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Soil urease activity and bacterial ureC gene copy numbers: Effect of pH

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ABSTRACT

Urea-N is ubiquitous in soils, having both natural and anthropogenic sources. The enzyme urease catalyzes its hydrolysis to NH_3 and is produced by plants and many soil microorganisms, but there are growing concerns related to possible urea-induced eutrophication of surface waters proximate to agricultural fields. Agronomic research has focused on the relationship between urea hydrolysis and soil physical or chemical properties, rather than on direct measurements of the microbial community and its population diversity, especially using quantification of genes that code for urease. We quantified bacterial and archaeal 16S rRNA, fungal ITS, and bacterial *ureC* gene copies as a function of physical and chemical soil properties. Soils were sampled from A and B horizons along a toposequence that comprised an agricultural field, a grassed field border, and a forested riparian zone in the Chesapeake Bay watershed of Maryland. The riparian zone soils contained the highest total number of genes among both A- and B-horizon soils. The soils were then experimentally altered in the laboratory to achieve a range of pH values between 3.1 and 7.1. Soil pH was chosen as a variable because it varies both naturally and due to agronomic practices, and it influences microbial community structure and function. Archaeal 16S rRNA extracted from the pH-adjusted soils did not show a consistent pattern of increase or decrease with changes in pH, while ITS was greatest at low pH and bacterial 16S and bacterial *ureC* were greatest at high pH. We measured higher urea hydrolysis rates and gene copy numbers in A-horizon soils than in B-horizon soils, and found that urea hydrolysis rate was significantly correlated with gene copies of bacterial 16S, *ureC*, and increased pH. This suggests that liming acid soils increases urea hydrolysis rates in part by encouraging the growth of microorganisms capable of producing urease.

1. Introduction

Urea is an organic N compound that is ubiquitous and naturally occurring in soils, and is a widely used fertilizer. It is a waste product in the excrement of mammals and a decomposition product of uric acid produced by all birds, some reptiles and amphibians, and most insects (Livingston et al., 1962; Nahm, 2003; Wright, 1995). Commercial production and use of urea fertilizer increased from approximately 0.3×10^6 to $>40 \times 10^6$ Mg from 1961 to 2002 (FAO, 2002), and urea is now estimated to make up almost 60% of the world's consumption of N fertilizer (Glibert et al., 2014). The increased use of urea fertilizer has raised concerns that this soluble, nonionic compound could be carried by runoff or leach through soils, contaminate surface waters, and support harmful algal blooms (Glibert et al., 2001; Glibert et al., 2005; Heil et al., 2007; Kudela et al., 2008; Li et al., 2010; Lomas et al., 2002).

Urea applied to soils usually results in rapid hydrolysis to NH_3/NH_4^+ and further oxidation to NO_3^- by soil bacteria and archaea (Wessen et al., 2010; Yadev et al., 1987). The availability of this N source to agricultural crops relies upon the activity of soil urease, the enzyme responsible for the hydrolysis to NH_3 [Eq. (1)] (Mobley and Hausinger, 1989).

$$CO(NH_2)_2 + H_2O + 2H^+ \rightarrow CO_2 + 2NH_4^+ \quad (1)$$

Many plants, bacteria, fungi, and archaea found in soils produce ureases, and the specific activities of their purified enzymes in laboratory assays range from 14.5 to 7100 μmol urea min^{-1} mg $enzyme^{-1}$ (Krajewska, 2009; Lu and Jia, 2013; Tourna et al., 2011; Witte, 2011). Urease exists both within cells and as an extracellular enzyme released into the soil upon cell death. Outside the cell, soil urease is stabilized by adsorption to soil colloids, particularly clays and organic matter, and can continue to function in this state (Krajewska, 2009). Thus, urease exits in soils in two pools: one intracellular in living cells; and the other, extracellular on clay-organic matter surfaces (Krajewska, 2009). The proportion of soil urease that is intracellular ranges from 37.1 to 73.1% of total urease activity in the soil, with the remainder being extracellular (Klose and Tabatabai, 1999). Despite this biological origin, the rate of urea hydrolysis in soils has traditionally been explained by variations in soil physical and chemical characteristics such as microbial biomass C and N, surface area and pH (Khakurai and Alva, 1995; Klose and Tabatabai, 1999; Singh and Yadev, 1981; Wali et al., 2003; Yadev et al.,

Abbreviations: PMA, phenylmercuric acetate; AG, agricultural field; GB, grassed field border; RZ, riparian zone; AOA, ammonia-oxidizing archaea.

