Short Communication

Effect of buckwheat (*Fagopyrum esculentum*) on soil-phosphorus availability and organic acids

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Abstract

As a cover crop, buckwheat (*Fagopyrum esculentum*) may increase soil-P availability. Buckwheat was grown in low-P and P-fertilized field plots, and organic anions were measured in rhizosphere soil. Soil-P availability was not affected by buckwheat, but the concentration of rhizosphere tartrate^{2-} was significantly higher ($p < 0.005$) in low-P *vs.* P-fertilized plots. This suggests that organic-anion root exudation may have a role in buckwheat-rhizosphere P dynamics.

Key words: sustainable agriculture / cover crops / nutrient management

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1 Introduction

In agroecosystems, sustainable management of limiting soil nutrients such as phosphorus (P) may be enhanced with cover-crop use (*Arcand* et al., 2010). For P, cover or rotation crops (*e.g., Lupinus albus, Vicia faba, Fagopyrum esculentum*) are selected because of ability to access and accumulate soil P, through mechanisms such as mycorrhizae, root acid, or enzyme exudation, and/or nutrient-scavenging behavior (*Ryan* et al., 2001; *El Dessougi* et al., 2003; *Steffens* et al., 2005). In particular, buckwheat (*Fagopyrum esculentum*) has been identified as such a P-efficient crop that putatively increases soil-P availability for the next crop rotation after buckwheat-biomass incorporation into the soil (*Teboh* and *Franzen*, 2011). While some studies have documented increased P availability after incorporation of buckwheat green manure, the agronomic significance of increases in P is questionable (*Arcand* et al., 2010). Further, the primary mechanism and potential magnitude of enhancing P availability with buckwheat is unknown. Thus, the efficacy of using buckwheat as a primary strategy to increase field P availability for subsequent crops requires further evaluation.

For some plants (*e.g., Brassica napus*), P solubilization and coupled uptake is enhanced through exudation of organic acid anions (*e.g.,* oxalate^{2-}, tartrate^{2-}) which interact with phosphate/metal complexes to increase free phosphate in the soil solution (*Ryan* et al., 2001). The exudation of oxalate^{2-} by buckwheat has been observed in conditions of Al toxicity (*Ryan* et al., 2001), and exudation of tartaric acid and oxalic acid also increased as a function of P deficiency in a laboratory experiment (*Kai* et al., 1999), suggesting that direct rhizosphere chemical alteration may also enhance P uptake by buckwheat in the field. In this study, the effect of buckwheat incorporation on bulk soil extractable P in control and added-P field plots was determined to assess the role of buckwheat in increasing P availability for subsequent crops. Further, organic acid anions in buckwheat rhizosphere soil from these plots were analyzed to investigate possible buckwheat soil-P acquisition mechanisms, specifically testing the hypothesis that buckwheat increases its access to soil P by exudation of organic acid anions.

2 Methods

Research was conducted between June and October 2009 at a certified organic vegetable farm (Kettle Pond Farm, Berkley, MA, USA). The field used for this study had recently been cultivated for vegetable production, with initial plowing in 2006 and shallow tillage in 2007, 2008, and 2009. In 2008, peppers were planted on this site. Study-site soil was characterized as Woodbridge fine sandy loam, a coarse-loamy, mixed, active, mesic Aquic Dystrudept with 0%–8% slopes (climatic conditions according to *Soil Survey Staff*, 2010). To characterize initial soil conditions, ten soil samples (∅ 0.8 cm, 18 cm depth) were collected randomly from each plot and homogenized. Initial soil conditions (pH, Al, P, K, Ca, Mg, N, and heavy metals) were determined by analysis at the UMass Amherst Soil and Plant Tissue Testing Lab (Amherst, MA). For pH, a 1:1 soil-to-water ratio was used. Aluminum, P, K, Ca, Mg, and heavy metals were extracted using modified Morgan's solution (dilute glacial acetic acid and NH_4 hydroxide) followed by analysis using an inductively-coupled-plasma (ICP) spectrometer (Spectro Analytical, Marlborough, MA). Organic-matter (OM) content was determined by loss

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on ignition (24 h at 450°C).The average percent OM content across all plots was (7.6 ± 5.4)%, with an average pH of 6.6 ± 0.1. Initial levels of soil P ([4.54 ± 0.8] μg g^{-1}), N ([10.67 ± 1.3] μg g^{-1}), K (874.38 ± 11.4] μg g^{-1}), and Al ([26.25 ± 6.3] μg g^{-1}) were low, low, low-medium low, and nontoxic, respectively.

