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Liming does not counteract the influence of long-term fertilization on soil bacterial community structure and its co-occurrence pattern

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ABSTRACT

Chemical fertilizer application is a common agronomic practice to improve crop productivity and liming is often used to counteract the soil acidification caused by long-term fertilization; however, it is unclear whether liming will counteract the effect of long-term fertilization on soil bacterial community structure and its co-occurrence pattern. Here, we examined the influence of long-term fertilization (NPKS) and liming (L) on soil bacterial community structure and its co-occurrence networks by sequencing 16S rRNA gene amplicons. Our results showed that liming counteracted the influence of long-term fertilization on soil pH, but not on soluble organic carbon (SOC) and electrical conductivity (EC). Long-term fertilization affected the abundance of Acidobacteriia, Deltaproteobacteria and Gammaproteobacteria in the 0–10 cm soil, and that of Deltaproteobacteria, Gammaproteobacteria and Gemmatimonadetes in the 10–20 cm soil; whereas liming affected the abundance of Acidobacteriia, Gammaproteobacteria, and Chloracidobacteria in the 0–10 cm soil and that of Deltaproteobacteria in the 10–20 cm soil. The bacterial community structure in soils with the NPKS-L treatment was different from that with other treatments, and was mainly affected by SOC, EC, and NO_3^- concentration. The link numbers in bacterial co-occurrence networks were decreased by long-term fertilization or liming alone, but were increased by the NPKS-L treatment. Notwithstanding the fact that liming alleviates fertilization-induced soil acidification, this study indicates that liming did not counteract the effect of long-term fertilization on soil bacterial community structure and its co-occurrence pattern, hence cannot recuperate the soil microbial functionality that is changed by long-term fertilization.

1. Introduction

Chemical fertilizer application is the most important input for maintaining crop productivity in intensively managed agricultural systems. Long-term fertilization often causes soil acidification (Guo et al., 2010), which induces other problems such as phosphorus (P) deficiency, aluminum (Al) toxicity, and reduction of biodiversity and soil productivity (Ahmad et al., 2013). Liming counteracts soil acidity, and helps to sustain and improve plant growth (Edmeades and Ridley, 2003), reduce Al toxicity, alleviate calcium (Ca) deficiency and increase the availability of P and other nutrients (Hamilton et al., 2007). Therefore, liming increases plant growth and organic carbon (OC) return to the soil, and potentially increases soil organic matter (OM) content and the long-term sustainability of the agricultural production system (Paradelo et al., 2015).

In cropping systems, soil bacteria drive biogeochemical processes of C and nutrients, influence crop growth, and govern the release of greenhouse gases (Nemergut et al., 2008). Soil pH changes caused by long-term fertilization and liming are critical factors that alter bacterial community composition and functionality (Pan et al., 2014). Soil pH is a key environmental factor that affects bacterial community composition from local to global scales (Fierer and Jackson, 2006; Griffiths et al., 2011). However, some studies suggest that soil pH indirectly drives the changes of bacterial community through influencing the availability of magnesium (Mg), nitrogen (N), Ca, and Al (Pan et al., 2014). Although liming counteracts the acidification caused by long-term fertilization, the complex interaction between fertilizer and lime in soils could induce changes of other soil properties, such as the soil OC and N concentrations (Manna et al., 2007). Liming can alleviate the nutritional stress in acidic soils and creates a better environmental

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condition for the development of acid-intolerant microorganisms, resulting in increased microbial biomass and soil respiration (Ahmad et al., 2013). Long-term fertilization and liming could also affect soil microbial communities by changing flux and stock of soil OC, which provides C resource for soil microorganisms. Chemical fertilization promotes oligotrophy and reduces OC mineralization (Xun et al., 2016), while liming increases OC mineralization due to a more favorable pH (Paradelo et al., 2015; Wang et al., 2016). However, both fertilization and liming increase soil OC input by increasing plant productivity and hence increasing returns of OM to the soil in the form of root exudates and crop residues (Paradelo et al., 2015). Moreover, soil fungal population and their activities would be promoted in acidic environments (Cheng et al., 2012). Accordingly, fertilization and liming would affect fungi activities in the soil (Guo et al., 2012; Kennedy et al., 2005) and would change the competition what the bacteria would be up against. However, there is a lack studies to elaborate the impact of liming on microbial community structure in soils with long-term fertilization.

Co-occurrence patterns of microbial populations are particularly important in understanding microbial community function by offering new insights into potential interaction networks and revealing niche spaces (Faust and Raes, 2012; Kara et al., 2013; Steele et al., 2011). Recent research has explored large, complex microbial community datasets and revealed previously unseen co-occurrence patterns, such as strong non-random associations and niche specialization (Faust et al., 2012), unexpected ecological relationships (Zhang et al., 2015), and deterministic processes (Chaffron et al., 2010). Topological-based analysis has been proved to be a powerful tool for understanding the characteristics of large co-occurrence networks (Lupatini et al., 2014). Changes in soil physicochemical properties caused by fertilization and liming would shape the niche structure in the soil ecosystem, but whether long-term fertilization and liming affect co-occurrence patterns of microbial communities is not known.

In this study, we examined the responses of soil bacterial community to 84 years of fertilization and 42 years of liming in the Classical Plots at Breton (in Alberta, Canada), which is the longest on-going experiment on Gray Luvisolic soils (Giweta et al., 2014). We sequenced 16S rRNA gene amplicons to characterize the bacterial community structure and to build co-occurrence networks. Given the complex interaction between fertilization and liming on soil bacterial community, we hypothesized that liming cannot counteract the influence of fertilization on the bacterial community structure and co-occurrence pattern in the studied long-term cropping system.