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1987; Zantua et al., 1977). There are conflicting reports in the literature about the optimum pH for urease activity, ranging from pH 5–8; and whether or not pH is correlated with urea hydrolysis (Fisher et al., 2016; Pettit et al., 1976; Zantua et al., 1977). These inconsistencies may be a result of sampling soils with different microbial community abundance and composition. Soil pH affects microbial biomass and activity, as well as the relative proportions of bacteria and fungi (Pietri and Brookes, 2008, 2009). However, the interactions among substrate availability, microbial competition, and soil pH in determining soil microbial community structure and function are complex (Rousk et al., 2010), and the resulting effect on rates of urea hydrolysis is unknown or speculative.

Molecular techniques have been used to investigate the link between functional microbial genes and N cycling in soils (Hallin et al., 2009; Morales et al., 2010; Placella and Firestone, 2013; Wu et al., 2012), although only a few studies have investigated the *ureC* gene, which codes for one of three structural subunits in the urease enzyme (Mobley et al., 1995). The majority of *ureC* work has been done with bacterial *ureC*, although very recent work has identified *ureC* in archaea and fungi as well (Cook et al., 2008; Lu and Jia, 2013). Of those studies looking at bacterial *ureC* (Yang et al., 2014; Yarwood et al., 2015), none were found that investigated the microbial community and bacterial *ureC* genes in agronomic soils, where the rate of urea hydrolysis is influenced by pH and is of interest for agronomic productivity and environmental protection.

In an experiment reported in Fisher et al. (2016), soils were sampled from A and B horizons along a Coastal Plain transect that included a site in an agricultural field, one in a grassed field border, and one within a riparian zone along the Wye River, a tributary of the Chesapeake Bay estuary in Maryland. The landscape transect provided a natural gradient in pH and in total C and N among the sites and soil horizons. The results showed that A horizon soils hydrolyzed urea more rapidly than did B horizon soils, and when soil pH was experimentally altered, urea hydrolysis increased with pH and was highest in the riparian zone A horizon soils. The results further identified that soil organic matter content was an important predictor of urea hydrolysis rates in the soils sampled across this transect. Based on that work, the objectives of this study were to 1) determine whether differences in the soil microbial community could explain differences in the previously measured rates of urea hydrolysis in the different soils and horizons sampled, and in which soil pH was experimentally altered, and 2) to correlate microbial gene numbers within the soil with urea hydrolysis rates. We hypothesized that microbial genes and urea hydrolysis rate would be highest in A-horizon soils where urea hydrolysis rates were highest; that bacterial 16S and bacterial *ureC* gene copy numbers would increase with pH and would be highest in the pH-adjusted riparian zone soils where urea hydrolysis rate was highest; and that urea hydrolysis rate would be correlated with bacterial *ureC* gene copy numbers.

