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Location-dependent impacts of liming and crop rotation on bacterial communities in acid soils of the Pacific Northwest

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ABSTRACT

The cropping systems of northern Idaho and eastern Washington are dominated by a cereal-based (winter wheat) rotation that is exclusively rainfed. Following the green revolution, the productivity of wheat increased dramatically with semi-dwarf varieties and inexpensive synthetic fertilizers. However, long-term and frequent use of ammonium-based nitrogen fertilizers has resulted in the gradual acidification of fields in the region, with reduced productivity and symptoms of aluminum toxicity in some locations. A micronized liquid formulation of lime (calcium carbonate) was evaluated at three locations to mitigate soil acidification in comparison to a no-lime control. To examine how liming affects bacterial community structure, soil samples were taken in the spring in wheat and pea rotations 7 and 19 months after lime application, DNA was extracted and the V1-V3 portion of the 16S rRNA gene was amplified and sequenced with Illumina MiSeq. pH was only increased ~0.2–0.3 units in most locations, and liming only had a significant effect on bacterial community composition at one location (Pullman). Liming had no consistent or significant effect on community richness or diversity. Location had a stronger effect on communities than liming or crop rotation, but some specific taxa increased in relative abundance in response to liming, including the families Cytophagaceae and Flavobacteriaceae (Phylum Bacteroidetes), A4b (Phylum Chloroflexi), and Opitutaceae (Phylum Verrucomicrobia). A number of operational taxonomic units (OTU) in Chitinophagaceae and Xanthomonadaceae were increased by liming at the Pullman location, which had 3–4 X more OTUs affected by liming than the other locations. Streptomycetaceae and Oxalobacteraceae were more abundant in root zone soil of wheat than pea. In conclusion, there was a strong interaction between liming and location in determining soil bacterial composition, but specific components of the bacterial community responded to even minimal increases in soil pH.

1. Introduction

Soil pH has been on the decline in northern Idaho and eastern Washington for several decades, primarily due to the use of ammonium-based nitrogen fertilizers (Mahler et al., 1985; Mahler and McDole, 1985). While very little activity to remediate this emerging issue has occurred, a renewed interest in soil acidification was sparked by the discovery of acute soil acidity and severe symptoms of aluminum toxicity in wheat crops in Spokane County, Washington (Koenig et al., 2011). Moreover, in no-till systems, where there is little soil disturbance, soil pH is often stratified and a band of pH near 4 can form in the seed zone (5–10 cm below the soil surface). Soil acidification is a serious problem for growers in this region, since at low pH, plant availability of many nutrients is reduced and aluminum is solubilized and reaches concentrations that become phytotoxic (Bojórquez-Quintal et al., 2017).

The soil pH is critically important for chemical, physical and biological processes that support plant and soil health. Diverse soil microorganisms are especially critical for many soil processes in agriculture, such as nitrogen fixation in legume crops, nitrogen mineralization and nutrient cycling, and general suppression of soil borne plant pathogens. Each of these microbial activities is sensitive to changes in soil pH, which is also intimately related to the composition, abundance, and diversity of bacterial communities (Fierer and Jackson, 2006; Lauber et al., 2009). As the soil pH reaches and drops below 5, aluminum becomes increasingly more soluble and can result in substantial root damage and reduced crop performance (de la Fuente-Martinez and Herrera-Estralla, 2000). As the pH falls, bacterial populations can decline substantially, while fungal populations can increase (Rousk et al., 2009). However, at a soil pH below 4.5 even the fungal populations will begin to collapse as free aluminum becomes more abundant (Rousk et al., 2009).

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Incorporation of lime ($CaCO_3$) into agricultural soils is being explored as a means to mitigate acidification in northern Idaho, a practice new to the region. Here, we explore the impacts of an ultramicronized liquid lime suspension and crop rotation on bacterial community composition and diversity in three locations in northern Idaho and eastern Washington. We conducted liming trials at three locations over two years, in a wheat and pea rotation, sampled soil in the spring following a fall lime application, and used Illumina MiSeq to explore the response of bacterial communities to liming. We hypothesize that 1) liming will significantly shift soil bacterial community structure and promote bacterial diversity; and 2) location and rotational crop identity (wheat vs. pea) will modulate the impacts of liming on soil communities.

2. Material and methods

2.1. Experimental design and sample collection

Field trials were established in the fall of 2014 at three locations in northern Idaho and eastern Washington. These included sites 3 km northeast of Potlatch, ID (46.945732° N, −116.866309° W), 4 km south of Winchester, ID (46.203600° N, −116.618350° W), and 6 km north of Pullman, WA (46.798440° N, −117.174157° W). All three locations had experienced significant acidification with a pH below 5.0 in the top 15 cm of soil. The soil in Potlatch is characterized as a Taney ashy silt loam with a soil pH of 4.9 in the top 15 cm. The Winchester soil is a Boles-Joel complex with a soil pH of 4.5 in the top 15 cm. The Pullman soil is a Palouse silt loam with a soil pH of 4.7 in the top 15 cm. At each location treatments consisted of no lime versus 2242 kg/ha of an ultramicronized calcium carbonate lime (NuCal, Columbia River Carbonates, Vancouver, WA). Lime was applied at each location in the October of 2014. After application of the fluid lime, the material was incorporated to a depth of about 10 cm using a tillage implement with sweeps. This was the only tillage applied to the plot for the duration of the study aside from the seed drill passing through the plot each year. Each plot was seeded with winter wheat cv. Madsen or spring pea cv. Banner and a winter wheat-spring pea 2-year rotation was maintained. The trial was organized into a randomized complete block design with four replications and each crop being seeded in separate, but adjacent portions of the field. Each individual plot was 4.9 m wide by 12.2 m long. All trials were managed using best management practices for fertility, weed management and pest control typical for production of soft white winter wheat and green pea in the region. Soil samples were collected in May of 2015 and 2016 for chemical and biological analysis. For chemical analysis, a sample was collected from each replicate of each treatment by crop combination. Each sample consisted of a composite of 10 soil cores (2.2 cm diameter) collected to a depth of 15 cm from each individual plot (total of 16 composite samples per location each year). Samples for community analysis were collected in a similar manner with a composite sample of 5 soil cores of the same diameter and depth removed from each replication of each treatment by crop combination. For the community analysis samples, the cores were removed from the root zone of the plants by slightly angling the corer next to the row of plants.

