 1b) except for microbial respiration for P2 and retention volume for P1 (Fig. 5).

When the temporal heterogeneity of the soil was explored analyzing consolidated samples of the three plots, by measuring microbial respiration, humidity, organic matter, and total phosphorous content at three different seasons, no statistical differences were found

(Fig. 6). These results could indicate, at least partially, that spatial and seasonal heterogeneities within the production units do not merit special attention in the future.

5 Conclusions

The biodegradation rate of chlorpyrifos, procymidone, and trifluralin in a periurban horticultural soil showed a notable increase compared to biodegradation in a close reference soil. This behavior could be explained by microbial community adaptation as a consequence of the intensive use of pesticides, *Pseudomonas* being the most abundant microorganisms isolated from the HS.

Although significant differences have been found between a horticultural soil under intensive production and a reference soil of the same class, specially in the organic matter (OM) and total phosphorous content, these variations could not explain by themselves the differences found in the pesticide degradation. When HS properties were spatially and seasonally analyzed, no meaningful differences were found.

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