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The millimetre-scale distribution of 2,4-D and its degraders drives the fate of 2,4-D at the soil core scale

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ABSTRACT

The biodegradation of organic compounds in soil is a key process that has major implications for different ecosystem services such as soil fertility, air and water quality, and climate regulation. Due to the complexity of soil, the distributions of organic compounds and microorganisms are heterogeneous on sub-cm scales, and biodegradation is therefore partly controlled by the respective localizations of organic substrates and degraders. If they are not co-localized, transfer processes become crucial for the accessibility and availability of the substrate to degraders. This spatial interaction is still poorly understood, leading to poor predictions of organic compound dynamics in soils. The objectives of this work were to better understand how the mm-scale distribution of a model pesticide, 2,4-dichlorophenoxyacetic acid (2,4-D), and its degraders drives the fate of 2,4-D at the cm soil core scale. We constructed cm-scale soil cores combining sterilized and "natural" soil aggregates in which we controlled the initial distributions of 2,4-D and soil microorganisms with the following spatial distributions: i) a homogeneous distribution of microorganisms and 2,4-D at the core-scale, ii) a co-localized distribution of microorganisms and 2,4-D in a single spot (360 mm^3) and iii) a disjoint localization of microorganisms and 2,4-D in 2 soil spots (360 mm^3) separated by 2 cm. Two sets of experiments were performed: one used radiolabeled ^{14}C-2,4-D to study the fate of 2,4-D, and the other used ^{12}C-2,4-D to follow the dynamics of degraders. Microcosms were incubated at 20 °C and at field capacity (−31.6 kPa). At the core scale, we followed 2,4-D mineralization over time. On three dates, soil cores with microorganisms and 2,4-D localized in soil spots, were cut out in slices and then in 360 mm^3 soil cubes. The individual soil cubes were then independently analysed for extractable and non-extractable ^{14}C and for degraders (quantitative PCR of *tfd A* genes). Knowing the initial position of each soil cube allowed us to establish 3D maps of 2,4-D residues and degraders in soil. The results indicated that microorganisms and pesticide localizations in soil are major driving factors of i) pesticide biodegradation, by regulating the accessibility of 2,4-D to degrading microorganisms (by diffusion); and ii) the formation of non-extractable residues (NER). These results also emphasized the dominant role of microorganisms in the formation and localization of biogenic NER at a mm-scale. To conclude, these results demonstrate the importance of considering micro-scale processes to better understand the fate of pesticides and more generally of soil organic substrates at upper scales in soil and suggest that such spatial heterogeneity should not be neglected when predicting the fate of organic compounds in soils.

1. Introduction

The biodegradation of organic compounds (of natural or chemical origin) in soil is a key process that influences chemical fertility, plant nutrition, structural stability, carbon storage, and also the dynamics of pollutants. One of the difficulties identified in the accurate prediction of the fate of organic compounds at the macroscopic scale is the lack of knowledge and understanding at the microscopic scale, and in particular, the spatial heterogeneity at this fine scale (Manzoni and Porporato, 2009; Dungait et al., 2012).

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Soil is a complex environment that generates a heterogeneous spatial distribution of microorganisms (Morris, 1999; Nunan et al., 2001) and their activities (Parkin, 1987; Parkin et al., 1987). The distribution of microorganisms can be clustered or more homogeneous (Pallud et al., 2004). For example, Vieublé Gonod et al. (2003, 2006) reported, that 2,4-D mineralization was organized into cm-sized hot spots. Substrates, whether indigenous or exogenous, are also heterogeneously distributed at the mm or cm-scales in soil. Gaillard et al. (1999) observed that straw residues induced mm gradients of C and N in soil. Vieublé Gonod et al. (2003) highlighted a variability of organic C and N content in individual mm aggregates. Similar results may be expected for xenobiotics such as pesticides that are heterogeneously distributed in soil when used to treat cultures because of their low concentration, their formulation, their mode of delivery and agricultural practices. Thus, organic substrates can be degraded by microorganisms in soil only when both are co-localized. Finally, when substrates and degraders are located in the same micro-environment, biodegradation in soils occurs only if organic substrates or metabolites are available (not irreversibly retained on soil constituents) to degraders (Strong et al., 1997; Chenu et al., 2001) and if local conditions such as soil oxygenation, water content, etc. are favourable to microbial degradation.

When organic compounds and degrading microorganisms are not co-localized in soil, transport processes determine the accessibility of the substrate to the microorganisms. For example, Dechesne et al. (2010) highlighted the impact of the spatial distribution of an inoculated bacterium on benzoate mineralization in a simple sand system. More recently, Ellergaard-Jensen et al. (2014) reported that a physical separation of bacteria and diuron reduced pesticide degradation.