2.2. DNA extraction and Illumina sequencing

For each sample (16 per location per year, total of 96 individual samples), DNA was extracted from 0.25 g of soil using the Powersoil DNA extraction kit (MoBio/Qiagen, Carlsbad, CA) following the manufacturers' instructions with bead-beating performed on a FastPrep bead beater (MP Biomedical, Santa Ana, CA) using the 'soil' protocol. DNA was quantified and quality checked with a NanoDrop spectrophotometer (Thermo Scientific) and shipped overnight on dry ice to the University of Minnesota Genomic Center (UMGC) for amplification of the V1-V3 region of the 16S rRNA gene with the template-specific primers (MN_27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and MN_534R (5′-ATTACCGCGGCTGCTGG-3′)) and subsequent Illumina sequencing (2x300 V3 chemistry) as described in Gohl et al. (2016). A single DNA extraction and sequencing run were performed for each soil sample.

2.3. Data processing

Illumina reads were paired using PEAR (v0.9.6; (Zhang et al., 2014)), followed by removal of primers and filtering of sequences (maximum expected error of 0.2, no ambiguous bases, and a minimum length of 350 base pairs) using cutadapt (version 1.9.1; (Martin, 2011)). Operational taxonomic units (OTUs) were clustered using the UPARSE pipeline (Edgar, 2013). Briefly, high-quality sequences for OTU clustering were generated by further error filtering of reads (maximum expected error of 1), dereplication, abundance sorting, and removal of singletons using Vsearch (Rognes et al., 2016). OTUs were clustered at 97% similarity using usearch (Version 8.1; Edgar, 2013) and raw reads were mapped to OTUs using vsearch to generate an OTU table. Taxonomy was assigned to OTU representative sequences (centroids) using the Ribosomal Database Project Naïve Bayesian Classifier (Wang et al., 2007) with a confidence threshold of 0.75 implemented in QIIME (version 1.9.1, Caporaso et al., 2010). OTUs identified as non-bacterial, mitochondria, or chloroplasts or those with < 10 total sequences were removed to ensure high-quality OTUs. OTU tables were rarefied to 10,662 sequences/sample using the vegan package of R (Oksanen et al., 2016) for all analyses unless otherwise noted.

2.4. Soil chemical analyses

Soil samples were passed through a 2-mm sieve and air dried for 72 h at 23 °C. All soil samples were sent to Best Test Analytical Services (Moses Lake, WA) for analysis. Except where indicated, the procedure was derived from the *Soil, Plant and Water Reference Methods for the Western Region* (Gavlak et al., 2005). Organic matter was measured using the Walkley-Black method (Walkley and Black, 1934) and pH was 1:1 (water:soil) slurry. The exchangeable bases (Ca, Mg, Na) and Al were measured by mass spectrophotometry after extraction with 1.0 N KCl (Bertsch and Bloom, 1996). Ammonia was also extracted with 1.0 N KCl and quantified colorimetrically using sodium phenate. The concentrations of SO_4-S, B, Zn, Mn, Cu and Fe were determined using mass spectrophotometry following extraction with diethylenetriaminepentaacetic acid. The quantities of K and P were determined using the Olson method, nitrate nitrogen was determined using the chromotrophic acid method, and the cation exchange capacity (CEC) was estimated using the ammonium replacement method.

2.5. Statistical analyses

Non-metric multidimensional scaling (NMDS) was used on Bray-Curtis distances to visualize community similarity among samples. Soil chemical characteristics were scaled to a mean of 0 and standard deviation of 1 and fitted to the NMDS ordination with the envfit function of vegan using 1000 permutations to determine significance. Permutational multivariate analysis of variance (PERMANOVA) was performed using the 'adonis' function of vegan. Richness, Shannon diversity (H′), and the inverse Simpson's index of diversity (1/D) of bacterial communities were calculated with vegan and compared among liming treatments and rotations using ANOVA and Tukey's Honest Significance post-hoc test using the agricolae R package. Differences in abundant (> 0.01% of sequences) bacterial families among treatments were compared using a ANOVA on $\log_{10}(1 + x)$-transformed sequence counts and a Benjamini-Hochberg correction of p-values for false discovery rate (FDR). OTUs that differed among rotations and treatments (DAotus) were identified using DESeq2 (Love et al., 2014), starting with an unrarefied OTU table. Briefly, unrarefied OTU tables were filtered to remove low abundance taxa (< 10 total