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Soil phosphorus mobilization in the rhizosphere of cover crops has little effect on phosphorus cycling in California agricultural soils

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ABSTRACT

Phosphorus (P) is a key limiting factor in many terrestrial ecosystems because most soil P is bound to soil minerals or organic matter. Increasing P cycling rates can increase P availability, including in agricultural soils that receive external P inputs. For example, cover crops may increase P cycling rates via plant uptake and P release during microbial decomposition. Cover crops and associated microbes may also change rhizosphere properties and stimulate soil P mobilization. We studied the potential of legume – fava bean (*Vicia faba*), vetches (*Vicia dasycarpa*, *Vicia sativa*, *Vicia benghalensis*) pea (*Pisum sativum*) – and cereal – rye (*Secale cereale*), wheat (*Triticum aestivum*), oat (*Avena sativa*) – cover crops to stimulate P cycling across management practices in two long-term systems trials in California. We measured cover crop biomass and nutrient content, P-mobilizing capacity (pH, organic acids, phosphatase activity) and soil P fraction changes in the rhizosphere. Cereals generally produced more biomass with similar P content compared to legumes, but higher C:P in cereal residues could favor microbial immobilization, delay residue mineralization and reduce P cycling rates. Legumes, especially fava bean, had the largest effect on rhizosphere properties by reducing pH and increasing organic acids concentrations and phosphatase activity. However, these changes in rhizosphere properties had a modest impact on soil P and did not increase soil P availability. Furthermore, we found no strong effect of management practices or soil P concentrations on soil P mobilization. Our results suggest that P mobilization in the rhizosphere of legumes is unlikely to increase P cycling rates in these soils, whereas P uptake and release in cereal biomass could have stronger effects.

1. Introduction

Phosphorus (P) is a key limiting nutrient in terrestrial ecosystems because most soil P is found in pools of low plant availability: bound to calcium, aluminum or iron minerals, or in low lability organic compounds (Frossard et al., 1995; Nelson and Janke, 2007). In intensive agriculture, external inputs such as mineral fertilizers, manures, and composts can increase phosphorus availability to crops, but a significant fraction of these inputs is not taken up by plants and ends up "fixed" in soil pools of low plant availability (Takeda et al., 2009; Richardson et al., 2011). Thus, stimulating P cycling to increase soil P availability could help utilize some of this fixed P.

Cover crops – crops for which the biomass is incorporated in the soil rather than harvested – could increase soil P availability by stimulating soil P cycling (Horst et al., 2001; Nelson and Janke, 2007). Soil P stored in pools of low plant availability can be converted into labile P at greater rates in the rhizosphere of cover crops than in bulk soils, especially in P-depleted soils (Kamh et al., 1999; Richardson et al., 2011). Cover crops also take up P, and residue decomposition will affect soil P cycling. Phosphorus release during microbial decomposition can be estimated based on residue %P and carbon (C) to P ratio (C:P), where residue P above 0.24% and C:P below 200:1 favor P release over microbial immobilization (Nachimuthu et al., 2009; Hasbullah et al., 2011; Alamgir et al., 2012). As plant species composition (Eichler-Loebermann et al., 2008) and soil P concentrations (Nuruzzaman et al., 2005) affect the stoichiometry of plant residues and microbial responses during decomposition, soil P cycling should vary in soils under different management conditions.

Cover crops also stimulate P cycling by mobilizing soil P that has accumulated in pools of low plant availability (Horst et al., 2001).

