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Diazotroph community structure and abundance in wheat–fallow and wheat–pea crop rotations

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ABSTRACT

Biological input of nitrogen (N) from the atmosphere by free-living diazotrophs can help alleviate fertilizer use in agricultural systems. In this study, we investigated the effect of N fertilizer and winter pea (*Pisum sativum* L.) crop on the community structure and abundance of free-living diazotrophs in a two year study of dryland winter wheat (*Triticum aestivum* L.) no-till production system in Eastern Oregon, USA. Based on quantification of the *nifH* gene, diazotroph abundance was strongly influenced by plant species and the crop year in which the soil samples were collected. A greater amount of *nifH* copies was recovered in 2012 compared to 2011 either as copies per gram soil or normalized to the abundance of bacterial 16S rRNA genes. The quantity of genes was greater under pea than wheat in 2012 although no difference was observed in the preceding year. The *nifH* gene abundance was positively correlated to ammonium concentration in 2011 and bacterial abundance in 2012. Nitrogen application did not influence diazotroph abundance in the top 0–5 cm; however the abundance was reduced by application at the lower 5–10 cm depth under wheat crop. The diazotroph community structure appeared to be influenced more by N fertilization rather than plant species with the exception of wheat in 2012. Changes in the community structure over the two years were greater for fertilized than unfertilized soil. Collectively, these data suggest that year-to-year variability had a greater influence on diazotroph communities rather than specific parameters of plant species, fertilization, total N, total organic C, or soil pH. Multi-year studies are necessary to define the specific drivers of diazotroph abundance, community structure and function.

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1. Introduction

Nitrogen (N) is a critical plant nutrient and crop requirements range from 20 to 50 kg N ton^{-1} grain yield for cereals (Myers, 1988; Sylvester-Bradley and Kindred, 2009). Nitrogen fertilizer application promotes plant biomass and improves grain protein yields; however, it also imparts significant cost to the producer and could become an environmental challenge. Environmental concerns for N fertilization include increased greenhouse gas emissions (Dalal et al., 2003), soil acidification (Barak et al., 1997; Gollany et al., 2005), and groundwater contamination (Ambus et al., 2001; Gollany et al., 2004). Alternatively, biological nitrogen fixation (BNF) is an important source of N in some ecosystems (Cleveland et al., 1999) and may help meet some of the plant nutritional needs.

Diazotrophs, or N_2-fixers, contribute to plant available N by reducing atmospheric N_2 to ammonium in the soil. Diazotrophs are highly diverse and include members of α-, β-, γ-, and δ-*Proteobacteria*, Firmicutes, Cyanobacteria, and Archaea (Dixon and Kahn, 2004; Rösch et al., 2002), most of which are uncultivated. Although the diversity and distribution of non-symbiotic diazotrophs suggest that most soils have a capacity for BNF (Izquierdo and Nüsslein, 2006; Poly et al., 2001), the ecological impact of free-living diazotroph activity is disputable with estimates of activity varying widely from 0 to 60 kg N ha^{-1} yr^{-1} in cropland and natural ecosystems (Cleveland et al., 1999; Day et al., 1975; Gupta et al., 2006).

Diazotroph activity, abundance and community structure have been attributed to numerous factors related to microbial biomass (Hayden et al., 2010), sampling season (Mergel et al., 2001; Pereira e Silva et al., 2011) and soil physical and chemical properties including soil water content (Brouzes et al., 1969; Limmer and Drake, 1996; Nelson and Mele, 2006; Roper, 1985), soil texture (Pereira e Silva et al., 2011; Riffkin et al., 1999), soil aggregate size (Poly et al., 2001), soil pH (Limmer and Drake, 1996; Nelson and Mele, 2006), electrical conductivity (Hayden et al., 2010), oxygen (Brouzes et al.,

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1969; Limmer and Drake, 1996, 1998), carbon (C) quality and quantity (Brouzes et al., 1969; Keeling et al., 1998; Limmer and Drake, 1996, 1998; Wakelin et al., 2010), and N availability (Hayden et al., 2010; Hsu and Buckley, 2009; Limmer and Drake, 1998; Nelson and Mele, 2006). Generally, some of the factors result in reproducible responses in N_2-fixation such as increased activity with decreased oxygen tension (Brouzes et al., 1969; Limmer and Drake, 1998) and increased soil water content (Brouzes et al., 1969; Roper, 1985), while others such as C (Brouzes et al., 1969; Limmer and Drake, 1996) and some soil physicochemical characteristics (Hsu and Buckley, 2009; Poly et al., 2001) produce inconsistent results. Although numerous factors may influence diazotroph populations, there is little knowledge regarding specific ecological drivers and soil properties do not appear to have similar effects on community structure and activity (Poly et al., 2001).

A better understanding of the drivers or influencing factors on the diazotroph communities and BNF will help improve N use efficiency in cropping systems and may lead to reductions in fertilizer use. An indirect approach to assess the potential for BNF is the characterization of the diazotroph populations by molecular methods. Diazotroph communities are often characterized by the *nifH* gene which encodes the iron subunit of the nitrogenase enzyme (Hayden et al., 2010; Hsu and Buckley, 2009; Mao et al., 2011; Nelson and Mele, 2006; Orr et al., 2011; Poly et al., 2001; Rösch and Bothe, 2005). The *nifH* gene is encoded by all diazotrophs and mimics 16S rRNA gene phylogeny making it an ideal candidate for ecological studies of community structure and/or composition. We compared the abundance and community structure of diazotroph populations in a long-term no-till wheat–fallow and adjacent wheat–pea rotation to examine whether crop rotation and fertilization influence the microbial communities in the dryland Pacific Northwest (USA).