2. Material and methods

2.1. Site description and soil sampling

The Breton Classical Plots were established in 1930 at Breton (53.07°N, 114.28°W; elevation 830 m above sea level), Alberta, to address production challenges on Gray Luvisolic soils in west-central Alberta (Giweta et al., 2014). The research site has a mean annual air temperature of 2.1 °C, a mean annual precipitation of 547 mm, and a mean annual potential evapotranspiration of 723 mm between 2009 and 2016. The natural vegetation in the area was a mixture of trembling aspen (*Populus tremuloides* Michx.), balsam poplar (*Populus balsamifera* L.), white birch (*Betula papyrifera* Marsh.), and white spruce [*Picea glauca* (Moench) Voss]. The soil at the Breton Plots was classified as an Orthic Gray Luvisol (Typic Cryobralf) of the Breton loam series.

The Classical Plots have eight soil fertility treatments with a 5-year wheat–oat–barley–hay–hay (WOBHH) rotation. The fertility treatments were intended to test which nutrient, N, P, potassium (K) or sulfur (S), is deficient in gray soils (Dyck et al., 2012). The present study used an unfertilized control treatment (CK) and a treatment received NPKS fertilizers (NPKS). From 1930 to 1979, N, P, K, and S were applied in the NPKS treatment at 10, 6, 16,10 kg ha^{-1}, respectively (Dyck et al., 2012). From 1980, the N application rate was changed to 90 kg ha^{-1} for wheat on fallow, 50 kg ha^{-1} for wheat after forage, 75 kg ha^{-1} for oats, 50 kg ha^{-1} for barley, and no N application for legume hay. The P, K and S application rates in the NPKS treatment were changed to 22, 46 and 5.5 kg ha^{-1}, respectively. Starting in 1972, lime was added to the east half of each plot whenever the soil pH measured was less than 6.0. Currently, lime is applied to restored soil pH to 6.5. Using these guidelines, lime application was done about once every 10 years. The treatments in this study make up an experiment with a split-plot design: 2 levels of fertilization [fertilized (NPKS) vs non-fertilized (CK)] applied to the whole plot (plot size ~270 m^2) level and 2 levels of liming [limed (L) vs non-limed (NL)] applied at the subplot (~135 m^2) level.

In September 2014, we collected soil samples from four of five-year rotation plots, excluding the plots with the second year of hay that was treated with biocide that year. Five soil cores (35 mm diameter) were randomly collected from each subplot (from a total of 16 subplots) from three depth increments: 0–10, 10–20, and 20–30 cm. The five samples from the same subplot were combined to form a composite sample for each depth. Between the subplots, the core sampler was cleaned using 70% ethyl alcohol. The samples were immediately transported back to the laboratory on ice in a cooler. Once in the laboratory, visible roots and fresh litter material were removed from the samples. About 10 g of each soil sample was stored at −20 °C before DNA extraction. The remaining soil samples were air dried and sieved to 2 mm, homogenized for the analysis of soil physical and chemical properties.

2.2. Analysis of soil properties

Soil pH was determined using a pH meter (Orion, Thermo Fisher Scientific Inc., Beverly, MA, USA) and EC using an AP75 portable waterproof electrical conductivity (EC)/TDS meter (Thermo Fisher Scientific Inc., Waltham, MA, USA) at a 1:5 soil weight to deionized water volume ratio. The OM content was determined the losing weight after burning for 16 h at 550 °C in a muffle furnace. Total C and N concentrations were determined with a CE440 Elemental Analyzer (Exeter Analytical, Chelmsford, MA, USA). The soluble organic C (SOC) and soluble organic N (SON) were extracted by 0.5 M KCl (1:5 v:v) for 1 h on a rotary shaker. The supernatant was filtered using a 0.45 μm mixed cellulose ester syringe filter and then analyzed for SOC and SON with a Shimadzu 5000A TOC analyzer (Shimadzu Corporation, Kyoto, Japan) and for NH_4^+ and NO_3^- + NO_2^- concentrations with a Lachat QuikChem 8500 Flow Injection Analyzer (Hach, Loveland, CO).

2.3. Analysis of bacterial community structure

Metagenomic DNA was extracted with the FastDNA SPIN kit for soil (MP Biomedicals, Solon, OH, USA). Sample processing followed the manufacturer's instructions. After isolation, the extracted DNA was eluted in 100 μL of elution buffer. The quality and purity of the isolated metagenomic DNA was confirmed by agarose gel-electrophoresis.

The library construction was performed using a 2-PCR method. The PCR1 (locus specific amplification) amplifies a region of the 16s rRNA gene using the tagged primers Glenn-F515 (5′- CAG TCG GGC GTC ATC AGT GCC AGC MGC CGC GGT AA -3′) and trp1-R806 (5′- CCT CTC TAT GGG CAG TCG GTG ATG GAC TAC VSG GGT ATC TAA T -3′) where Glenn is a universal tag (Glenn, 2011), trP1 is the Ion Torrent truncated P1 adaptor (Life Technologies, Inc), and F515 and R806 are previously published locus specific primers (Barberán et al., 2012). The PCR2 (Barcode and Ion Torrent specific adaptor attachment) uses the diluted PCR1 product as a template. Here the forward primer is, from the 5′ end, Ion Torrent A adapter sequence including the 4bp key (Life Technologies, Inc), barcode (Life Technologies, Inc) and the Glenn tag (Glenn, 2011). The reverse primer is trP1 which is the Ion Torrent truncated P1 adaptor sequence (Life Technologies, Inc). This amplification, which was verified using agarose gel electrophoresis, results in amplicons which contain Ion Torrent specific adaptors, barcode and the targeted region of the 16s rRNA gene. Amplified samples that are all