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Clomazone influence soil microbial community and soil nitrogen cycling

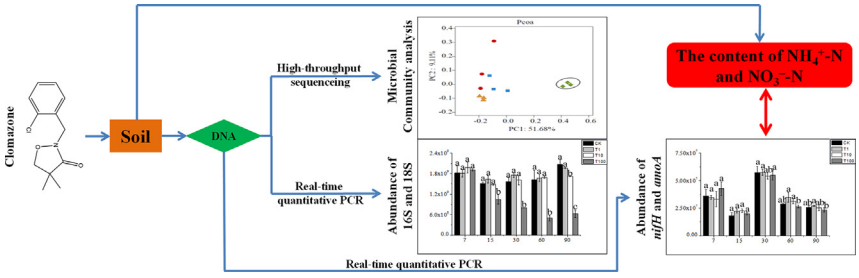
Pengqiang Du [a,b], Xiaohu Wu [a,c,*], Jun Xu [a,c], Fengshou Dong [a], Xingang Liu [a], Ying Zhang [d], Yongquan Zheng [a]

[a] State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, No. 2 Yuanmingyuan West Road, Beijing 100193, China
[b] College of Chemistry, Central China Normal University, No. 152 Luoyu Road, Wuhan 430079, China
[c] Key Laboratory of Control of Biological Hazard Factors (Plant Origin) for Agri-product Quality and Safety, Ministry of Agriculture and Rural Affairs of the People's Republic of China, China
[d] Institute of Plant Protection, Guizhou Academy of Agricultural Sciences, Guiyang 550006, China

HIGHLIGHTS

- The effects of clomazone on soil microorganism and N_2-cycling have been studied.
- High dosage clomazone decreased bacterial abundance and changed the composition.
- Clomazone could increase fungal abundance.
- High dosage clomazone significantly impact the abundance of *nif*H and *amo*A.

GRAPHICAL ABSTRACT



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ABSTRACT

We designed an indoor mesocosm experiment to investigate the long-term effects of exposure to clomazone, a widely used herbicide, on soil microbial communities and their nitrogen (N) cycling functions. Clomazone was applied to two typical soils from China at three concentrations: 0.8 (the recommended dosage), 8 and 80 mg kg^{-1} soil dry weight, and the mix was incubated for 90 days. Samples were removed periodically for assay with several techniques. The half-lives of clomazone in this experiment were 11–126 d. Results were significant only for the highest clomazone concentration. Next-generation sequencing of the 16S and 18S rDNA genes revealed that bacterial diversity significantly decreased whereas fungal abundance increased after day 60 but with no detectable effect on the microbial community. Hierarchical cluster and principal coordinates analysis revealed that the bacterial community structure was negatively impacted. Linear discriminant analysis of effect size identified *Sphingomonas* and *Arthrobacter* as the predominant bacterial species. Finally, we measured soil NH_4^+ and NO_3^- concentrations and used real-time PCR to analyze the abundance of the N-cycling genes, *nifH* and *amoA*. In the first 30 days, the NO_3^--N content and the number of ammonia-oxidizing bacteria increased. N_2-fixing bacteria were inhibited after 60 days, but the NH_4^+-N concentration remained unchanged and was likely provided by ammoniation.

1. Introduction

With the development of modern intensive agriculture, pesticides are important and common agents used to guarantee agricultural production; however, the biological, physical and chemical properties and non-target effects of pesticides have caused animal death, environmental problems, and ecological imbalances (Li and Jennings, 2018; Sanchez-Bayo, 2014). Direct application of herbicides to soil affects

* Corresponding author at: State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, No. 2 Yuanmingyuan West Road, Beijing 100193, China.
E-mail address: xhwu@ippcaas.cn (X. Wu).

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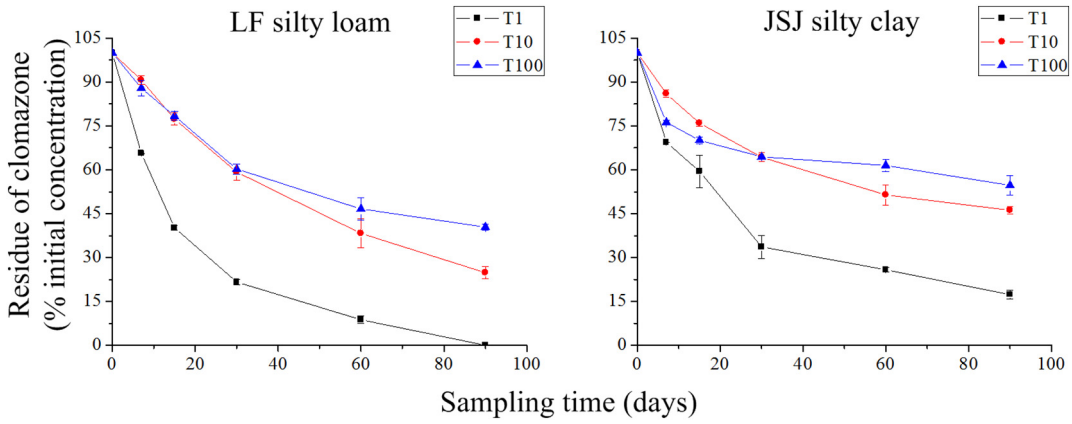

Fig. 1. Dynamics of clomazone degradation in LF silty loam and JSJ silty clay. Residue of clomazone: expressed as the percent of the initial concentration for each sampling time.