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Cover crops and associated microbes can mobilize rhizosphere P by lowering pH to dissolve calcium-P (Ca–P) minerals, increasing organic acids content to desorb P bound to aluminum and iron oxides (Al/Fe–P), and mineralize organic P (P_{org}) by stimulating phosphatase enzyme activity (Kamh et al., 1999, 2002). Plants may affect their rhizosphere directly or stimulate P mobilization via changes in microbial activity and species composition (Wang et al., 2012). Cover crops, especially legumes, effectively modify their rhizosphere, mobilize soil P, and provide yield benefits to subsequent cash crops in low-input farming systems located on old, high P-fixing soils (Horst et al., 2001; Pypers et al., 2007). However, as P mobilization rates often decrease at higher soil P concentrations (Pearse et al., 2006; Li et al., 2007), P mobilization may be much lower in less-weathered soils that have a lower P-fixing capacity. As a result, cover crops could have a smaller impact on P cycling in these soils (Kuo et al., 2005; Takeda et al., 2009).

Our main objective was to compare the potential of cereal and legume cover crops to stimulate P cycling in intensive agricultural systems located on less-weathered soils in California. We measured cover crop P content, rhizosphere properties and soil P fractions to determine how cover crops affect soil P dynamics in soils that differ in P availability due to management practices. We hypothesized that:

1) Cereals would produce more biomass with less P than legumes, resulting in lower mineralization potential in cereals vs. legumes;
2) Legumes would increase rhizosphere P mobilization via stronger changes to rhizosphere properties resulting in higher soil P availability compared to cereals;
3) Soil P mobilization would occur at higher rates when soil P was lower – no manure, compost or fertilizers added.

2. Material and methods

2.1. Study systems

2.1.1. Russell Ranch Sustainable Agricultural Facility (RR) – Davis (CA, USA)

We used 18 plots (0.4 ha) from the Long-Term Research in Agricultural Systems (LTRAS) experiment at the Russell Ranch Sustainable Agricultural Facility (RR – est. 1993) to study one cover crop mixture across three management types: organic, mixed (conventional with cover crops), and unfertilized. Winter cover crops, grown between October and March, consisted of hairy vetch (*Vicia dasycarpa* Ten. – 47.4 kg seed ha^{-1} yr^{-1}) and pea (*Pisum sativum* L. – 87.0 kg seed ha^{-1}) from 1993 to 2006, and fava bean (*Vicia faba* L. – 89.6 kg seed ha^{-1} yr^{-1}), hairy vetch (22.4 kg seed ha^{-1}) and oat (*Avena sativa* L. – 28.0 kg seed ha^{-1}) from 2007 to 2013. Volunteer winter wheat (*Triticum aestivum* L.) and weeds also grew in the cover crop mixture during most years.

The organic and mixed plots are irrigated and fertilized two-year grain-tomato (*Solanum lycopersicum* L.) rotations, where fertility is based on N and provided as mineral fertilizers only in the mixed plots (75 kg N ha^{-1} yr^{-1} on average) or composted poultry manure in the organic plots (135 kg N ha^{-1} yr^{-1} on average). Each treatment is replicated in six plots, and at the time of sampling, three replicates were under winter wheat (no cover crops during the winter) and three were under tomato (with cover crops during the winter).

The unfertilized plots consist of a rainfed two-year wheat-cover crop rotation with six replicates: three plots under winter wheat and three under cover crops for each given year. Fertility is only supplied via N-fixation inputs during the cover crop phase (estimated at 75 kg N ha^{-1} yr^{-1} in years when cover crops are grown – J. Six, unpublished data).

Phosphorus inputs are highest in organic plots (101 kg P ha^{-1} yr^{-1}), lowest in unfertilized plots (0 kg P ha^{-1} yr^{-1} for all years except 49 kg P ha^{-1} yr^{-1} in 1999), and intermediate in mixed plots (11 kg P ha^{-1} yr^{-1} for all years except 60 kg P ha^{-1} yr^{-1} in 1999). All plots are randomly allocated across two very similar soil types: Yolo silt loam (fine-silty, mixed nonacid, thermic Typic Xerothents) and Rincon silty clay loam (fine, montmorillonitic, thermic Mollic Haploxeralfs).

