Applied Soil Ecology 111 (2017) 48–56

Contents lists available at ScienceDirect

Applied Soil Ecology

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

Quantification of bacterial non-specific acid (*phoC*) and alkaline (*phoD*) phosphatase genes in bulk and rhizosphere soil from organically managed soybean fields

Tandra D. Fraser[a], Derek H. Lynch[b], Jonathan Gaiero[c], Kamini Khosla[c], Kari E. Dunfield[c,*]

[a] Centre for Agri-Environmental Research, School of Agriculture, Policy and Development, University of Reading, UK
[b] Department of Plant and Animal Science, Dalhousie University, Canada
[c] School of Environmental Science, University of Guelph, Canada

ARTICLE INFO

Article history:
Received 27 June 2016
Received in revised form 16 November 2016
Accepted 21 November 2016
Available online 27 November 2016

Keywords:
Phosphatase
Bacteria
P-scavenging genes
Available phosphorus
Plant P uptake

ABSTRACT

Phosphorus (P) is a limiting nutrient in many environments but plants and microbes have evolved with mechanisms for acquiring soil P, including the excretion of phosphatase enzymes. Molecular analysis of bacterial phosphatase genes can provide insight into biological P transformations and the contribution to soil P availability and plant uptake. To assess these relationships, soil and plant samples were collected from 12 organically-managed soybean fields varying in pH, labile P concentration, and potential phosphatase activity (pH 6.5) across Prince Edward Island, Canada. Real-time PCR was used to quantify bacterial phosphatase genes (*phoC* and *phoD*) in bulk and rhizosphere soil. Primers targeting class A (*phoC*) of the bacterial non-specific acid phosphatases (NSAPs) were designed and confirmed as effectively targeting *phoC* genes through sequencing, and phylogenetic comparison with acid phosphatase genes from Genbank. Across all sites, we found that labile P in bulk soil was negatively correlated with *phoC* and *phoD* gene abundance and phosphatase activity. In addition, phosphatase activity was consistently higher in rhizosphere compared to bulk soil and was significantly correlated with *phoC* (bulk soil only) and *phoD* (rhizosphere soil only) gene abundance. A positive relationship was observed between phosphatase activity, nodule weight, and plant P uptake. Quantification of bacterial genes involved in organic P transformations has been limited, with this study providing the first attempt at quantifying *phoC* genes in field soils.

1. Introduction

Soil P is an essential nutrient for plant growth but is often limiting in high yielding agricultural production systems. In many regions of Canada, low levels of bioavailable P have been reported in soils under organic management as a result of large-scale production systems and/or limited access to manure (Entz et al., 2001; Knight et al., 2010; Main et al., 2013; Martin et al., 2007; Roberts et al., 2008). The provisioning of nutrient cycling in these soils through biological activity can be an essential component of productive and sustainable organic farming systems.

Plants and soil organisms play a vital role in transforming soil P into bioavailable forms by various mechanisms, including the excretion of extracellular enzymes. Acid (ACP, EC 3.1.3.2) and alkaline phosphatases (ALK, EC 3.1.3.1) are non-specific phosphohydrolases capable of hydrolysing simple phosphate monoesters to acquire orthophosphate (Nannipieri et al., 2011; Ragot et al., 2015) and are often described by their optimal pH activity (Eivazi and Tabatabai, 1977). Phosphatase activity is typically highest in the rhizosphere (defined as the soil surrounding a plant root), with higher levels of microbial activity compared to bulk soil (Hinsinger, 2001; Marschner et al., 2004). While excretion of ACP by plant roots (George et al., 2006; Li et al., 2002; Spohn et al., 2015; Tarafdar and Claassen, 1998), nodules (Li et al., 2012; Penheiter et al., 1997a), and soil microbes (Nannipieri et al., 2011; Spohn et al., 2015; Tarafdar and Claassen, 1998) has been well documented, production of ALK has been primarily reported from soil bacteria and some fungi (Nannipieri et al., 2011). Previous studies have confirmed the presence of a phosphate regulon (Pho) in some bacteria, consisting of a suite of genes responsible for phosphate acquisition induced under conditions of phosphate starvation, including phosphatase production (Apel et al., 2007; Krol and Becker, 2004; Lidbury et al., 2016; Vershinina and

* Corresponding author.
E-mail addresses: dunfield@uoguelph.ca, kdunfield@gmail.com (K.E. Dunfield).

http://dx.doi.org/10.1016/j.apsoil.2016.11.013
0929-1393/

Znamenskaya, 2002). Some experiments have demonstrated increased phosphatase activity under phosphate-limited conditions (Fraser et al., 2015a,b; Kier et al., 1979; Zhang et al., 2012), although others have found no correlation (Fraser et al., 2015b).

Soybean fields under organic management on Prince Edward Island, Canada were sampled in an attempt to improve our understanding of the potential contribution of bacteria to extracellular phosphatase production and P availability. We predict that in these systems, the turnover of organic P by microorganisms is essential for maintaining long-term productivity. In a previous study, we demonstrated a general correlation between the abundance of an ALK gene (*phoD*) and ALK (pH 11.0) activity in a heavy clay soil (pH 7.4_{H20}) from the Northern Great Plains near Winnipeg, Canada (Fraser et al., 2015a) and a negative correlation with labile P (Fraser et al., 2015a). However, we predict that in soils with a neutral or lower pH that the bacterial *phoC* genes will be more predominant than the *phoD* genes. In addition, we foresee that there will be higher phosphatase activity in the rhizosphere compared to the bulk soils.