soil health and its function (Lupwayi et al., 2010), such as chlorothalonil, chlorimuron-ethyl and metham could decrease the abundance of genes involved in N cycling (Li et al., 2017a; Tan et al., 2013; Yang et al., 2014; Zhang et al., 2016), myclobutanil could decrease ammonium nitrogen (Ju et al., 2016). Soil plays a pivotal role in most ecosystems, including those involved in agricultural production, regulation of greenhouse gases, decomposition of dead creatures and excreta, degradation of organic contaminants, and control of groundwater quality (Hartman et al., 2008; Sandra et al., 2006; Tilman et al., 1997; Xu et al., 2017). Soil function is closely related to soil microbial communities (Da C Jesus et al., 2009; Hartman et al., 2008; Tian et al., 2017a). Chemical pollution has been shown to affect soil microbial communities and functions (Vázquez et al., 2017), and these effects have been shown in several studies. In the study of Li et al. (Li et al., 2017b), chloropicrin could impact soil bacterial community and decrease ammonia-oxidizing archaea (AOA). Wu et al. (Wu et al., 2014) found fomesafen impact soil microbial community by phosphor lipid fatty acid. In Zhang's study (Zhang et al., 2016), chlorothalonil had impact soil N cycling. Several microbial groups that supplement nitrogen (N) soil reserves, including ammonia-oxidizing bacteria (AOB), AOA, and N_2-fixing bacteria, are sensitive to pesticides (Ahtiainen and Vanhala, 2003; Zhang et al., 2016). The negative effects of pollution on soil bacterial and fungal communities will influence soil health and function (Lupwayi et al., 2010), and the negative impacts on key subgroups of AOB, AOA, and N_2-fixing bacteria will perturb the N supplementation and influence soil fertility and hence agricultural production.

Clomazone [2-[(2-chlorophenyl)methyl]-4,4-dimethyl-1,2-oxazolidin-3-one] belongs to the isoxazolidinone chemical group and is a selective herbicide for many crops. For example, it is used to control annual Gramineae and broadleaf weeds including paddy field rice, soybean, maize, tobacco, cotton, sugarcane and cassava (Cabral et al., 2017). Owing to its effectiveness for weed control, clomazone has been used worldwide, and its consumption has gradually increased. However, few studies have focused on its impact on soil microbial communities and N cycling (Cycon and Piotrowska-Seget, 2007; Tomco et al., 2013). Additional studies are thus needed to better understand the response of soil microorganisms to clomazone exposure. We must further characterize clomazone-related properties such as its degradation in different soils, impact on soil bacterial and fungal communities, and consequences on N cycling.

The aim of this work was to better evaluate the impact of clomazone exposure on two different agricultural soils. We used several approaches to assess ecosystem responses. We estimated the bacterial and fungal biomass by sequencing 16S and 18S rDNA genes, respectively. We used real-time PCR to analyze the abundance of two genes that are important in N cycling, namely *nifH*, which encodes the nitrogenase reductase subunit, and *amoA*, which encodes ammonia monooxygenase that participates in ammonia oxidation. Finally, we assayed soil N content by directly measuring NH_4^+ and NO_3^- concentrations. To our knowledge, this is the first comparative analysis in two different soil types of the ecotoxicity of clomazone on the diversity and structure of bacterial and fungal communities, the abundance of *nifH* and *amoA*, and N cycling.

2. Materials and methods

2.1. Soil, experimental design, and sample collection

Two types of soil (0–15 cm plough layer) were collected from the LF experimental plot of the Chinese Academy of Agricultural Sciences (N39°82′, E116°70′) and the JSJ reclamation area (N47°27′, E132°64′). The physicochemical properties of the soils are listed in Supplementary Table S1. Soil samples were sieved through 2-mm mesh filters and then pre-incubated for 2 weeks (OECD, 2000). The moisture content of the soil was adjusted to 50% of the maximum water-holding capacity with deionized water and the temperature was 25°C. Analytical-grade clomazone (purity, 98.4%) was purchased from Beijing Qinchengyixin Technology Development Co., Ltd., (Beijing, China). Of the three treatment doses prepared, the lowest concentration, T1, is the recommended dose for field application assuming an effective soil depth of 10 cm with a bulk density of 1.5 g cm^{-3} (GB/T31270.1-2014, 2014). T10 and T100 correspond to, respectively, 10 and 100 times the manufacturer's recommended dose. A control treatment was performed using an equal volume of acetone. For sample preparation, soil (50 g) and the clomazone solution (dissolved in acetone) were first combined in brown wide-mouth bottles and mixed thoroughly with a rotary mixer at full speed. The mixture was kept in darkness for 24 h at 25 °C to make the acetone volatilize. And then 200 g soil was added and mixed, and finally, water was added to 50% of the maximum water-holding capacity. A rotary mixer (Vortex Genie 2; Scientific Industries, New York, USA) was used to ensure uniform mixing of all samples.

Table 1
Kinetic parameters given by the bi-exponential model of clomazone degradation for the three different initial concentrations in LF silty loam and JSJ silty clay.

Soil type	LF silty loam			JSJ silty clay		
Parameters	T1	T10	T100	T1	T10	T100
A	0.3110	4.579	33.33	0.4301	2.451	15.209
$k1$	0.09821	0.02011	0.03069	0.07327	0.05302	0.2103
B	0.4607	0.1294	0.3606	0.3979	0.6218	0.7126
$k2$	0.03066	0.002304	0	0.008772	0.003427	0.002818
$t_{1/2}$ (d)	11.43	40.94	49.63	18.06	69.28	125.70
r^2	0.9989	0.999	0.9981	0.9895	0.9994	0.9971

Clomazone degradation in soil was described by the bi-exponential model: $PC(t) = Ae^{-k1\,t} + Be^{-k2\,t}$, where $PC(t)$ = pesticide concentration at time t; A, B: Constants. k_1, k_2: Dissipation kinetic constants for the first and second component of the curve and t = time. $t_{1/2}$: Half-life, or time required for a 50% dissipation of the initial concentration.