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Nitrogen fertilizer rate affects root exudation, the rhizosphere microbiome and nitrogen-use-efficiency of maize

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ABSTRACT

The composition and function of microbial communities in the rhizosphere of crops have been linked to edaphic factors and root exudate composition. We examined the effect of N fertilizer (urea) rate on maize root exudation, the associated rhizosphere microbial community, and nitrogen-use-efficiency. Increasing N rate had a significant effect on root exudate quantity and composition. Specifically, the total abundance of sugars, sugar alcohols, and phenolics was positively correlated with N rate ($p < 0.005$). Similarly, the abundance of rhizosphere bacteria (16 S rRNA copies g^{-1} soil FW) was enhanced with increasing N rate. Using PICRUSt, we also explored the metagenomic contribution of bacterial OTUs to the abundance of N cycle-related genes in the maize rhizosphere. On a relative abundance basis, the nitrifying-(*pmoA-amoA*) and denitrifying-genes (*nirK* and *nosZ*) were significantly influenced by N rate ($p < 0.05$); whereas, the nitrogen fixing (*nifD* and *nifH*) and urease (*ureC*) genes were not influenced by N rate ($p > 0.05$). However, on a total abundance basis (gene copies g^{-1} soil FW) all N-cycle genes increased significantly with increasing N rate ($p < 0.05$). Percent N recovery from both soil and fertilizer sources showed a curvilinear response that was highest at intermediate N rates; whereas, fertilizer N lost from the system increased significantly at the two highest N rates ($p < 0.05$). In summary, our results show high N rates increase both root exudation and the abundance of soil bacteria, which may help explain the decline in fertilizer-use-efficiency and loss of N from the system at higher N rates.

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1. Introduction

Maize (*Zea mays* L.) is one of the main crops widely used worldwide for food and biofuel. Increasing nitrogen (N) fertilizer applications has been a major management strategy to obtain high grain and biomass yields (Guo et al., 2010). However, N surplus combined with low N use efficiency have had negative consequences to the environment such as soil acidification, environmental pollution and decreased soil microbial activity (Chen et al., 2014; Guo et al., 2010; Ramirez et al., 2012). Thus, the reduction of N fertilizer inputs and improved N use efficiency are crucial for sustainable production of maize.

One alternative to reduce the application of N fertilizer could be the use of free-living N-fixing bacteria in maize agricultural systems (Cocking, 2003), which could improve crop production, reduce the overuse of synthetic fertilizers, and decrease greenhouse emissions (Kennedy et al., 2004). Benefits from N fixation have been demonstrated in sugarcane, rice, and graminaceous forage plants (App et al., 1984; Boddey and Victoria, 1986; Urquiaga et al., 1992). Many N-fixing bacteria have been reported in association with maize including *Azospirillum*, *Bradyrhizobium*, *Herbaspirillum*, *Ideonella*, *Klebsiella*, *Pantoea*, *Bacillus*, *Rhizobium etli*, *Burkholderia* and *Raoultella* (Chelius and Triplett, 2000; Palus et al., 1996; Di Cello et al., 1997; Perin et al., 2006; Dobbelaere et al., 2002; Roesch and Olivares, 2006; Roesch et al., 2008; Montanez and Sicardi, 2013). In addition, inoculation of maize with free living N-fixing bacteria has been shown to enhance crop yields through supplemental N input to the soil (Jacoud et al., 1998; Riggs et al., 2001; Sharma and Johri, 2003; de Garcia Salomone et al., 1996; Dobbelaere et al., 2002; Hungria et al., 2010).

The composition and function of microbial communities present in the rhizosphere can be affected by biotic and abiotic

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factors, including soil N content, soil type and host plant (Pennanen et al., 1999). High levels of N-fertilization have been shown to negatively affect members of the diazotrophic community (Allison and Martiny, 2008; Roesch et al., 2008). For example, the activity of *Azospirillum lipoferum* and *Gluconacetobacter diazotrophicus* was inhibited by the supplementation of large quantities of N (Muthukumarasamy et al., 1999; Tsagou et al., 2003).

Other studies indicate that plants predominantly drive and shape the surrounding microbiome through the secretion of exudates that specifically stimulate or repress distinct microbial members of the soil (Haichar et al., 2008; Grayston et al., 1998; Badri et al., 2013; Hartmann et al., 2009; Broeckling et al., 2008; Chaparro et al., 2012). Moreover, the secretion of root exudates is dependent on plant physiological status and nutrient availability. High nutrient availability favors the release of root exudates, whereas low nutrient availability restricts the allocation of plant resources towards root exudation (Neumann and Römheld, 2007). And, other studies have determined the effect of limited plant nutrition on root exudates and rhizosphere microbiology (Yang and Crowley, 2000; Liu et al., 2011). For example, maize secreted lower amounts of amino acids through roots under N deficiency, and these exudates suppressed numerous genes involved in protein synthesis in *Bacillus amyloliquefaciens* (Carvalhais et al., 2011, 2013). However, the effect of surplus N fertilization on root exudation and rhizosphere microbial communities in maize is not fully understood. Beginning to understand this complex process could reveal new methods for N application that would optimize the N balance through minimizing the losses of N and increasing microbe biological N fixation. In this study, we tested the effect of increasing levels of urea fertilization applied to maize on root exudation, the rhizosphere microbiome, and nitrogen-use-efficiency. We hypothesized that as N rates increase, root exudation will be modified along with (i) changes in microbial composition in the rhizosphere and a (ii) decrease in nutrient-use-efficiency.

