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Effects of chlorimuron-ethyl application with or without urea fertilization on soil ammonia-oxidizing bacteria and archaea

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HIGHLIGHTS

- Chlorimuron-ethyl applied alone or in combination with urea in soil.
- Investigated the changes of the population sizes of AOB and AOA.
- Employed Real-time PCR to measure the abundance of functional genes.
- Chlorimuron-ethyl inhibited nitrification and denitrification.
- AOB and AOA occupy separate ecological niches

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ABSTRACT

Chlorimuron-ethyl (CE) has been widely used in modern agriculture, but little is known regarding the influence of CE on ammonia-oxidizing bacteria (AOB) and archaea (AOA) populations in soils. In this study, microcosm incubation of aquic brown soil was conducted for 60 d. Associated changes in the population sizes of AOB and AOA in response to CE application with or without urea fertilization were examined via quantitative real-time PCR (qPCR) assays of the ammonia monooxygenase gene (*amoA*). The half-life of CE ranged from 11.80 d to 14.54 d in the tested soil. Compared to the untreated control, the application of CE alone had no strong effects on soil pH, and urea fertilization temporarily increased soil pH in the first 7 days. The abundance of the AOA *amoA* gene was greater than the abundance of the AOB *amoA* gene in all treatments, but both were significantly suppressed by CE application in a dose-dependent manner. Urea fertilization generally increased AOB and AOA *amoA* gene abundances, except that the AOA *amoA* gene level was slightly reduced at the early stage of the incubation period. AOB and AOA preferred different N levels for growth, with AOB only growing significantly at high NH_4^+ levels and AOA growing substantially at low NH_4^+ levels. The stimulation effects of urea fertilization on AOA and AOB *amoA* gene abundances were strongly suppressed by the CE application. This study indicated that the CE application substantially suppressed soil nitrification via inhibiting the AOB and AOA population regardless of urea fertilization, which resulted in significant changes in the soil NH_4^+-N and NO_3^--N levels. Furthermore, AOB and AOA inhabiting separate ecological niches with different NH_4^+ levels played various roles in N cycling.

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1. Introduction

The sulfonylurea family of herbicides is widely used in modern agriculture. Sulfonylurea is a potential weed inhibitor that suppresses the plant- and microbial-specific enzyme acetolactate synthase (ALS). ALS catalyzes the biosynthesis of branched-chain amino acids such as valine, leucine and isoleucine; therefore, its inhibition by sulfonylurea leads to the rapid cessation of plant cell division and growth [1–3]. Research has previously indicated that sulfonylurea herbicides are toxic to microorganisms [4–7]. For example, metsulfuron methyl inhibited the growth of a large proportion of tested fluorescent *Pseudomonas* strains, even at low concentrations [8]. High levels of bensulfuron-methyl dramatically decreased the colony forming units (CFUs) and the diversity of soil microbial communities [9]. In addition, bensulfuron-methyl has been found to significantly suppress nitrification for 1–4 weeks post-application [10].

As a member of the sulfonylurea family, chlorimuron-ethyl (CE) is commonly used as a herbicide to control weeds in wheat, rice, soybean, barley, potato, and corn [2,11]. This common application is largely due to its broad-spectrum, low use rate (ca. 2–75 g ha^{-1}), good crop selectivity and low toxicity to animals [3,12]. In a soybean field in northeast China, CE has been extensively applied to control pre- and post-emergence gramineal weeds since 1993 [13].

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CE persists in the soil for a long time (over 100 days); therefore, its application to soil causes a substantial accumulation of residual compounds. Consequently, the long-term use of CE may cause serious damage to sensitive rotational crops, particularly in alkaline soils because of the decreased hydrolysis rate of CE with increased pH, resulting in greater residual concentrations of CE [3]. In addition, CE has a low K_{ow} value and a high water solubility; therefore, it can easily leach from soils and contaminate groundwater [3,11,14,15]. Despite many reports on CE regarding its mode of action, toxicology, degradation pathway and detection method [16–18], few studies have investigated the influence of CE on soil microorganisms [5,19,20], particularly the organisms associated with the nitrogen (N) cycle.

Nitrification is a key process in the global soil N cycle. As the first and rate-limiting step of nitrification in soil, oxidation of ammonia to nitrite is catalyzed by the key ammonia monooxygenase (AMO). Previously, autotrophic ammonia-oxidizing bacteria (AOB) of beta- and gamma-proteobacteria were considered to be the most important contributors to ammonia oxidation. However, the recent development of cultivation-independent molecular techniques and metagenomics has led to the discovery of *amoA* genes that encode the alpha-subunit of AMO, which raises questions about the role of AOB. An increasing number of studies have recently shown that ammonia-oxidizing archaea (AOA) that are widely distributed in terrestrial and aquatic systems potentially represent the most important and abundant group of ammonia oxidizers [21–24]. The population sizes and community structure of AOB and AOA are shifting in response to temperature, pH, fertilizer levels and altitude [12,21,25]; however, little is known regarding the influence of sulfonylurea herbicides on AOB and AOA populations in soil.