The objective of this study was to quantify bacterial acid (*phoC*) and alkaline (*phoD*) phosphatase gene abundance in rhizosphere and bulk soils and link gene abundance with potential phosphatase activity and bioavailable P. A novel set of primers were designed to target the promiscuous *phoC* gene in bacteria that contributes to acid phosphatase production, a key enzyme in the hydrolysis of labile phosphomonoesters into orthophosphate.

2. Materials and methods

2.1. Site description

Soil and plant samples were collected at 12 locations across Prince Edward Island, Canada during the 2010 growing season (Table 1). Twelve certified organic (>five years) field sites planted to soybean were chosen with various management histories and a wide range of available P (24–184 mg P kg^{-1} soil) and pH (5.1–6.7). Previous cropping rotation was not considered since it was not possible to find 12 sites with identical management histories for such a survey.

Precipitation and temperature were recorded by Environment Canada at Charlottetown Airport Station (Latitude 46°17′19″N; Longitude 63°01′43″W; 48.8 m elevation). Total precipitation was 1354.0 mm in 2010, with 548.8 mm occurring May through September. Mean climatic data (1981–2010) indicates that the island receives an average of 1158 mm of annual precipitation and a yearly mean temperature of 5.7 °C, with a minimum of −12 °C in January and maximum temperature of 23.3 °C in July.

2.2. Soil and plant sampling

An initial site characterization was conducted to determine differences in soil properties resulting from geographical variations and/or past management. Soil samples (n = 20; 0–15 cm) were collected from a representative area of each field approximately 25 m × 25 m with a hand core probe (r = 2.5 cm) and combined into one sample. Composite samples were put through 4 mm sieves, air-dried and sent to the Nova Scotia Department of Agriculture lab for soil analysis (Table 1). Mehlich 3-extractable nutrients (Mehlich, 1984) were measured by inductively coupled plasma (ICP) spectroscopy.

Soil and plant samples were collected in July 2010 at soybean pod initiation. In each of the 12 fields, two random 10 m by 10 m sampling areas were identified. Soil samples (n = 20; 0–15 cm) were collected from each sampling area as described above. We collected nine soybean plants and separated the shoots and roots, being cautious to conserve the root-attached soil. The rhizosphere soil, defined as soil adhering to roots after shaking, was separated from the roots in the lab.

2.3. Soil and microbial biomass phosphorus (P) determination

Bulk and rhizosphere soil samples were passed through 2 mm sieves and air-dried before analysis of Mehlich 3-extractable P using 3 g soil in a 1:10 soil:solution ratio (Mehlich, 1984). Samples were passed through Whatman No. 42 filter paper and the molybdate reactive P in the extracts analysed by molybdate-blue colorimetric (Murphy and Riley, 1962) and measured at 845 nm on a NovaSpec 4049 spectrophotometer (LKB Biochrom, Cambridge, England). Molybdate reactive P was assumed to represent inorganic P in the extract.

Microbial biomass P was determined on bulk soil by chloroform fumigation-extraction technique in triplicate (Brookes et al., 1984; Voroney et al., 2008). Fresh soils (10 g) were incubated at 40% field capacity at 25 °C for five days. Three samples were fumigated with chloroform for 24 h, three samples remained unfumigated and three were used as a non-fumigated spike to test for P recovery of the extraction. Samples were shaken with 200 mL of 0.5 M $NaHCO_3$ for 30 min and passed through Whatman no. 42 filter papers. The extracts were acidified for 30 min to dissolve any remaining organic matter, centrifuged for 10 min (5000 rpm, 0 °C), neutralized and P in the extracts determined as described above. All samples were analysed within 24 h of extraction. Microbial biomass P was calculated as described in Eq. (1.1) where P_F represent P from the fumigated samples, P_{UF} from the unfumigated samples, R is the percent recovery (100*[(P spiked soil − soil P_{UF})/P_i

Table 1
Soil characteristics at 12 PEI farm sites. Standard soil tests were completed by Nova Scotia Agriculture on composite soil samples (n = 20; 0–15 cm) taken prior to seeding in 2010.

Site	Location	Texture (%)			OM	pH	CEC	P	K	Ca	Mg	Al	Fe	Mn
		Sand	Clay	Silt	%			mg kg^{-1} soil						
1	Saint Nicolas	50	17	33	4.1	6.0	12.1	24	252	1447	122	1568	203	18
2	Saint Nicolas	62	11	26	3.8	5.9	9.8	77	246	1300	90	1330	193	185
3	Saint Nicolas	57	14	30	4.0	5.9	10.3	89	224	1067	129	1451	188	45
4	Saint Nicolas	53	19	27	3.5	6.7	12.7	72	148	1765	175	1169	211	212
5	Freetown	64	13	24	3.0	5.5	7.7	184	242	558	55	1782	162	25
6	Freetown	56	16	29	3.2	5.4	8.6	179	216	630	73	1801	207	35
7	Winslow	48	12	40	3.9	6.2	9.9	32	379	1322	71	1098	303	30
8	Winslow	50	13	37	3.7	6.1	9.9	42	343	1136	111	1331	256	43
9	Waterside	42	17	40	3.3	5.9	9.5	30	125	1146	108	1132	329	82
10	Waterside	47	14	39	3.0	5.3	7.0	143	124	667	101	1321	337	74
11	Waterside	46	17	38	3.4	5.6	7.4	67	155	788	118	1436	234	51
12	Waterside	50	17	34	2.8	5.1	6.4	78	100	700	56	1216	328	53

CEC, cation exchange capacity meq/100 g.