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Assessing the dynamic changes of rhizosphere functionality of *Zea mays* plants grown in organochlorine contaminated soils

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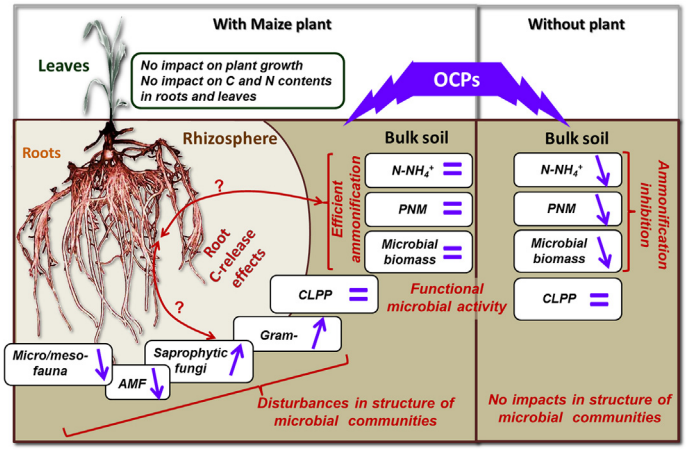
HIGHLIGHTS

- OCP inhibited ammonification step in bulk soils.
- Plants enabled efficient C- and N-turnover in soils even under OCP stress.
- OCP exposure did not alter the microbial community-level physiological profiles.
- With OCPs, maize stimulated microbial functionality leading to potential remediation.

GRAPHICAL ABSTRACT



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ABSTRACT

The persistent organochlorine pesticides (OCPs) in soils are suspected to disturb soil biogeochemical cycles. This study addressed the dynamic changes in soil functionality under lindane and chlordecone exposures with or without maize plant. Decreases in soil ammonium concentration, potential nitrogen mineralization and microbial biomass were only OCP-influenced in bulk soils. OCPs appeared to inhibit the ammonification step. With plants, soil functionality under OCP stress was similar to controls demonstrating the plant influence to ensure the efficiency of C- and N-turnover in soils. Moreover, OCPs did not impact the microbial community physiological profile in all tested conditions. However, microbial community structure was OCP-modified only in the presence of plants. Abundances of gram-negative and saprophytic fungi increased (up to +93% and +55%, respectively) suggesting a plant stimulation of nutrient turnover and rhizodegradation processes. Nevertheless, intimate microbial/plant interactions appeared to be OCP-impacted with depletions in mycorrhizae and micro/meso-fauna abundances

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(up to −53% and −56%, respectively) which might have adverse effects on more long-term plant growth (3–4 months). In short-term experiment (28 days), maize growth was similar to the control ones, indicating an enhanced plasticity of the soil functioning in the presence of plants, which could efficiently participate to the remediation of OCP-contaminated soils.

1. Introduction

Organochlorine pesticides (OCPs) are an important class of xenobiotics listed as priority environmental pollutants by the Stockholm Convention [1]. Lindane (γ-hexachlorocyclohexane, γHCH) and chlordecone (decachloropentacyclodecan-5-one, CLD)-extensively used to control a wide range of agricultural pests- are well-known as being highly persistent in terrestrial ecosystems, highly bioaccumulated/biomagnified [2] and suspected to be carcinogenic and endocrine disruptors [3,4]. Although γHCH and CLD have been banned of used (C.N.524.2009.TREATIES-4, Stockholm Convention, 22/05/2001), they are nevertheless major contaminants of soils and by dissipation of food-crops, waters and trophic webs [5]. The soil contamination with OCPs is heterogeneous, with hot spots in agronomical soils containing 0.1–43 mg kg^{-1} (up to 4000 mg kg^{-1} in dumping sites) for γHCH [1,6] and 1.8–10 mg kg^{-1} (up to 10,000 mg kg^{-1} in industrial sites) for CLD [7,8].