2.1.2. Salinas Organic Cropping Systems experiment (SOCS) – Salinas (CA, USA)

We used two of the eight treatments from the Salinas Organic Cropping Systems experiment (SOCS – est. 2003) that monitored a double-cropping production system of lettuce (*Lactuca sativa* L.) and broccoli (*Brassica oleracea* L.) or spinach (*Spinacia oleraceae* L.) under certified organic management (Brennan and Boyd, 2012). The SOCS experiment is under tillage-intensive management that is typical for farms in this region. Both treatments (four 240 m^2 replicate plots per treatment) were fertilized equally during the production of vegetables with pelleted chicken manure and feather meal as well as liquid fertilizers (22 kg N ha^{-1} for spinach, 56–74 kg N ha^{-1} for lettuce, 134–170 kg N ha^{-1} for broccoli) from 2003 until 2011 (Brennan and Boyd, 2012). The first treatment ("compost") received winter cover crops and yard compost every year (15.2 Mg ha^{-1}, C/N ~ 22, 1.5% N, 0.25% P) while the other treatment ("no compost") received winter cover crops every fourth year and no yard compost from 2003 until 2011 (Brennan and Boyd, 2012). The cover crop mixture was identical in both treatments: 'Merced' rye (*Secale cereale* L. – 42 kg seed ha^{-1}), fava bean (147 kg seed ha^{-1}), 'Magnus' pea (105 kg seeds ha^{-1}), common vetch (*Vicia sativa* L. – 63 kg seeds ha^{-1}), and purple vetch (*Vicia benghalensis* L. – 63 kg seeds ha^{-1}). The various seed components were mixed together with N-dure *Rhizobium* inoculum (INTX Microbials, LLC Kentland, IN) and planted simultaneously. All plots were converted to strawberries in the fall of 2011 (no cover crops), and planted with the aforementioned cover crop mixture during the fall of 2012. The soil type is a Chualar loamy sand (fine-loamy, mixed, superactive, thermic Typic Argixerol).

2.2. Analytical methods

2.2.1. Sample collection

We collected samples (plants and soils) in late February and early March 2012 (only in RR) and during February 2013 (RR and SOCS).

In RR, we sampled three 20.25 m^2 microplots located randomly within each 0.4 ha plot. In each microplot, we used a 0.36 m^2 quadrat to collect aboveground biomass separated as cereals (wheat and oat combined because they were difficult to differentiate at sampling), fava bean, vetch, or weeds (all other species). Aboveground biomass was sampled several weeks before cover crops were terminated, thus our biomass measurements underestimate final cover crop biomass. Near these microplots, vigorous individual plants were excavated to sample rhizosphere soil, where we first removed loosely adhering soil by shaking plants and used a toothbrush to sample the soil that was strongly adhering to roots. We also sampled bulk soil from a zone without plants. Plant debris and roots were removed from samples, and soils (not dried or sieved) were refrigerated until use. A subsample of each soil was dried at 105 °C for 72 h to determine dry weights, and we measured pH on field-moist soils in distilled water (soil:solution = 1:5) within a week of collection.

Soils were sampled with the same procedure in SOCS within each plot (there are no microplots in SOCS), and biomass was sampled just before cover crop termination (i.e. sampled biomass ≈ final biomass), as described in Brennan and Boyd (2012).

2.2.2. Organic acids

Within 12 h of collection, we extracted organic acids from each sample by shaking 2 g of soil with 10 mL of 0.2 mM $CaCl_2$ for 30 min (Nuruzzaman et al., 2006). Samples were centrifuged, filtered (0.22 μm), acidified with sulfuric acid (H_2SO_4) and frozen at −20 °C prior to analysis. Samples were processed using a modified version of Cawthray (2003) for reverse-phase UPLC, with a Waters Acquity H-Class instrument (Waters Corp., Milford, MA). We used a 20 mM KH_2PO_4 mobile phase (pH = 2.2) at a flow rate of 0.09 mL min^{-1} with a Synergi Hydro-RP C18 column (50 mm * 2 mm, 2.5 μm particle size – Phenomenex, Torrance, CA) used at room temperature (25 °C). We used a UV detector at 210 nm for organic acid identification, and mixed standards (Bio-Rad Laboratories Inc., Hercules, CA and Sigma–Aldrich, St-Louis, MO) for quantification of malate, malonate, citrate and fumarate. We used samples spiked with 1 mM citrate to determine potential interactions of organic acids with sample matrix and estimate recovery from a known source. We used a ULTRA UPLC C18 guard column (Phenomenex, Torrance, CA) and a gradient elution method (from 100% mobile phase to 40% mobile phase and 60% methanol) after every 8th sample to maintain column performance.

