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Heterogeneous soil conditions influence fungal alkaline phosphatase activity in roots of *Lotus corniculatus*

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ABSTRACT

Extremes in soil water, temperature, pH and inorganic nutrient availability can highly influence mycorrhizal formation and nutrient acquisition. However, the influence of soil heterogeneity and seasonal soil changes on fungal alkaline phosphatase (ALP) activity in mycorrhizal roots is unknown. Whether the activity of intraradical fungal ALP is influenced by soil texture heterogeneity, soil depth, nutrient concentrations and root density during the growing season were addressed in this research. The root system of *Lotus corniculatus* grown on a reclamation site was analyzed for intraradical mycorrhizal ALP activity in 0–10, 10–20 and 20–30 cm depth increments of sandy soil and in clay–silt fragments (mostly localized in 10–20 cm) from May to September. Root length density and water soluble nutrient contents in sandy soil and clay–silt fragments were determined for each sample. Soil water content and soil temperature data of a nearby site were used for climate conditions on sampling dates. Fungal ALP activity in roots growing in clay–silt fragments was higher than in those growing in sandy soil, independent of sampling date. Fungal ALP activity in sandy soil increased with soil depth which is related to decreasing root densities and lower nutrient concentrations. However, nutrient dense clay–silt fragments significantly positively influenced local mycorrhizal ALP activity in *L. corniculatus* roots, suggesting that the ability of mycorrhizae to selectively take up phosphorus and other nutrients from those nutrient hot spots is enhanced and could play a significant role in successful establishment of pioneer plant species on infertile reclamation sites.

1. Introduction

Over 80% of terrestrial plants form symbiotic associations with arbuscular mycorrhizal fungi (AMF). It is therefore the most abundant and widespread form of mycorrhizal symbiosis worldwide (Smith and Read, 2008). Under nutrient limited conditions, symbiosis of plants with mycorrhizal fungi is necessary for plant growth as it can increase efficiency of plants to take up water and nutrients. Low available phosphorus uptake is particularly facilitated by mycorrhizal fungi (Entry et al., 2002; Smith and Read, 2008) is essential for plants growth (Smith and Read, 2008).

Various studies address the impact of extremes in water, temperature, soil pH and inorganic nutrient availability on mycorrhizal formation and nutrient acquisition (Entry et al., 2002). However, less is known about the influence of soil heterogeneity and seasonal soil water content and temperature variations on arbuscular mycorrhizal activity. We are not aware of any field study addressing response of intraradical fungal ALP activity to soil heterogeneity.

Boldt-Burisch et al. (2013), studying a post mining site in the Lusatian Mining District (East Germany), found that in the initial stage of ecosystem development after reclamation, nutrient rich clay–silt fragments within a nutrient poor sandy soil created a high soil textural heterogeneity which was very suitable to study effects of soil heterogeneity on mycorrhizal activity. Those clay–silt fragments were highly rooted by a commonly occurring pioneer plant species, *Lotus corniculatus* L. (bird's foot trefoil). Root parts in clay–silt fragments had greater nutrient concentrations relative to those in the sandy soil and recent studies by Boldt-Burisch and Naeth (unpublished) showed that precision root allocation in those fragments significantly improved plant development and that increased fungal activity in roots in clay–silt fragments could contribute to local increased nutrient uptake and transfer to the plant.

To assess mycorrhizal activity in roots, measurements of fungal enzyme activities are traditionally used. These include histochemical staining of succinate dehydrogenase (Ezawa et al., 2001) representing metabolically active fungus; acid phosphatase staining representing a

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process responsible for hydrolysis of vacuolar polyphosphate after which phosphorus could be released by the fungus into the plant fungal interface (Ezawa et al., 2001; van Aarle et al., 2002); and histochemical staining of fungal ALP (fluorescent or non-fluorescent) (Tisserant et al., 1993; Vierheilig et al., 1998). ALP activity is considered a physiological marker for analyzing efficiency of mycorrhizae, as there is strong evidence that ALP of the intraradical mycelium is part of phosphorus efflux from the fungus to the plant (Gianinazzi et al., 1979; Tisserant et al., 1993; Ezawa et al., 1995; Kojima and Saito, 2004).

We used histochemical staining of intraradical fungal ALP in *L. corniculatus* roots to assess changes in intraradical mycorrhizal ALP activity in the field. The study objectives were to determine whether the activity of fungal ALP within roots of *L. corniculatus* was influenced by soil texture heterogeneity (sandy soil versus clay–silt fragments), soil depth, soil nutrient concentrations and root density during the growing season. As earlier observations of *L. corniculatus* roots at this research site showed increased mycorrhizal colonization intensity of roots in fragments (data not published), we hypothesized that root parts growing in clay–silt fragments would have increased intraradical fungal ALP activity relative to root parts in sandy soil. We further hypothesized that fungal ALP activity would be strongly dependent on root density.

2. Material and methods

2.1. Study site description

The research was conducted in 2016 on an experimental reclamation site in the Lusatian coal mining area of the active lignite mine Welzow Süd (Brandenburg, Germany). The site was constructed in 2006 by dumping and contouring Pleistocene, calcareous, sandy overburden embedded with fine textured clay–silt fragments (mean volume 131 cm^3). Both materials were retrieved from the fore field of the open cast lignite mining area. Sandy soil was 95.6% sand, 2.5% silt and 1.9% clay; silt–clay fragments were 51% sand, 14.6% clay and 32.4% silt (details and other soil parameters in Boldt-Burisch et al., 2013). Plants investigated were naturally established on this site (no seeding).

