Applied Soil Ecology 131 (2018) 89–98

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Foliar application of Fe resonates to the belowground rhizosphere microbiome in Andean landrace potatoes

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ARTICLE INFO

Keywords:
Fungal community
Iron
Mycorrhizae
Glomus
Rhizosphere
Soil

ABSTRACT

Iron (Fe) is a crucial nutrient for plant growth (e.g. chlorophyll production), and though it is one of the most abundant elements in soil, very low bioavailability can limit plant growth. Studies indicate that many soil bacteria and fungi (e.g. mycorrhizal) play a role in Fe nutrient cycling and plant production, but the evidence for fungal support of plant growth is overwhelmingly correlative and in need of experimental corroboration. An Andean native potato landrace was grown in a greenhouse under Fe limitation and using three levels (Low, Medium, High) of foliar fertilization (FeEDDHA). Application occurred at 45, 60 and 70 days of growth corresponding to periods where Fe limitation is expected to be greatest. The rhizosphere soils were sampled at the flowering stage (80 days). Soil bacterial and fungal communities were examined using high-throughput sequencing of 16S and ITS regions of ribosomal RNA gene, respectively, followed by analysis using Quantitative Insights Into Microbial Ecology (QIIME v1.8). Multivariate data analyses showed that Fe fertilization of leaves significantly ($p < 0.05$) influenced the beta diversity of fungi but not bacterial communities in the rhizosphere. Using our novel approach, it was expected and confirmed that fungal communities would shift and mycorrhizal genera (*Glomus*) would be altered, however, the degree to which community change was observed was more than expected. *Glomeromycota* (~16.3%) related to the family *Gigasporaceae* accounted for 2.8% of OTU and were 2–3 times greater in the rhizosphere of high relative to medium and low Fe conditions. Overall, the results indicate that foliar addition of Fe influences plant Fe and resonates into the root system to affect rhizosphere fungal communities. Potato Fe status thus appears to impact potato root-fungal interactions potentially mediated through mycorrhizal fungi.

1. Introduction

Iron (Fe) limitation can decrease biomass and yield production in crops such as tomato (*Solanum lycopersicum*) and others (Briat et al., 2015). Furthermore, Fe-deficiency anemia has been reported to be a highly prevalent malnutrition problem, affecting over 30% of the world's population (Bouis, 1995; WHO, 2017). High amounts of Fe are found in soil; however, soluble Fe in its naturally occurring forms of hydroxides, oxyhydroxides and oxides is extremely low in cultivated soils, especially when pH is greater than 6 (Marschner, 2012). Because of its importance in the human diet, greater Fe levels in crop production may help support healthier and more productive plants, but also human health, especially in regions of the world with limited consumption of animal protein.

Many research organizations worldwide are investing in the genetic potential of crop plants to improve Fe bioavailability in common staple food crops through both traditional plant breeding and transgenic approaches (Trijatmiko et al., 2016; Velu et al., 2014). These approaches have their advantages, but because of the importance that soil microbes and microbial-root interactions play in plant nutrient status, integration of their activities into breeding or management are warranted (Govindasamy et al., 2009; Ryu et al., 2005). Recently, inoculation of soil with several types of microbes were shown to differentially increase plant Fe uptake in wheat, white lupin and cucumber plants (de Santiago et al., 2009; Pii et al., 2015; Zhang et al., 2009; Zhao et al., 2014). Hence, microbial type does have an effect on Fe status.

In the rhizosphere, the concentration of bioavailable Fe in solution is decreased due to uptake by roots and microbes (Marschner et al., 2011). As a result, Fe deficiency together with plant rhizodeposits could lead to the selection of microbial populations that aid plant Fe status.

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https://doi.org/10.1016/j.apsoil.2018.08.006
Received 15 May 2018; Received in revised form 14 August 2018; Accepted 15 August 2018
Available online 24 August 2018

These microbial populations may promote plant health by suppressing soil-borne pathogens through siderophore-mediated microbial antagonism and eliciting plant defensive capacity called induced systemic resistance. Microbial driven nutrient availability have probably been most studied via mycorrhizal fungi (Lehmann and Rillig, 2015), but phosphate-solubilizing bacteria (*Pseudomonas* spp.) have been shown to also support plant-available P (Bünemann et al., 2012; Oberson et al., 2001; Oberson and Joner, 2005). Chemically P and Fe and elemental phosphates can combine to form insoluble complexes, and so microbial driven increases in bioavailable plant P may also support increasing Fe availability, and vice-versa (Borggaard et al., 1990; Hinsinger, 2001; Tomasi et al., 2008). There is strong scientific support that soil microorganisms play an important role in nutrient availability, but less is known about iron.

Plant-microbial interaction involving Fe acquisition has been demonstrated in a few previous studies (Robin et al., 2007, 2006; Yang and Crowley, 2000). Red clover and maize Fe content, for example are associated with Fe sequestering microbial siderophores (Carvalhais et al., 2011; Jin et al., 2010). Nevertheless, direct evidence that microbes and their interactions with plants function to support Fe acquisition has not been clearly established.

As for potato, Fe fertilizer by foliar application before and during the flowering stage has been shown to increase tuber weight and result in higher concentrations of Fe in the harvested tubers (Al-Jobori and Al-Hadithy, 2014; Hadi et al., 2014). These influences may result directly from the release of plants from Fe limitation, but if soil microbes are involved in an interaction with plants, the application of iron to leaves and the reduction of iron limitation would likely resonate down the shoots, to the roots, and alter root-microbe interactions and influence the rhizosphere microbial community. In this greenhouse study, foliar application of Fe was conducted to investigate whether plant-microbial communities would be altered by changing plant Fe status. The objective was, therefore, to examine the impact of plant Fe nutritional status on the rhizosphere bacterial and fungal communities and to assess the soil microbes that may be impacted by potato Fe acquisition. It was hypothesized that plant Fe treatment of leaves would translate to roots and affect bacterial and fungal communities in the rhizosphere. Specifically, it was expected that high Fe amendment would alter microbial communities that support Fe acquisition, and in particular the relative abundance of *Glomeromycota* in Fe-limited soil.

