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Humic acids buffer the effects of urea on soil ammonia oxidizers and potential nitrification

Lianhua Dong [a,b], Ana Lucía Córdova-Kreylos [b,1], Jinshui Yang [a], Hongli Yuan [a,*], Kate M. Scow [b]

[a] State Key Lab for Agrobiotechnology, College of Biological Sciences and Center of Biomass Engineering, China Agricultural University, No. 2 Yuanmingyuan West Road, Beijing 100193, PR China
[b] Department of Land, Air and Water Resources, University of California, Davis, CA 95616-5200, USA

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ABSTRACT

Humic acids (HAs) play an important role in the global nitrogen cycle by influencing the distribution, bioavailability, and ultimate fate of organic nitrogen. Ammonium oxidation by autotrophic ammonia-oxidizing bacteria (AOB) is a key process in ecosystems and is limited, in part, by the availability of NH_4^+. We evaluated the impact of HAs on soil AOB in microcosms by applying urea (1.0%, equal to 10 mg urea/g soil) with 0.1% bHA (biodegraded lignite humic acids, equal to 1 mg/g soil), 0.1% cHA (crude lignite humic acids) or no amendment. AOB population size, ammonium and nitrate concentrations were monitored for 12 weeks after urea and HA application. AOB densities (quantified by real-time PCR targeting the *amoA*) in the Urea treatments increased about ten-fold (the final abundance: 5.02×10^7 copies (g of dry soil)$^{-1}$) after one week of incubation and decreased to the initial density after 12 weeks incubation; the population size of total bacteria (quantified by real-time PCR with a universal bacterial probe) decreased from 1.12×10^{10} to 2.59×10^9 copies (g of dry soil)$^{-1}$ at week one and fluctuated back to the initial copy number at week 12. In the Urea + bHA and Urea + cHA treatments, the AOB densities were 4 and 6 times higher, respectively, than the initial density of approximately 5.07×10^6 copies (g of dry soil)$^{-1}$ at week 1 and did not change much up to week 4; the total bacteria density changed little over time. The AOB and total bacteria density of the controls changed little during the 12 weeks of incubation. The microbial community composition of the Urea treatment, based on T-RFLP using CCA (canonical correspondence analysis) and pCCA (partial CCA) analysis, was clearly different from those of other treatments, and suggested that lignite HAs buffered the change in diversity and quantity of total bacteria caused by the application of urea to the soil. We hypothesize that HAs can inhibit the change in microbial community composition and numbers, as well as AOB population size by reducing the hydrolysis rate from urea to ammonium in soils amended with urea.

1. Introduction

Large amounts of N fertilizer are applied annually to agricultural soils with serious consequences for climate change and water quality (Clinton et al., 1995). Any ammonia not absorbed by plants is rapidly oxidized by autotrophic ammonia-oxidizing archaea (AOA) and bacteria (AOB). Ammonia oxidation, the chemolithoautotrophic oxidation of NH_3 to NO_2^- via hydroxylamine (Wood, 1987), is limited in many ecosystems by the availability of NH_3 (Belser, 1979; Laanbroek and Woldendorp, 1995). In soils where ammonia oxidizers compete with plants and heterotrophic bacteria for available NH_3 (Bodelier et al., 1998), humic acids (HAs) may play a role in regulating the soil availability of NH_3, due to their adsorption properties (Mackowiak et al., 2001).

HAs are polyelectrolytic macromolecular compounds originating from chemical and biological degradation of plant and animal residues, and microbial cells (Hayes and Wilson, 1997). HAs play an important role in the global nitrogen cycle through their influence on the distribution, bioavailability, and ultimate fate of sedimentary organic nitrogen (Davies and Ghabbour, 1998; Lichtfouse et al., 1998; Stankiewicz and Van-Bergen, 1998; Christl et al., 2000). Humic substances can incorporate nitrogen into their structure either directly through chemical reactions or indirectly through microbial activities and subsequent decomposition of microbial biomass (Clinton et al., 1995). Several reports indicate that ammonia–N may be abiotically fixed to soil organic matter, lignin, peat or coal (Nommik and Vathras, 1982; Lapierre et al.,

* Corresponding author. Tel.: +86 10 62733464; fax: +86 10 62734008.
E-mail address: hlyuan@cau.edu.cn (H. Yuan).
[1] Present address: Marrone Organic Innovations, 2121 Second St., Suite 107B, Davis, CA 95618, USA.

