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Combined acid rain and lanthanum pollution and its potential ecological risk for nitrogen assimilation in soybean seedling roots☆

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ABSTRACT

Rare earth elements (REEs) are used in various fields, resulting in their accumulation in the environment. This accumulation has affected the survival and distribution of crops in various ways. Acid rain is a serious global environmental problem. The combined effects on crops from these two types of pollution have been reported, but the effects on crop root nitrogen assimilation are rarely known. To explore the impact of combined contamination from these two pollutants on crop nitrogen assimilation, the soybean seedlings were treated with simulated environmental pollution from acid rain and a representative rare earth ion, lanthanum ion (La^{3+}), then the indexes related to plant nitrogen assimilation process in roots were determined. The results showed that combined treatment with pH 4.5 acid rain and 0.08 mM La^{3+} promoted nitrogen assimilation synergistically, while the other combined treatments all showed inhibitory effects. Moreover, acid rain aggravated the inhibitory effect of 1.20 or 0.40 mM La^{3+} on nitrogen assimilation in soybean seedling roots. Thus, the effects of acid rain and La^{3+} on crops depended on the combination levels of acid rain intensity and La^{3+} concentration. Acid rain increases the bioavailability of La^{3+}, and the combined effects of these two pollutants were more serious than that of either pollutant alone. These results provide new evidence in favor of limiting overuse of REEs in agriculture. This work also provides a new framework for ecological risk assessment of combined acid rain and REEs pollution on soybean crops.

1. Introduction

Because of their special catalytic, optical and magnetic properties, rare earth elements (REEs) are used in many fields, such as industry, agriculture, medicine and environmental protection, resulting in their continuous accumulation in the environment (Hu et al., 2006; Kim et al., 2015; Loell et al., 2011a; Wei et al., 2001). According to previous reports, REEs affect the survival and distribution of plants in various ways (Asher, 1995; Ippolito et al., 2010, 2011; Tyler, 2004). Acid rain is known as a serious and harmful global environmental problem and its impact on the environment is multifaceted (Wu et al., 2016). For example, acid rain can cause the corrosion of various building materials and the collapse of ecosystems (Fang et al., 2013; Menz and Seip, 2004). China, a big agricultural country, has become the third largest zone of acid rain pollution in the world after North America and Europe (Fang et al., 2013; Menz and Seip, 2004). Worryingly, there are vast agricultural ecosystems in regions of China impacted by acid rain and REEs (Bian et al., 2013; Larssen et al., 2006). Thus, combined pollution with acid rain and REEs has become a new factor affecting crop safety.

Some effects of combined acid rain and REEs on crops have been reported and the majority of previous studies have focused on seed

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germination, plant growth, yield and quality, and physiological and biochemical processes (Liang and Wang, 2013; Sun et al., 2013b; Wang et al., 2014; Wen et al., 2011; Zhang et al., 2015). However, the compound effects of these two pollutants on crop root nitrogen assimilation are rarely known. Roots are vital for long-term plant adaptation to terrestrial conditions (Zhang et al., 2007), and they have important functions, such as the absorption and transportation of water and nutrients in soil, synthesis and storage of nutrients and secondary metabolism products, environmental perception, and signal transduction (Rogers and Benfey, 2015). The growth of plant roots is controlled by genes but is also affected by environmental factors to a large extent, and it is an important goal to observe the responses of plants to environmental changes, which are also closely related to plant growth and yield formation (Rogers and Benfey, 2015). Nitrogen, the most important plant nutrient, is essential to normal plant growth and development and is an important component of amino acids, proteins, nucleic acids, phospholipids, coenzymes, and cofactors in the plant cell (Buchanan et al., 2000). Plant roots convert absorbed nitrogen to organic compounds for amino acid metabolism by assimilating NO_3^- and NH_4^+ (Buchanan et al., 2000). During this process, the activities of enzymes will affect amino acid metabolism of plant roots and then affect the growth and development of roots and in turn, the whole plant (Xu et al., 2012). Therefore, understanding nitrogen assimilation in plant roots is very important in studying the effect of typical environmental pollutants on plant biology.

Soybean (*Glycine* max L.), a common food crop that usually grows in regions where acid rain pollution and REEs occur simultaneously, is an important crop material recommended for toxicological research by the United States' Environmental Protection Agency (2012). Lanthanum (La), a typical REE, is ubiquitous in soil (Tyler, 2004). In the present study, the combined impact of acid rain and lanthanum ion (La^{3+}) on the NO_3^-, NH_4^+, amino acid and soluble protein contents and the key enzyme activities related to nitrogen assimilation in soybean seedling roots were determined. The aim of this study is to deeply and clearly identify the effects of combined acid rain and REEs pollution and its mechanism on nitrogen assimilation in crop roots. Moreover, this work aims to provide a framework for assessing the potential ecological risks of REEs to crops, thereby supporting the limitation of REEs in agriculture.