Two treatments (buckwheat with and without P fertilizer) were each assigned to four replicate 2 m × 1 m plots in a randomized block design separated by 0.5 m wide aisles. Tennessee Brown Phosphate (TBP), a mineral amendment material derived from superphosphate processing (0–3–0, with 27% of total P as orthophosphate) (Fedco Seed Company, Waterville, ME), was applied to added-P treatments at the recommended application amount (450 kg TBP according to 13.5 kg P per hectare) on June 30, 2009. No other plant nutrients (*e.g.,* N, K, Ca, S, *etc.*) were applied above trace levels. TBP was incorporated to a depth of 1 cm by surface raking. Buckwheat seed (Fedco Seed Company, Waterville, ME) was applied by broadcast seeding at the recommended density of 122 kg seed ha^{-1} (*Björkman* et al., 2008) on July 4, 2009. The buckwheat germinated evenly 6 d after seeding, and buckwheat culture continued for 21 d, until flowers, but not mature seeds, were visible. Two centimeters of water was applied on two occasions to supplement rainfall (% normal precipitation: Jun = 137%, Jul = 253%) (*Mass DCR*, 2009).

To determine differences in buckwheat growth among the treatments and observe possible organic-anion exudation, all buckwheat plants and surrounding soil (≈ 2 cm distance from root) in 0.25 m × 0.25 m randomly placed quadrats were collected in each plot 21 d after germination. Root and shoot tissue were separated. From each quadrat, the roots of five plants were rinsed thoroughly, and ≈ 1 g of rhizosphere soil (1 mm of soil surrounding roots) was retained for organic acid analysis following *Fan* et al. (1997). The collected rhizosphere soil was lyophilized immediately to prevent microbial degradation, rehydrated in 1 mL deionized water, and syringe-filtered for organic acid analysis. Roots and shoots from each quadrat were oven-dried (24 h at 50°C) and weighed. Immediately after sampling, plots were subdivided and either mowed with a push lawn mower, or tilled to a depth of ≈ 20 cm using a rototiller.

Analysis of organic acids in rhizosphere soil aqueous extracts was completed using a Waters 2695 Separations module liquid-chromatography instrument and Waters dC18 IS column (4.6 × 20 mm, 5 μm), with a 5 mM Na phosphate (NaH_2PO_4) aqueous mobile phase (pH 3.8, flow rate 0.3 mL min^{-1}) and detection using a PDA detector and a Micromass ZQ mass spectrometer (Waters Associates., Inc., Milford, MA). Regent-grade oxalic (99.999%), tartaric, citric, and lactic acid standards (Sigma Aldrich, St. Louis, MO) were used without further purification. Organic acids were detected at 210 nm or by extracting the mass of interest from the total ion chromatogram.

In addition to initial sampling, soil samples for P analysis were collected (1) before buckwheat germination (Jul 6, 2009), (2) at buckwheat sampling (Jul 31, 2009), and (3) 2 weeks after mowing/tilling (Aug 18, 2009), as described above. Available soil P was extracted by HCl/NH_4-fluoride Bray P extraction (*Bray* and *Kurtz*, 1945), with associated gravimetric moisture content evaluated by difference in mass after oven drying (24 h at 110°C). The extractant was frozen until spectrophotometric analysis with a Smartchem 200 Discrete Chemistry Analyzer (Westco Scientific Instruments, Brookfield, CT).

All data were analyzed for normality and homogeneity and transformed logarithmically as needed. One-Way ANOVA was used to analyze differences in buckwheat biomass, rhizosphere tartaric acid concentration, and extractable soil P at time of buckwheat harvest between added-P and no-added-P plots. Changes in P availability over time and as a function of buckwheat treatments were analyzed using Repeated Measures (RM) ANOVA, with pairwise analysis sampling dates using Tukey's Multiple Comparison Test.

3 Results and discussion

On the second and third sampling dates, average Bray soil P levels (μg [g dry soil]$^{-1}$) across all treatments did not differ from each other or the initial levels ($p > 0.05$). Phosphorus levels declined significantly (1.3-fold decrease) in all treatments between the third (at buckwheat harvest) and fourth (after mowing or tilling) sampling dates ($p < 0.05$). No significant differences in extractable soil P as a function of buckwheat treatment over time ($F_{2,47} = 0.59$, $p = 0.57$), interaction between buckwheat treatments and time ($F_{6,47} = 0.46$, $p = 0.77$), or post-buckwheat manipulation treatment ($F_{1,23} = 3.14$, $p = 0.09$) were detected. At the time of buckwheat harvest, there were no significant differences in extractable soil P, buckwheat dry shoot, or dry root mass between control and added-P plots (Table 1), suggesting that, if P availability was limiting for growth, compensatory P acquisition by buckwheat was present in unamended plots.