Soils exposed to long-term OCP contamination represent extreme challenges to find remediation solutions. It was demonstrated that plants constitute an important sink for OCPs, as they are able to retain them efficiently in their tissues (mainly in underground tissues as roots or tubers) by partition/diffusion processes [9–11]. Moreover, plant roots are able to maintain a diversity of microbial communities in the adjacent root-surface soil which are liable to bioaccumulate and/or biodegradate OCPs [12–15]. Kidd et al. [16] demonstrated a greater biodegradation of HCH isomeres in the soil-plant-microbe system of *Cytisus striatus* and *Holcus lanatus* than in bulk soil. These previous studies highlighted the role of the rhizosphere which is the soil volume that is physically, chemically, or biologically altered by the presence of plant roots [17], as a key core system which might be implicated in the remediation of OCPs [18,19]. The prerequisite for the efficiency of such a remediation strategy is the sustainability of the rhizosphere in terms of volume/density, functionality, microbial abundance/biodiversity [20]. Previous studies have shown that plants without rhizospheric microbial association showed OCP-dose dependent toxic effects [21,22]. Blondel et al. [23] have demonstrated that the maize root metabolome is altered by γHCH and CLD in terms of quality and relative quantity of metabolites (some of them are constitutive of exudates). Plant exudates provide readily available organic carbon sources to the surrounding microbes, which profoundly affect their catabolic activities and the structure of microbial communities [24,25]. Therefore, the OCP effects might impact directly and/or indirectly (*e.g.*, *via* exudates changes) microbial biomass and/or biodiversity [9,16,26,27]. However, changes in microbial community structure do not necessarily imply changes in microbial functions due, for instance, to functional redundancy [28]. Another fundamental ecological process is described as the "microbial loop" model, through which soil microbes from the rhizosphere mobilize nutrients, primarily nitrogen, then microbial turn-over release these nutrients in forms that are bioavailable to plants [29,30]. The OCPs could have potential effects on rhizosphere functionality by disturbing nutrient turnover and availability [31], and possible negative effects on plant nutrient acquisition, growth and carbohydrate allocation may be suspected [32,33]. Thus, characterizing plant-microbial functionality in OCP-contaminated soils remains an important goal of fundamental ecotoxicologic understandings, with potentially significant inputs in remediation developments.

In this study, we used maize (*Zea mays*) as one of the major crops worldwide which is able to cope with excessive levels of OCPs [21,22,34–36]. A pot experiment was conducted to study the changes in the rhizosphere functionality of maize plants grown in γHCH and CLD contaminated soils at relevant environmental doses (48 mg kg^{-1} and 6.86 mg kg^{-1}, respectively). The specific objectives of this study were to monitor the temporal variation in (1) soil parameters (moisture, pH, C- and N-contents, potential nitrogen mineralization), (2) microbial biomass, catabolic activities (Biolog EcoPlates® designed for ecological study of whole microbial community level physiological profile; CLPP) [37] and community structure changes (Fatty acid methyl ester profiles; FAME), and (3) plant growth (leaf and root functional traits).

2. Materials and methods

2.1. Chemicals

Lindane (γ-HCH) and chlordecone (CLD) were purchased at Sigma-Aldrich (Germany) as pure standard (99% of purity).

2.2. Sterilization of maize seeds

Maize seeds (*Zea mays* L.,Golden Bantam; Graines Baumaux, France) were surface sterilized in ethanol:water (*v/v* 8:2) for 30 s and then placed in sodium hypochlorite:water (*w/v* 5:100) for 2 min before washing several times in sterilized distilled water [22].

2.3. Soil conditioning and experimental design

Standardized and certified organic potting soil (1.1% N, 35.6% C, 74.3% soil organic matter content, pH6.2; Botanic, France) was sieved at 5.6 mm and divided in pots of 70 g fresh weight (FW). The soils were firstly treated with 30 mL of water containing 0.05% of ethanol for control pots (used to dissolve OCPs) and containing γHCH (7 mg L^{-1} water solubility) or CLD (1 mg L^{-1} water solubility) and mixed. Afterwards, all pots were watered every two days with these solutions to maintain the soil moisture at 60–70% during 45 days under controlled conditions (16 h/8 h (day/night); 25 ± 2 °C; 250 μE m^{-2} s^{-1} photosynthetically active radiation). The final soil concentrations were 48 mg kg^{-1} γHCH and 6.86 mg kg^{-1} CLD. This time was the Time 0 (T0) of the experiment (Fig. S1). Sterile seeds pre-germinated in sterile Petri dishes were planted in soils (1 seed per pot) not-contaminated or contaminated with OCPs. All pots were maintained under controlled conditions for 28 days. After 14 and 28 days (T14 and T28, respectively), plantlets were harvested randomly and dissected (triplicates/treatment/time/pot type). The bulk soils were entirely collected, mixed and stored at −80 °C (triplicates/treatment/time/pot type). To collect the rhizosphere, roots were manually shacked and then introduced in a phosphate buffer solution (PBS) during 1 h under stirring [15]. Afterwards, solutions were centrifuged at 9000 rpm during 10 min at 4 °C. Supernatants and pellets were stored separately at −80 °C (triplicates/treatment/time/pot type).