2. Materials and methods

2.1. Crop culture and treatment

In order to eliminate pathogenic microorganisms and prevent soybean pests and diseases, the soybean seeds (Zhonghuang 25, Wuxi Seed Co., Ltd., China) were surface sterilized for 5 min (Sinclair, 2010). Then, their germination and growth were referred to our previously published methods: the soybean seeds were germinated and grown at 25 ± 5 °C, 300 μmol $m^{-2}\cdot s^{-1}$ light intensity, and 16/8 h day/night cycles in a greenhouse. Fifteen days later, seedlings were cultured in half-strength Hoagland's solution. The air supply in the culture solution during hydroponic cultivation was provided by an aquarium air pump. The nutrient solution was renewed every 3 d to stabilize the pH value (Wen et al., 2011; Sun et al., 2013c). The simulated acid rain solutions at pH 4.5 and 3.0, La^{3+} solutions (1.20, 0.40 and 0.08 mM), and combined solutions of acid rain and La^{3+} were prepared as our previously published method (Sun et al., 2013c). When the seedlings were twenty-five days old, they were treated with the above acid rain and La^{3+} solutions in 3 × 4 groups: (1) In the control treatment, soybean seedlings were cultured in the -P nutrient solution (pH 7.0) and sprayed with distilled water until the drops began to fall; (2) in the La^{3+} treatment, soybean seedlings were cultured in the -P nutrient solution with La^{3+} (0.08, 0.40 or 1.20 mM, pH 7.0), and then sprayed with distilled water until the drops began to fall; (3) in the acid rain treatment, soybean seedlings were cultured in the acidic -P nutrient solution (pH 3.0 or 4.5) and sprayed with acid rain at the same pH with an acidic -P nutrient solution (pH 3.0 or 4.5) on foliage until the drops began to fall; (4) in the combined treatment with La^{3+} and acid rain, soybean seedlings were cultured in the acidic -P nutrient solution with La^{3+} (0.08, 0.40 or 1.20 mM, pH 3.0 or 4.5) and then sprayed with acid rain at the same pH with an acidic -P nutrient solution (pH 3.0 or 4.5) until the drops began to fall (Wen et al., 2011; Sun et al., 2013c). All treatments were repeated 5 times. After 7 days of treatment, the fresh roots were collected to determine the following experimental indicators.

2.2. Determination of the NO_3^- content

The NO_3^- content was determined according to the published methods with slight modifications (Delhon et al., 1995; Sun et al., 2013a). Fresh soybean seedling roots (1 g) were cleaned with distilled water, ground with 20% acetic acid solution and transferred to a scaled tube to a final volume of 20 mL. Then a powder mixture (0.4 g) containing 10 g $MnSO_4$, 100 g $BaSO_4$, 2 g zinc powder, 75 g citric acid, 2 g α-naphthylamine and 4 g sulfanilic acid was added. Shaken the tube for 3 min, and stranded it for 10 min followed by the centrifugation at 4000 × *g* relative centrifugal force (RCF) for 5 min. Then the absorbance at 520 nm (A_{520}) was determined. The NO_3^- content was calculated with reference to a standard curve prepared with $NaNO_3$ of different concentrations and expressed as $\mu g\cdot g^{-1}$ fresh weight (FW).

2.3. Determination of NR activity

The nitrate reductase (NR) activity was determined according to the published method with some modifications (Ogawa et al., 1999). Fresh soybean seedling roots (1 g) were first cleaned with distilled water, ground in an ice bath with 6 mL pre-cooled phosphate buffered saline (PBS, pH 7.2), and homogenized at 4 °C followed by the centrifugation at 10000 × g RCF for 20 min. The supernatant was retained as the enzyme extract. Next, 50 μL enzyme extract was added to a mixed solution [10 mM KNO_3, 5 mM $NaHCO_3$, 25 mM PBS, 0.2 mM nicotinamide adenine dinucleotide (NADH)]. After these two solutions reacting fully at 25 °C for 30 min, put the reaction solution (500 μL) in a test tube, and then 1% sulfonamide reagent (250 μL) and 1% α-naphthylamine reagent (250 μL) were added to react for 20 min. Then A_{520} was determined, and the NO_2^- content was calculated according to a standard curve prepared with different concentrations of $NaNO_2$. The NR activity was expressed as the amount of NO_2^- generated from the reaction with 1 g of fresh roots in 1 h (μg $NO_2^-\cdot g^{-1}$ FW$\cdot h^{-1}$).

2.4. Determination of the NiR activity

The nitrite reductase (NiR) activity was determined according to the amount of NO_2^- reduction (Debouba et al., 2006; Lea and Miflin, 2003; Ogawa et al., 1999; Sánchez et al., 2004). The NiR extraction process was as same as that for NR. The enzyme extract (50 μL) was first added to react with a mixture [0.5 mM $NaNO_2$, 50.0 mM Tris HCl buffer solution (pH 7.5) and 1 mM methyl violet fine] and was diluted to a final volume of 0.9 mL. Next, 100 μL solution (0.2 M $NaHCO_3$ and 0.12 M $Na_2S_2O_4$) was added to react at 30 °C for 60 min, and 100 μL of 1 M zinc acetate was added followed by the centrifugation at 6000 × *g* RCF for 10 min. Then, A_{520} was measured using the supernatant, and the NO_2^- content was calculated according to a standard curve prepared with $NaNO_2$ of different concentrations. The NiR activity was expressed as the amount of