Denitrification, which is responsible for the reduction of nitrogen oxides into gaseous products, is an important step in the environmental N cycle. The reduction of nitrite (NO_2^-) to nitric oxide (NO) is catalyzed by two different types of nitrite reductases (Nir); one type utilizes cytochrome cd1 and is encoded by the *nirS* gene, and the other type utilizes Cu and is encoded by the *nirK* gene [26]. Both *nirS* and *nirK* were harbored by denitrifiers only; therefore, these two genes were frequently used as gene markers to analyze the denitrifier community [27,28].

Our previous study showed that CE shifted the NH_4^+-N and NO_3^--N levels in treated soil [29]. The aim of present study was to examine how the application of CE with or without urea fertilization influences soil AOB and AOA populations, which are the two most important ammonia oxidizers in soil. We studied these effects via laboratory incubation of aquic brown soil and quantitative real-time PCR (qPCR) assays to determine the expression of *amoA* genes. Changes in the population sizes of AOB and AOA in response to different levels of CE in the presence or absence of urea were determined, and the relative roles of AOB and AOA were evaluated at different N levels. Both *nirS* and *nirK* gene levels were also determined to assess the effects of chlorimuron-ethyl on denitrifiers and to comprehensively estimate the effects on the N cycle. The results contribute to the accurate ecological risk assessment of CE application to soils.

2. Materials and methods

2.1. Soil sampling

Surface soil (0–20 cm) was collected in mid-October 2010 from abandoned land (41°31′N, 123°22′E), which had not been treated with pesticide or fertilizer for 14 years, in the National Field Research Station of Shenyang Agroecosystems. The soil was classified as aquic brown soil and contained 16.5 mg kg^{-1} organic matter, 25.2 mg kg^{-1} available N, 9.37 mg kg^{-1} available phosphorus (P), and 148.9 mg·kg^{-1} available potassium (K) with a pH value of 6.25. The soil sample was air-dried at room temperature and passed through a 2-mm sieve. Sieved soil was stored at 25 °C in the dark for one week for conditioning.

2.2. Experimental design

The CE solution was made by dissolving a CE standard (Shenyang Research Institute of Chemical Industry, China) in 80:20 (v/v) water:methanol, and the urea solution was made by dissolving urea (SCRC, China) in water. Then, the CE and urea solutions were sprinkled into soil in 8 treatments on a dry soil basis: (A) blank control, 20 μg kg^{-1} CE (field equivalent rate), 200 μg kg^{-1} CE, and 2000 μg kg^{-1} CE and (B) 120,000 μg kg^{-1} urea-control (field equivalent rate), 120,000 μg urea + 20 μg CE, 120,000 μg urea + 200 μg CE, and 120,000 μg urea + 2000 μg CE. A blank control was prepared that was not treated with CE or urea. The soil moisture content was adjusted to 20% after treatment. Each treatment, as well as the control, was performed in three replicates.

For microcosm incubation, 1 kg of soil (dry weight basis) was weighed in a plastic pot (20-cm diameter) with two aeration holes (0.5-cm diameter each) in the lid and then randomly placed in an incubator and incubated at 25 °C for 2 months. To maintain the moisture content, sterile water was weighed and injected into the microcosm every two days. During the incubation period, soil subsamples were taken at specific time intervals (1, 7, 15, 30, 45 and 60 days post-treatment) for soil biochemical analysis within one week. A set of subsamples were stored at −80 °C prior to DNA extraction for molecular biological analysis.

2.3. The determination of residual CE and pH in soils

The residual CE in soils was determined by high-performance liquid chromatography (HPLC) as previously described [5]. Briefly, 50.0 g of fresh soil was put into a centrifuge tube, soaked in 50 ml of a methanol solution (methanol: water = 8:2, *v*/*v*), vortexed for 1 min, and sonicated for 5 min, and the liquid, and solid phases were separated via centrifugation at 4000 rpm for 10 min. The extraction process of each sample was repeated three times. All liquid was combined and extracted with CH_2Cl_2 three times in a separate funnel for each sample, dried with a slight N_2 stream, and then suspended in methanol prior to HPLC.

CE (99.7% purity) was purchased from Sigma–Aldrich (Shanghai, China) as the standard. The HPLC analytical column was equipped with a Zorbax SB-18 ODS Spherex column (4.6 μm × 250 mm) and DAD array detector. The operating conditions were as follows: mobile phase, methanol and 0.1% *v*/*v* acetic acid in water (70:30, *v*/*v*); ultraviolet detection wavelength, 254 nm; flow rate, 1 ml min^{-1}; injection volume, 10 μl; and column temperature, 25 °C.

The soil pH was determined for a 1:2.5 soil/water suspension using a glass electrode.

2.4. DNA extraction

Total genomic DNA was extracted from 0.5 g of fresh soil using the FastDNA SPIN Kit for soil (MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions with minor modifications (e.g., homogenization in the FastPrep instrument for 15 s at a speed setting of 4.0). The quality and quantity of DNA extracted were checked on a 1% agarose gel and a Nanodrop spectrophotometer (NanoDrop, Wimmington, DE, USA). The extracted DNA was stored at −20 °C prior to being used in the qPCR assay.

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