2. Methods

2.1. Site description and sample collection

The Pacific Northwest Columbia Plateau has a Mediterranean climate with cool, wet winters and hot, dry summers producing optimal conditions for dryland small grain production. Cropping practices are constrained in the region by limited annual precipitation and typically follow a two-year rotation with a fallow period of 12 or more months preceding winter wheat (W) which is planted in fall and harvested in July/August of the following year. The field site was located 15 km northeast of Pendleton, Oregon, USA (45° 72′ N, 118° 62′ W, elevation 458 m). Annual precipitation occurs mostly during the winter months and yearly averages were 447 mm, 571 mm, and 368 mm for crop years 2010, 2011, and 2012 (CY10, CY11, CY12). The soil is Walla Walla silt loam (coarse-silty, mixed, superactive, mesic Typic Haploxeroll) developed in loess overlying basalt (Wuest and Gollany, 2013). Treatments were arranged in a 2 by 2 factorial design with crop rotation as main plots and fertilization as subplots. The winter wheat–fallow rotation (W–F) main plot (NTA experiment) was established in 1982. The winter wheat-fallow rotation (NTB experiment) was initiated in 1997 and was converted to winter wheat–winter pea rotation (W–P) in 2010 by replacing the fallow period with Austrian pea. The NTB experiment is 7.62 m north of NTA and both are strictly no-till cropping systems. Nitrogen fertilizer was applied as urea at either 0 or 180 kg N ha^{-1} (160 lbs N ac^{-1}). Urea was banded at 10 cm depth during wheat planting and treatments referred to as “fertilized” pea and “fertilized” fallow received N only during the wheat phase of the rotation (Table 1). The CY11 and CY12 were the first pea harvests from each plot in the NTB experiment. In CY12, the winter wheat did not establish, therefore it was subsequently sprayed and re-planted to spring wheat on April 13, 2012. The naming convention for the plots lists first the crop under which the sample was taken, followed by the rotational crop and amount of the N fertilizer applied with wheat (e.g., W0-F for wheat of unfertilized W–F rotation and P-W180 for pea of W–P rotation in which wheat received 180 kg N ha^{-1}).

Soil sampling was initiated on June 28, 2011 and July 10, 2012 from the wheat and fallow plots of NTA and the pea plots of NTB and was completed within 2–3 days. In each plot, five soil cores were collected from the top 0–5 cm, 5–10 cm and 10–20 cm depths using a 1.85-cm diameter soil probe. Cores from the individual depths were pooled and homogenized by hand. Approximately 10–20 g of soil was transferred in the field into small zippered bags for DNA analysis and stored on ice until transfer to a −20 °C freezer upon arrival in the lab. The remaining soil was dried at 40 °C for chemical analyses. Three additional cores were retained for bulk density and soil water content analyses.

2.2. Soil chemical and physical properties

Soil water content and bulk density were calculated according to standard protocols. Dried soils were extracted for 30 min in 1 M potassium chloride prior to quantification of nitrate (NO_3–N) and

Table 1
Soil chemical properties under different crop rotations during wheat and fallow phase of wheat–fallow (W–F) rotation in the NTA experiment and pea phase of the wheat–pea (W–P) in the NTB experiment.

Properties	Wheat–fallow (NTA)[a]				Wheat–pea (NTB)		Pearson Coefficient[d]	
Rotation	W–F		W–F		W–P			
Crop, N	W0-F	W180-F	F-W0	F-W180	P-W0	P-W180	*nifH*	16S
Total Nitrogen (%)[b]								
2011	0.134^{BC}	0.166^{A}	0.133^{C}	0.165^{AB}	0.138^{ABC}	0.150^{ABC}	NS	NS
2012	0.122^{C}	0.147^{ABC}	0.128^{C}	0.164^{AB}	0.123^{C}	0.138^{ABC}	NS	NS
Total Organic Carbon (%)[c]								
2011	1.809^{AB}	2.092^{A}	1.691^{AB}	1.978^{AB}	1.815^{AB}	1.826^{AB}	NS	NS
2012	1.593^{B}	1.861^{AB}	1.706^{AB}	2.087^{A}	1.703^{AB}	1.916^{AB}	NS	+
pH								
2011	5.48^{A}	5.08^{B}	5.53^{A}	5.50^{A}	5.32^{AB}	5.45^{A}	NS	NS
2012	5.47^{A}	5.32^{AB}	5.52^{A}	5.32^{AB}	5.37^{AB}	5.23^{AB}	NS	NS

[a] Winter wheat–fallow (W–F) and winter wheat–winter pea (W–P) rotations were managed under no-till farming. All samples were collected in the summer during the crop listed first in the rotation (e.g. W–F sampled under wheat and F–W sampled under fallow). Nitrogen was applied as urea at either 0 or 180 kg N ha^{-1} rate to the plots during planting of the wheat phase of the rotation and is indicated after wheat (i.e., W0-F for unfertilized wheat and F-W180 for fallow crop previously fertilized under wheat).
[b] Values for soil properties followed by the same letter are not significantly different at $P < 0.05$ level over both years.
[c] Values are the same as total carbon since inorganic carbon measured was below detection.
[d] Pearson correlation coefficient of gene copy number and soil chemical properties; NS, not significant; ±, significant positive or negative correlation at $P < 0.10$.