2.2.3. Phosphatase activity

Within seven days of collection, samples were processed for phosphatase activity using a modified version of the protocol described in Nuruzzaman et al. (2006). We extracted 2 g of soil with 30 mL of 40 mM sodium maleate buffer (pH = 6.5 for RR, pH = 6.3 for SOCS) and incubated samples with 5 mM of p-nitrophenol phosphate (Sigma–Aldrich, St-Louis, MO) for 1 h at room temperature (25 °C) under agitation. After stopping the reaction with 1 M sodium hydroxide (NaOH), we determined phosphatase activity by quantifying p-nitrophenol release via colorimetry at 410 nm.

2.2.4. Soil C, N and P

We determined different soil P fractions (Hedley fractions) using a modified version of Tiessen and Moir (2007) on RR soils. Samples of 0.5 g were sequentially processed with 1) distilled water and anion-exchange resins charged with 0.5 M sodium bicarbonate ($NaHCO_3$) 2) 0.5 M $NaHCO_3$ 3) 0.1 M NaOH 4) 1 M hydrochloric acid (HCl) 5) concentrated H_2SO_4 and hydrogen peroxide (H_2O_2). The first four steps were carried with samples extracted with 30 mL of liquid for 16 h (15 rpm end-over-end), followed by centrifugation at 7000 rpm (~7000 g). Phosphorus content in the supernatant was determined by colorimetry at 880 nm (ascorbic acid method). Extracts made with $NaHCO_3$ and NaOH were digested with potassium persulfate and 0.9 M H_2SO_4 and subjected to colorimetry to determine total P. Organic P in these fractions was calculated as the difference between the digested and undigested samples, the latter being corrected for native color by measuring the absorbance with all reagents except ascorbic acid. The final digest step was carried out at 360 °C using 5 mL of concentrated H_2SO_4 and repeated 0.5 mL H_2O_2 additions until the color in the sample disappeared. Phosphorus content was determined by the ascorbic acid method (Tiessen and Moir, 2007).

Soil C and N content for 2013 samples was determined by combustion on a Carlo-Erba NA 1500 analyzer; tin capsules were filled with 15–30 mg of dried soil material and combusted at 1050 °C. Atropine was used as a standard.

2.2.5. Plant C, N, and P

Plant samples were dried at 65 °C for 72 h, ground in a Wiley mill (mesh 20) for P determination, and ball-milled prior to C:N determination. In RR, plants from all microplots were processed for P content but only two microplots per plot were processed for C and N. All plots were analyzed for C, N and P in SOCS.

Phosphorus content was determined using a similar digestion as the final step of the Hedley procedure, where 50 mg of ground plant tissue were digested at 360 °C with 5 mL H_2SO_4 and multiple additions of H_2O_2 until the color disappeared. Phosphorus content was determined by the ascorbic acid method.

Plant C and N content was determined by combustion on a Carlo-Erba NA 1500 analyzer; tin capsules were filled with 5 mg of plant material and combusted at 1050 °C.

2.3. Statistical analyses

Because of within and among-plot variations, we computed a change – denoted as delta or Δ – between rhizosphere and bulk soils to account for this variation when analyzing soil data. For RR soils, we averaged the data from the three microplots within each plot and used this average for statistical analyses, as data from microplots were not independent.