2. Materials & methods

2.1. Site description and soil collection

This study was conducted using soils sampled in October 2012 at the Wye Island Natural Resource Management Area in Queen Anne's County, Maryland (38°54′11.97″N, 76° 8′12.20″W), located within the Coastal Plain physiographic province of the Mid-Atlantic region of the USA. Soils were sampled along a transect consisting of three locations running from an agricultural field (AG) actively farmed in a typical Maryland two-year grain rotation of corn (*Zea mays* L.) followed by winter wheat (*Triticum aestivum* L.) with a double-crop of soybeans (*Glycine max* L.), through a grassed field border (GB) of tall fescue (*Festuca arundinacea* Schreb.), to a forested riparian zone (RZ) of sassafras (*Sassafras albidum* Nutt.), southern red oak (*Quercus falcata* Michx), black cherry (*Prunus serotina* Ehrh.), red maple (*Acer rubrum* L.), and black walnut (*Juglans nigra* L.), with under story vegetation composed of multiflora rose (*Rosa multiflora* Thunb.), sumac (*Rhus* sp. (L.), trumpet creeper (*Catalpa radicans* L. Seem.), common greenbrier (*Smilax rotundifolia* L.), Virginia creeper (*Parthenocissus quinquefolia*), and honeysuckle (*Lonicera japonica* Thunb.). The soils were sampled both from the A horizon (0–15 cm) and at a depth representative of the B horizon. In the AG, this depth was between 45 and 60 cm, and in the GB and RZ sites, the sample was taken between 65 and 80 cm due to horizon differences observed in the soil profile. The AG soil was sampled from the Ingleside mapping unit (38°54′11.97″N, 76° 8′12.20″W), the GB soil sampled from the boundary of Ingleside and Longmarsh and Indiantown mapping units (38°54′10.37″N, 76° 8′13.79″W), and the RZ soil was sampled from the Longmarsh and Indiantown mapping unit (38°54′9.98″N, 76° 8′14.70″W); all of which were similar to the Ingleside series (coarse-loamy, siliceous, mesic Typic Hapludult). Sampling soils in the autumn may have resulted in soils with lower seasonal urease activities compared to spring (Kang et al., 2009), and therefore, the rates of hydrolysis discussed in this work may be lower than those that would be measured in soils sampled in the spring under warm, humid Mid-Atlantic climatic conditions. However, autumn also is the season when groundwater recharge begins to occur in the Coastal Plain, and transport of urea and mineral N species may take place when the water table is at or near the surface during the winter.

A 7.6-cm diameter, open-faced soil auger was used to sample four profiles from four different locations within a 6 m^2 area at each transect point. These four samples were combined to form one composite sample from each depth at each point along the transect. The BC horizon sample in the RZ, however, consisted of three auger holes because a proliferation of tree roots prevented additional sampling. The soils were sampled on the same day and stored in a cooler for fewer than 5 h during transport to the laboratory and being allowed to equilibrate to room temperature (21–23 °C). The soils were sieved to pass a 4-mm screen, and kept field-moist (approximately −10 kPa matric water potential) in double plastic bags in closed plastic buckets for 8 to 10 months during experimentation. Previous work monitored the rate of urea hydrolysis in soils stored over a period of this length (Fisher, 2014) and found that a distinct decrease in the urea hydrolysis rate was not measured until after 8 to 10 months of storage. These findings are consistent with those of Zantua and Bremner (1977), who determined that urease activity was not affected by storing field-moist soils for up to six months at temperatures ranging from −10 to 40 °C.

2.2. Determination of pH effects on urea hydrolysis

The determination of pH effects on urea hydrolysis is described in detail in Fisher et al. (2016). Briefly, soils were treated with a solution of 18 MΩ water (control), 0.5 or 1.0 M HCl, or dry $CaCO_3$ and were brought to a moisture content approximately equivalent to −10 kPa using 18 MΩ water or HCl solution, as necessary for the treatment. The soils were allowed to equilibrate at room temperature (21–23 °C) for approximately 4 weeks until the adjusted pH stabilized between 3.1 and 7.1, depending upon the treatment. After pH equilibration, the moist equivalent of 2.5-g oven-dried soil (105 °C) from each pH treatment was frozen at −20 °C for future DNA extraction. The rate of urea hydrolysis in each soil × pH treatment was determined in a multi-day incubation experiment. Triplicate, moist soil samples equivalent to 2.5 g oven-dried soil from each treatment were weighed into 37.5-mL polycarbonate centrifuge tubes in a randomized complete block design in the laboratory using the variables of transect location, horizon, and pH. The soils were mixed with 22.5 mL of a 7.94×10^{-4} M urea-N solution (equivalent to 100 mg urea-N kg $soil^{-1}$, a concentration equal to the most N a farmer might apply in a fertilizer application for maize production in Maryland) in a background solution of 0.01 M $CaCl_2$ and placed on an orbital shaker set at 800 cycles min^{-1} that shook the tubes for 30 min each h. This treatment followed the method of Greenan and Mulvaney (1995) with modifications. Specifically, soils were incubated in a solution free of phenylmercuric acetate (PMA) to allow for microbial activity to take place during the incubation. At pre-