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1994; Bosatta and Agren, 1995) when the C/N ratio of plant residue during humification is higher than 10 (Knicker et al., 1997). Thorn and Mikita (1992), using ^{15}N and ^{13}C NMR techniques, detected that ^{15}N-labeled ammonia was incorporated into HAs in the laboratory incubation and that the average N content of HAs increased from 0.88 to 3.17%.

It is important to note that HAs can vary in the chemical characteristics and properties based on their origin. Weathered lignite contains 40–85% HAs, while soils on average contains only 1–5% HAs. Lignite HAs contain more carbonyl carbon (about 16%) and less aliphatic carbon (27%) than soil HAs with about 11% carbonyl carbon and 31% aliphatic carbon (Zheng, 1991). HAs made from low grade coal, such as lignite, has a long history of use as a fertilizer in combination with urea. In China alone, 350,000 tons HAs are used in agriculture every year (Zheng, 1991; Jiang and Zhang, 2002; Liang et al., 2007). It has been shown in previous studies that lignite HAs can increase crop yields relative to urea-only treatments (Zheng, 1991), indicating a synergistic effect between the two compounds, However, little is known regarding the mechanism by which lignite HAs increase the benefits of urea application. Based on earlier reports (Thorn and Mikita, 1992; Clinton et al., 1995) and our previous study (Dong et al., 2006), two possible mechanisms are suggested: 1) part of the ammonium generated from urea mineralization is incorporated into lignite HAs, this reducing the net loss due to volatilization, 2) lignite HAs inhibit the activity of urease, which decomposes urea to NH_3, resulting in a lower rate of urea hydrolysis. This reduced rate of hydrolysis reduces the loss of NH_3, increasing urea availability for plants. The increased availability of NH_3 could in turn affect the structure or population size of AOB communities in the soil (Bollmann and Laanbroek, 2001).

Other potential effects of HAs on microbial communities are structure stabilization: buffering the changes in size or abundance of some microbial groups by chelating unavailable nutrients (thus making them available) and buffering pH (Mackowiak et al., 2001; Pertusatti and Prado, 2007). Additionally, HAs may reduce negative effects of direct application of urea and other chemical fertilizers on soil bacteria or fungi. The buffering of pH is an important determinant of AOB and total bacteria community structure (Frostegård et al., 1993; Pennanen et al., 1998; Kelly et al., 1999; Enwall et al., 2007). HAs have been shown to buffer pH between 5.5 and 8.0 (Pertusatti and Prado, 2007). So we hypotheses that HAs can buffer the community change caused by increasing or decreasing pH.

With the present study, we aimed to clarify the mechanisms by which lignite HAs amplify the effects of urea on crop yields. To test this effect, we measured the effects of lignite HAs on microbial community structure and population size, and more specifically on AOB and total bacteria in urea-amended soil. We assumed that lignite HAs could either decrease the AOB population size or change the AOB community composition, and stabilize the diversity of soil total bacteria after the application of urea. Compared to the original formulation extracted from crude lignite (cHA), biodegraded lignite HA (bHA) has a relatively higher nitrogen content and lower molecular mass, with greater potential to stimulate biological activity in soil (Dong et al., 2006; Yuan et al., 2006). Soils were treated with two different kinds of HAs (cHA: crude lignite humic acids, and bHA: biodegraded lignite humic acids) after urea application in microcosms. Changes in the microbial community structure were monitored by Terminal Restriction Fragment Length Polymorphism (T-RFLP) and the population sizes of total bacteria and AOB were measured by real-time PCR. Other parameters measured during the incubation included pH, ammonium and nitrate concentration, potential nitrification and urease activity.