All data were analyzed using two-way ANOVAs with management and plant species as fixed factors, followed by a Tukey HSD test ($\alpha = 0.05$). We analyzed RR data from 2012 and 2013 in separate analyses. We verified that data met normality and homoscedasticity conditions and applied transformations (square root or log) when necessary. We considered that the difference between rhizosphere soils of a given plant and bulk soils was significant if the 95% confidence interval of the mean did not overlap with zero.

We computed Pearson correlations between selected variables and verified the significance of correlations using a mixed model where plot identification within a treatment or plant was used as a random effect. The mixed model never changed the significance of the correlations, thus we present the Pearson correlation values because mixed models do not allow computing a straightforward correlation coefficient. All statistical tests were carried out using R (version 2.12.2, 2011).

3. Results

3.1. Plant biomass and nutrient content

Cereals dominated the biomass of cover crop mixtures in RR – legumes contributed less than 20% of total biomass in 2012 and less than 40% in 2013 – and biomass was lowest in unfertilized plots (Table 1). In SOCS, rye produced more biomass than individual legume species, although it dominated total cover crop biomass only when compost was added (Table 1).

Cover crop P uptake was highest in organic plots and in cereals for RR, and highest in rye with no effect of compost in SOCS (Table 1). Plant %P was highest in organic plots and lowest in cereals (2012) or fava bean (2013) for RR, and lowest in fava bean for SOCS, whereas plant C:P followed the opposite trend (Table 1). Cereals and plots without fertilizer, manure or compost additions had less plant N, i.e. lower %N and N:P and higher C:N (Supplementary material Table S1).

3.2. Soil pH, phosphatase activity and organic acid concentrations

In both sites, soil pH was consistently lower in rhizosphere soils of fava bean compared to other plants and bulk soils, and management did not affect patterns among plants (Fig. 1).

In RR, phosphatase activity was higher in rhizosphere soils – legumes > cereals for 2012 vs. fava bean > cereals only in mixed plots for 2013 (Figs. 2A and B). Phosphatase activity was positively correlated to plant %N (2012: $r = 0.59$, $p < 0.01$; 2013: $r = 0.39$, $p < 0.05$) and N:P (2012: $r = 0.45$, $p < 0.05$; 2013: $r = 0.74$, $p < 0.001$) in RR. In SOCS, phosphatase activity was marginally higher in fava bean vs. rye rhizosphere, and compost addition had no impact (Fig. 2C).

Table 1
Cover crop biomass (kg ha^{-1}), %P, P uptake (kg P ha^{-1}) and plant C:P at the Russell Ranch Sustainable Agricultural Facility (RR) in 2012 and 2013 and at the Salinas Organic Cropping Systems (SOCS). Mean and 95% confidence intervals of the mean are presented. Different letters represent statistical differences computed with a Tukey HSD test performed after a two-way ANOVA. For comparison among plants, letters should be interpreted only within a given row, where management and system are constant (e.g., within organic management in RR-2012). Interaction terms are presented only if significant. n.s. = $p > 0.05$,* = $p < 0.05$,** = $p < 0.01$,*** = $p < 0.001$.