2. Methods

2.1. Plant materials and experimental design

An Andean native potato landrace (*Solanum tuberosum* L. CIP 703580) was imported from CIP (International Potato Center). To make identical plants for replication, subcultured *in vitro* plants were grown in test tubes (25 × 150 mm) containing 20 mL of MS basal medium (Murashige and Skoog, 1962) (PhytoTechnology Laboratories, Shawnee Mission, KS, USA) with 0.7% agar (Sigma-Aldrich, St. Louis, MO, USA) in a growth chamber under a 16 h photoperiod with a light intensity of 500 $\mu M\, m^{-2}\, s^{-1}$ at 22 °C (day)/16 °C (night) for 3 weeks. Then the subcultured potato plantlets were transplanted to 15 pots (3.8 L) containing a soil mixture with relatively low Fe concentration (5 ppm, pH ≈ 7) in the greenhouse at Virginia Tech in Blacksburg in 2015 spring (Supplementary Table 1). The soil mixture was composed of 30% field soil collected from agricultural land at Kentland Farm, Montgomery County, VA (37.20 N, 80.56 W), 0–20 cm cultivated with soybean plants, 40% sand and 30% perlite. The field soil had been air-dried and passed through a 2-mm sieve. During plant growth, the soil moisture was maintained using an automatic irrigation system delivering tap water as needed. To help ensure Fe deficient conditions, the soil pH was checked weekly to ensure it was maintained above 6. Every week, to ensure adequate nutrient supply, plants were fertilized with half strength Hoagland solution (pH 7) without Fe. Greenhouse conditions were maintained at 28 °C (day), 22 °C night, and a relative humidity of ~65%. The daily light schedule (intensity 370 $\mu M\, m^{-2}\, s^{-1}$) consisted of 15 h light and 9 h of darkness.

Preliminary experiments were conducted to assess level of plant Fe limitation. The potato plants were misted with a foliar spray on both the adaxial and abaxial leaf surface using chelated Fe fertilizer (FeEDDHA, Sprint 138 iron chelate) in deionized water until full wetting. For the low Fe treatment, only deionized water was applied. For moderate and high Fe treatments, 200 mg/L and 600 mg/L of FeEDDHA solution (pH of 5.5) were prepared. Foliar applications contained 0.1% (v/v) Tween80 as a surfactant. Soil of each pot was covered with plastic wrap during application to avoid Fe fertilization of soil. Misting occurred every other week (3 times) up to flowering stage. Fe spraying occurred from 6 to 7 pm to prevent leaf damage. Three treatments with five replicate pots were used to create a complete randomized experimental design.

2.2. Sampling and growth parameters

Rhizosphere soil sampling was done 80 days following transplantation into pots, at flowering. The plant shoots were cut near the soil surface. Roots and rhizosphere soil were sampled by inverting the pots while firmly holding the stems. Non-root associated soil fell from the pots. Roots were further shaken to remove loosely attached soil. Rhizosphere soil adhering firmly on the root surface after gently shaking (Supplementary Fig. 1) was collected and put in sterile conical tubes and stored at −80 °C. The DNA was then extracted from the soil and further used for ITS and 16S rRNA gene-based Illumina sequencing.

Plant length and number of branches were determined. Whole plants and tubers were harvested. The total plant biomass and fresh tuber yield in terms of number of tubers and tuber weight were recorded. For Fe concentration measurement, the shoot tops of each plant were harvested until the third leaf from the apex. Relatively young root tips were also harvested. These shoots and roots were washed of soil using tap water, followed by rinsing with deionized water. Shoots and roots were then placed into paper bags and oven dried at 60 °C for 3 days. The total dry weight of both shoots and roots were similarly measured. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used to measure soil and plant Fe concentration (Soil Testing Laboratory at Virginia Tech). For ICP-AES sample preparation, the dried shoots and roots were ground and digested in 10 mL 70% HNO_3 overnight, and then digested in a microwave acid digestion system (MARS 6, CEM corporation, NC, USA) for 30 min and diluted to 50 mL with deionized water.

Analysis of variance (ANOVA) was conducted to assess differences in plant and root properties across treatments. This was accomplished using JMP statistical software (SAS Institute Inc., Cary, North Carolina). Means were compared by *t*-test at $p < 0.05$ in all cases.

2.3. DNA extraction and PCR amplification

A 0.5 g subsample of moist and homogenized rhizosphere soil was used for microbial community DNA extraction using PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA) according to the manufacture's protocol. DNA quality was checked on a 0.8% (w/v) agarose gel. DNA concentrations were determined by fluorometric quantification using the Qubit1 2.0 platform with Qubit dsDNA HS Assay Kit (Life Technologies). DNA was diluted to 5 ng/μL and stored in the −20 °C freezer during the time preceding amplification of 16S rRNA and ITS gene regions.

Extracted DNA was used for Illumina high-throughput sequencing of the 16S rRNA gene and the ITS region for bacterial and fungal community analyses, respectively. The 16S rRNA gene and the ITS region were targeted using the metagenomic sequencing library preparation protocol described by Illumina (2013) with some modification. Briefly, two stages of PCR were applied for amplifying region of interest and