2. Material and methods

2.1. Lignite sample and HA extraction

Lignite was collected from the Huolingele Minerals Administration coal mine, Inner Mongolian Autonomous Region, Northwest China. Air-dried crude lignite or biodegraded lignite was pulverized and passed through a 70-mesh sieve. cHA and bHA were extracted according to a previously described protocol (Dong et al., 2006). Briefly, lignite or biodegraded lignite was suspended in a NaOH solution and stirred at 20 °C for 24 h, then centrifuged at 6000 × *g* for 15 min. The supernatant was filtered and the pH was adjusted to 2.0 with HCl. The solution was centrifuged to precipitate HA which was washed with distilled water three times and dried at 60 °C.

2.2. Experimental design

2.2.1. Soil sampling

In April 2006, soil cores from depths of 0 to 10 cm were collected at the China Agriculture University farmlands in, Beijing, China. A corn crop had been harvested in October 2005, and no vegetation was present at the time of sampling. Physical and chemical characterization of the soil yielded the following results: pH, 7.47; organic matter, 10.83%; total N, 0.53%; inorganic N, 69 mg/kg; soluble P, 9.83 mg/kg; bulk density, 1.32 g cm^{-3}. Based on its chemical and physical properties the soil at this site is classified as a Cinnamon soil (Institute of Soil Science, CAS). The soil was air dried, passed through a 1-mm sieve and stored at 4 °C.

2.2.2. Microcosm preparation

Four soil treatments were added as solutions of (1) Urea (1.0% urea, equal to 4.7 mgN/g dry soil), (2) Urea + bHA (1.0% urea + 0.1% bHA), (3) Urea + cHA (1.0% urea + 0.1% cHA), and (4) untreated control (only water). Urea and HAs were added to the soil as described by Venterea and Rolston (2000) to minimize spatial stratification. The influent solutions were prepared as follows: 1 g HA dissolved in 1 L 0.01 M NaOH, and pH was adjusted to 7.0 with 0.1 M HCl. After that, 10 g urea was added to the HA solution. Soils were flushed slowly with their respective HA + urea solutions until effluent urea–N concentrations (measured by spectroscopic photometry (Peiqi and Mile, 1991) were equal to the influent level. The flushed solution volumes used were 2000, 2015 and 2011 ml for the Urea, Urea + bHA, and Urea + cHA treatment respectively. Soils were then drained with a pressure plate to equilibrate to 0.05 MPa. The control was flushed with 2000 ml dH_2O instead of the Urea + HAs solution.

Soil microcosms were constructed in 5 L plastic pots. A total of 12 pots (four treatments in triplicate) with 2 kg soil each were incubated at 28 °C. Samples were collected from each pot after 0, 2, 3, 5, 7, 9, 12, 16, 28 and 84 days of incubation and analyzed for pH, NH_4^+–N, NO_3^-–N, urease activity and potential nitrification. Additionally, five-gram soil from each pot was collected at 1, 4 and 12 weeks of incubation. These samples were stored at −80 °C for subsequent DNA extraction.

2.3. Chemical measurements

Soil pH was determined using a combined glass electrode in 1:1 (w:v) ratios of soil with distilled 1 M KCl. To measure ammonium and nitrate concentrations, 100 ml of 0.01 M $CaCl_2$ was added to 10 g of soil, shaken for 1 h on a reciprocating shaker and centrifuged at 4100 × *g* for 5 min at 5 °C. The supernatant was collected and stored at −20 °C until analysis. NH_4^+ and NO_3^- concentrations in the extracts were determined by a continuous flow analytical system (TRAACS 2000, Bran & Lubbe, Norderstedt, Germany).