Table 1: Mean (± SD) soil-P availability, dry buckwheat plant biomass, and rhizosphere tartaric acid concentration at the time of buckwheat harvest (21 d after germination). $N = 4$ for each treatment with exception of rhizosphere tartaric acid concentration ($N = 3$). Within the same variable, means with different letters differ significantly ($p < 0.05$); dm = dry matter.

Variable	Added P	No Added P	Control	Statistic$_{(d.f.)}$	*p* value
Soil-P availability / μg (g dry soil)$^{-1}$	3.90 (± 0.59)a	4.59 (± 0.66)a	4.31 (± 0.39)a	$F_{2,11} = 1.65$	0.245
Buckwheat shoot biomass / g dm plant^{-1}	0.34 (± 0.04)a	0.33 (± 0.06)a	–	$F_{1,7} = 1.39$	0.285
Buckwheat root biomass / g dm plant^{-1}	0.26 (± 0.08)a	0.26 (± 0.07)a	–	$F_{1,7} = 0.009$	0.927
Rhizosphere tartaric acid concentration / μg g^{-1}	0.24 (± 0.07)a	0.56 (± 0.03)b	–	$F_{1,6} = 53.05$	< 0.005

Oxalic acid (protonated oxalate^{2-} at mobile phase pH, 3.8), if present in the samples, was not detected (with instrumental detection limit for oxalic acid of 0.5 μg mL^{-1}). However, tartaric acid ($C_6H_4O_5$, MW = 150 g mol^{-1}) was detected in rhizosphere-soil extractions by its retention time (6.7 min) and its mass spectrum (mass 149, negative ion mode). Tartaric acid was 15 times greater in rhizosphere samples from control plots ([0.30 $\pm$ 0.07] μg [mL soil solution]$^{-1}$) than added-P plots ([0.02 $\pm$ 0.05] μg mL^{-1}), a significant difference (Table 1).

The absence of oxalic acid (protonated oxalate^{2-}) in buckwheat rhizosphere soils could be attributed to sufficient P availability. However, the presence of tartaric acid in rhizosphere soil adds additional information to the understanding of buckwheat–rhizosphere soil interactions. While not identified by *Ryan* et al. (2001) as a buckwheat exudate, *Zhang* et al. (1997) identified tartaric acid as a primary component of radish (*Raphanus sativus* L.) root exudates, with 15 to 60 times higher production under P-deficient conditions. In this study, increased exudation of tartrate^{2-} as a possible physiological response to low soil-P availability in unamended soils was observed. While statistically significant differences in extractable soil P were not observed as a result of P addition, this may not reflect actual plant P availability, which could have increased enough to reduce tartrate^{2-} exudation by buckwheat in fertilized as compared to unamended plots.

Because no significant difference in extractable soil P during buckwheat culture or after its incorporation into the soil was found, the results of this study do not support the hypothesis that buckwheat immediately increases P availability in field conditions. Future studies may benefit from extended sampling after buckwheat incorporation, allowing for detection of increased extractable soil P due to continued residue decomposition. Further, analysis of buckwheat tissue N and P content may clarify the degree of P deficiency. However, significant differences in rhizosphere tartrate^{2-} concentration between low-P and fertilized plots suggest that this acid anion may be produced by buckwheat as a response to low soil-P availability. Lack of difference in buckwheat biomass between the treatments suggests that this may be a compensatory mechanism to enable greater P uptake. However, enhanced uptake may not be directly correlated with increased P availability in the bulk soil for a subsequent crop in the immediate short term, as evidenced by lack of difference in extractable P following buckwheat incorporation. Consequently, this study reveals a possible new mechanism for the enhanced P-uptake capabilities of buckwheat documented in other studies (*e.g., Teboh* and *Franzen*, 2011) that have also questioned the magnitude and significance of this for subsequent crop growth. Nonetheless, the identification of tartrate^{2-} exudation as a potential P-uptake mechanism in buckwheat may guide development of improved buckwheat varieties and cover crop-management strategies for more sustainable soil-P management in agro-ecosystems.

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