2. Material and methods

2.1. Experimental design and growth conditions

Two separate experiments were designed to test the effect of increasing levels of urea fertilization applied to maize on root exudation and the rhizosphere microbiome as well as nitrogen-use-efficiency. For the rhizosphere microbiome and nitrogen-use-efficiency analysis, maize plants ($n = 15$) were grown at six different nitrogen (N) rates (0, 10, 30, 50, 80, and 160 mg N L^{-1} soil). The N rates used in this study were determined based on a preliminary experiment that showed the biomass of corn was maximized between 50 and 80 mg N L^{-1} soil (data not shown). The conversion of fertilizer rates between pot studies and field application rates is not a trivial matter; however, if we assume a plow layer of soil weighs 2000 t per ha, then our highest N rate is approx. 320 kg ha^{-1}. Two independent trials were conducted for both the maize biomass and pyrosequencing analyses. Values for C and N analysis of shoot and soil in the present study were derived only from the second trial. For maize root exudate analysis, a separate trial was conducted with maize plants grown at four N rates (0, 30, 80, and 160 mg N L^{-1}). Each N rate was applied to 18 pots, the soil leachate from six replicate pots from each treatment were later pooled to generate three independent replicates per N rate. For all the above treatments, N was supplied as 90% urea (99%) and 10% urea-^{15}N (99.7 atom%) to each pot (1 l soil pot^{-1}) as a single pre-planting application. The soil consisted of a mix of two parts sand (Play sand obtained from US Mix, Denver CO) and one part native soil collected from a Ponderosa pine forest near Young's Gulch, CO (pH 6.3; 1.4% organic matter; nutrient content: 2.6 mg/kg NH_4—N, 3.6 mg/kg NO_3—N, 3.0 mg/kg P, 136 mg/kg K, 1.1 mg/kg Zn, 16.7 mg/kg Fe, 2.5 mg/kg Mn, 1.3 mg/kg Cu). We selected a natural soil to rule out the potential interference of previous N fertilizer applications typical to agricultural soils. Two maize seeds [variety P1151HR (GMO) Pioneer] were sown in each pot. Once seedlings were established (~7 days) the plants were thinned to one plant per pot. All pots were placed into a rack in the greenhouse (12 h photoperiod with temperatures maintained below 35 °C) and rotated every week. Plants were irrigated once initially with 20 ml of full strength Hoagland's modified solution (Nitrogen-free medium) and then twice a week with water throughout the course of the experiment. When the maize plants reached the four leaf stage, they were sampled as described below.

2.2. Root exudates collection for GC–MS analysis

Root exudates were collected and prepared according to the method of Badri et al. (2013) with a few modifications. Briefly, 200 ml of water was put into each pot and the flow-through was collected. A total of approx. 400 ml soil leachate was collected from each group (i.e., 6 pots) at the four leaf stage, similar to the biomass experiment above.

Root exudates were filtered by using 0.45 μm filter paper and 300 ml root exudates per replicate were freeze-dried into powder and weighed. The dried powder was dissolved in 5 ml of 80% methanol and vortexed thoroughly to make sure that all powder dissolved completely. The solution was centrifuged at 8000 rpm for 15 min to pellet the undissolved particles; the clear liquid was transferred into new glass tubes and dried under nitrogen gas. Then, 1 ml of 80% methanol was added into the dried tube and vortexed thoroughly to dissolve all components and transferred into 1.5 ml Eppendorf tubes, which was centrifuged at 13,000 rpm for 15 min. Subsequently, the clear liquid was transferred into HPLC (brown) vials and dried under nitrogen gas. The samples were subjected to GC–MS analyses at the Genome Center Core Services, University of California Davis to identify the compounds present in the root exudates. Briefly, the root exudates were derivatized as described by Sana et al. (2010). A gas chromatograph (Agilent 6890, Santa Clara, CA) was used to run the samples controlled by Leco ChromaTOF software Version 2.32 (St. Joseph, MI). The resulting data files were exported to the data server with absolute spectra intensities and further processed by a filtering algorithm implemented in the metabolomics BinBase database (Skogerson et al., 2011). Quantification was reported as peak height using the unique ion as default (Fiehn et al., 2008). Metabolites were unambiguously assigned by the BinBase identifier numbers using retention index and mass spectrum as the two most important identification criteria. Additional confidence criteria were used by giving mass spectral metadata using the combination of unique ions, apex ions, peak purity, and signal/noise ratios. All data entries in BinBase were matched against the Fiehn mass spectral library (http://fiehnlab.ucdavis.edu/Metabolite-Library). Data normalization was performed as described in Fiehn et al. (Fiehn et al., 2008) by using vector normalization.

For the GC–MS data of root exudates, we normalized the peak height of each compound with the sum of all peak heights for all identified metabolites for each sample. Relative peak heights were then used to calculate compound-specific concentrations (μg $plant^{-1}$) using the total exudate dry weights. Based on the chemical nature of the root exudates these were divided into seven categories (sugars, sugar alcohols, amines [amino acid, amino alcohols, etc.], carboxylic acids, polyols, phenolics, lipids [fatty acids, fatty alcohols, etc.], and others) with each value representing the sum of all compounds present in any given category. Significant differences in compound concentrations were analyzed using a one-way ANOVA using SigmaPlot 12.5 (Systat Inc.) and passed the