The motility of bacteria is rather reduced in soil and particularly in soils with low matric potentials (Grundmann et al., 2007; Dechesne et al., 2010). Other ways for microorganisms to colonize new environments are diffusion, transfer *via* other soil living organisms (Banitz et al., 2011a, 2011b) and soil particles or over shorter distances, extension with growth (Gammack et al., 1992). Degradation is also controlled by the transport of substrates by diffusion or convection towards microorganisms.

Some studies have underlined the impact of the transport processes on biodegradation. Harms (1996), working with naphthalene and a pure bacterial strain, demonstrated a negative correlation between the substrate-bacteria distance and bacterial growth. Grundmann et al. (2007) also concluded that diffusion-controlled isoproturon mass flow towards microbial hot spots was one of the most important processes enabling increases in isoproturon mineralization. In addition, some studies have shown that homogenizing the soil and thus increasing the contact between substrates and degrading microorganisms improved the biodegradation of pollutants (Rijnaarts et al., 1990).

Transport of hydrophilic organic substrates by diffusion is positively correlated with the soil water content. Higher soil water content leads to higher microbial growth and higher degradation, as shown by Harms (1996) and Dechesne et al. (2010), except near saturation. The soil water content also has a strong impact if the degradation of pollutants involves different microbial populations (metabolic cooperation) and these populations are not co-localized. In this case, intermediary metabolites must move into the soil to come into contact with the different degraders. Monard et al. (2012) showed that an increase in soil water content led to a reduction in the spatial heterogeneity of pesticide mineralization, presumably by increasing the probability of degrader and substrate encounters.

The few studies addressing the impact of the spatial distribution of microorganisms and substrates on microbial degradation have used simplified media such as glass beads or sand and/or inoculated strains and targeted the cm-scale. Wong and Griffin (1976) observed that the rate of movement of microorganisms differed in artificial and natural soils. Studies performed with natural soil and its autochthonous microflora and focussing on the mm-scale are scarce.

Therefore, the objective of this work was to better understand how the spatial contact between degraders and organic substrates impacts biodegradation in soil. More specifically, we studied the impact of different spatial distributions of the pesticide 2,4-D and soil microorganisms at the mm-scale on the fate of 2,4-D and the colonization of microorganisms in repacked soil cores. We selected 2,4-D as a model substrate because of the numerous published results for this pesticide and because molecular genetic tools were available for the detection and quantification of its degrading microorganisms.

2. Materials and methods

2.1. Soil, sampling and sterilization

Soil was sampled from the ploughed layer (0–30 cm) of an agricultural field located in the INRA experimental site of "La Cage" in Versailles (France) in March 2011. The soil is classified as a Luvisol with the following characteristics: 27% sand, 17% clay, 56% silt, 10 mg g^{-1} soil and 1.01 mg g^{-1} for total carbon and nitrogen contents, respectively, C/N ratio equal to 9.9 and pH of 7.4. The plot was cultivated with wheat and has not received 2.4-D application in the last 15 years. The water content of soil at sampling was 0.18 g g^{-1} soil. The soil was sieved at this water content, and only aggregates with diameters of 2–3.15 mm were kept. A sub-sample of these aggregates was sterilized by gamma-irradiation at a dose of 45–75 kGy (Ionisos, Dagneux, France).

2.2. Substrate

^{14}C-ring-labelled 2.4-dichlorophenoxyacetic acid (2,4-D) (specific activity: 1371 MBq $mmol^{-1}$, radiochemical purity: 97.5%) was purchased from Izotop (Budapest, Hungary) and used for the experiments dealing with the fate of 2,4-D in soil. ^{12}C-2,4-D (Sigma, purity: 95%) was used for the experiments quantifying 2,4-D degraders in soil. Solutions of 2,4-D were sterilized by autoclaving (20 min at 120 °C) before use.

2.3. Core construction and incubation conditions

The soil cores were prepared from 4 pools of aggregates: sterilized aggregates amended or not amended with 2,4-D and non-sterilized aggregates with indigenous microorganisms amended or not amended with 2,4-D (Fig. 1). The final water content of aggregates was adjusted with sterilized water or 2,4-D solution to 0.21 g H_2O g^{-1} soil ($\psi = -31.6$ kPa, pF = 2.5) with a micropipette (to reduce the size of the droplets and bring the substrate or water as homogeneous as possible) before core construction. Soil cores with final dimensions of 5 cm diameter and 3 cm height were progressively assembled layer per layer (6 in total) and consistently compacted with a hydraulic press to obtain a final dry bulk density of 1.3 g cm^{-3}, a density commonly observed in the ploughed layer of cultivated soil (Défossez et al., 2003). The dry mass of the soil was 76.6 g per core.

In the soil cores, we initially localized the soil microorganisms and 2.4-D according to three different spatial distributions, as shown in Fig. 1:

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