The temperate, slightly continental climate of this region is characterized by high summer temperatures and pronounced droughts in the growing season (Gerwin et al., 2011). Mean annual precipitation in 2016 in the area was 489.2 mm, mean annual air temperature was 11.8 °C (measurements: January to October 2016; weather station: Chicken Creek, Brandenburg University of Technology).

2.2. Air temperature and soil water content

Soil water content and temperature at the experimental site on sampling dates (May through September) were estimated from data procured with soil sensors from an adjacent research site (Chicken Creek) with the same soil conditions and vegetation. Data are from 10 and 30 cm soil depth. Soil water content was measured with a Delta-T/FDR probe ML2x (AT Delta-T Devices, Cottbus, Germany). Soil temperature was measured with an ecoTech/pF-meter (Eco Tech Umweltmesssysteme, Bonn, Germany). Data logged several times daily from three site locations were used to calculate mean values (Table 1).

2.3. Plant species, soil and root sampling and chemical analyses

The legume *Lotus corniculatus* L. is a key pioneer species in the Lusatian post mining landscape and can establish a pronounced root system that is highly heterogeneous in distribution, with a high root surface area, particularly in the upper 30 cm soil depth (Boldt et al., 2012). A 60 by 60 m area within the experimental site with predominantly individual *L. corniculatus* plants was selected for sampling. Sampling was conducted every four weeks from May through September 2016; at each sampling time there were 6 replicates from each of the depth increments in sandy soil and 6 replicates from rooted silt–clay fragments. The clay–silt fragments were mostly found in the 10–20 cm depth increment.

Table 1
Soil volumetric water content and temperature from May through September 2016 in 10 and 30 cm of sandy soil at a research site (Chicken Creek Catchment) adjacent the experimental site.

	Soil water content (vol.%)		Soil temperature (°C)	
	10 cm	30 cm	10 cm	30 cm
May	13.4 (0.8)	15.6 (1.0)	18.1 (0.8)	16.3 (0.3)
June	11.0 (0.5)	12.2 (0.6)	23.1 (1.1)	21.3 (0.6)
July	15.0 (1.0)	16.1 (0.9)	21.7 (1.2)	21.1 (0.5)
August	15.2 (1.1)	14.8 (1.1)	19.1 (1.0)	20.0 (0.3)
September	10.2 (0.7)	11.1 (0.7)	13.0 (2.9)	16.0 (0.3)

Values are means with standard deviation. $n = 3$.

At each sampling date individually growing *L. corniculatus* plants of similar size (approximately 30 cm height) were selected for sampling locations. Plant shoots were cut and removed. Soil–root samples were taken with a soil corer (diameter 5.7 cm, length 40 cm) by pushing the corer directly on the cut shoot into the soil to a depth of 35 cm. The intact core was cut with a knife into 0–10, 10–20 and 20–30 cm depth increments, each with a volume of 255 cm^3. All clay–silt fragments and their roots (Fig. 1a–c) within the soil core depth increments were separated from the sandy soil by hand. When no clay–silt fragments occurred in the soil core, or they were too small to obtain enough roots, the soil immediately around the plant was further excavated with a small shovel to find larger rooted clay–silt fragments. Total volume of clay–silt material derived from fragments was determined in a measuring cup after roots were removed. Once in August, non-rooted fragments were taken from areas with no plants to compare nutrient concentrations of rooted and non-rooted clay–silt fragments. All samples were stored separately in plastic bags in a cooler filled with ice for 2–4 h until further processing in the laboratory. Single sandy soil samples were transferred to a 2 mm sieve to separate roots from soil. Roots were immediately washed in ice cold water, then stored in plastic tubes on ice until staining. Roots from clay–silt fragments were carefully separated by breaking fragments by hand and collecting roots with forceps. Further treatment was the same as for roots from sandy soil.

The following analyses were performed for each sandy soil from each depth increment and for clay–silt fragment samples at each sampling time and for non-rooted silt–clay fragments from the August sampling. Analyses were made on the particle-size fraction < 2 mm (air-dried). Water soluble potassium, calcium, magnesium, manganese and phosphorus was determined in a water extract from soil water solution ratio of 1–2.5. Samples were mechanically shaken for 1 h, stored for 16 h at room temperature, centrifuged for 5 min at 3000 rpm, then the supernatant was filtered through 0.2 µm Whatman filter paper (512 ½ folding filter, Whatman; Dassel, Germany) (Schlichting et al., 1995) and analyzed with inductive coupled plasma spectrometry (iCAP 6000 series, Thermo scientific, Germany). Total soil nutrient concentrations were determined from August samples. From each sample, 200 g soil was ground to a fine powder with mortar and pestle, then treated with 2 ml nitric acid (HNO_3, 65%) and digested in a pressure vessel which were placed in an oven at 160 °C for 7–9 h at approximately 12 bar for complete digestion. Samples were filtered through 0.2 µm (Schleicher & Schuell, Dassel, Germany, filter 512, phosphorus-free) and filled up to 25 ml with ultra pure water and analyzed with inductive coupled plasma spectrometry (iCAP 6000 series, Thermo scientific, Germany).

Soil pH (only from August samples) was determined from 5 g soil in 12.5 ml water, mechanically shaken for 5 min, incubated for 1 h, then centrifuged for 5 min (3000 rpm); supernatant was assessed with a MultiLab 540 pH electrode (WTW, Weilheim, Germany).