System	Variable	Management		Plant				ANOVA		
				Cereals[a]	Fava bean	Vetch	Pea/Weeds[b]	Factor	F	Sig
RR-2012	Biomass (kg ha^{-1})	Organic		1351 ± 429 A	96 ± 35 B	77 ± 39 B	379 ± 288 B	Plant	76.0	***
		Mixed		1282 ± 423 A	64 ± 4 B	35 ± 18 B	73 ± 84 B	Management	1.6	n.s.
		Unfertilized		1085 ± 343 A	146 ± 61 B	90 ± 12 B	50 ± 49 B			
	%P	Organic	a	0.44 ± 0.02 C	0.47 ± 0.01 B	0.48 ± 0.01 B	0.75 ± 0.02 A	Plant	43.3	***
		Mixed	b	0.36 ± 0.03 B	0.41 ± 0.03 B	0.43 ± 0.03 B	0.60 ± 0.15 A	Management	23.8	***
		Unfertilized	b	0.30 ± 0.03 B	0.37 ± 0.03 AB	0.47 ± 0.02 A	0.49 ± 0.10 A	Interaction	2.8	n.s.
	P uptake (kg P ha^{-1})	Organic	a	6.0 ± 0.5 A	0.4 ± 0.1 B	0.4 ± 0.2 B	2.8 ± 2.1 A	Plant	73.4	***
		Mixed	b	4.6 ± 1.2 A	0.3 ± 0.0 B	0.2 ± 0.1 B	0.3 ± 0.1 B	Management	15.3	***
		Unfertilized	b	3.2 ± 1.1 A	0.5 ± 0.2 B	0.4 ± 0.1 B	0.2 ± 0.2 B	Interaction	8.3	***
	C:P	Organic	c	96 ± 4 A	87 ± 3 A	91 ± 4 A		Plant	56.2	***
		Mixed	b	119 ± 7 A	104 ± 5 B	101 ± 6 B		Management	55.3	***
		Unfertilized	a	138 ± 8 A	114 ± 7 B	91 ± 3 C		Interaction	14.7	***
RR-2013	Biomass (kg ha^{-1})	Organic	a	1081 ± 213 A	398 ± 118 B	190 ± 51 C	185 ± 118 C	Plant	59.6	***
		Mixed	a	1276 ± 260 A	503 ± 130 B	197 ± 92 C	8 ± 3 C	Management	11.5	***
		Unfertilized	b	796 ± 659 A	160 ± 71 B	41 ± 13 C	13 ± N/A[c] C			
	%P	Organic	a	0.42 ± 0.03 BC	0.38 ± 0.02 C	0.45 ± 0.01 B	0.79 ± 0.06 A	Plant	77.6	***
		Mixed	c	0.31 ± 0.03 B	0.22 ± 0.02 B	0.29 ± 0.02 B	0.49 ± 0.13 A	Management	76.4	***
		Unfertilized	b	0.38 ± 0.03 A	0.31 ± 0.00 B	0.40 ± 0.01 A	0.44 ± N/A[c] A	Interaction	3.7	*
	P uptake (kg P ha^{-1})	Organic	a	4.5 ± 0.7 A	1.5 ± 0.5 B	0.9 ± 0.2 C	1.3 ± 0.8 C	Plant	66.9	***
		Mixed	b	3.8 ± 1.8 A	1.1 ± 0.4 B	0.5 ± 0.2 C	0.0 ± 0.0 C	Management	23.6	***
		Unfertilized	c	2.8 ± 1.7 A	0.5 ± 0.2 B	0.2 ± 0.0 C	0.1 ± N/A[c] C			
	C:P	Organic	c	101 ± 8 A	105 ± 5 A	96 ± 2 A		Plant	23.1	***
		Mixed	a	130 ± 6 B	187 ± 21 A	149 ± 15 B		Management	91.8	***
		Unfertilized	b	105 ± 10 B	126 ± 6 A	104 ± 5 B		Interaction	6.9	**
SOCS	Biomass (kg ha^{-1})	No compost		1699 ± 373 A	1227 ± 385 AB	1036 ± 281 AB	1013 ± 182 B	Plant	40.0	***
		Compost		2933 ± 262 A	901 ± 317 B	682 ± 208 B	747 ± 369 B	Management	0.4	n.s.
								Interaction	12.4	***
	%P	No compost		0.45 ± 0.10 A	0.28 ± 0.04 B	0.51 ± 0.04 A	0.49 ± 0.08 A	Plant	19.4	***
		Compost		0.40 ± 0.03 A	0.28 ± 0.01 B	0.46 ± 0.07 A	0.48 ± 0.05 A	Management	1.8	n.s.
	P uptake (kg P ha^{-1})	No compost		7.8 ± 3.0 A	3.4 ± 0.7 B	5.3 ± 1.7 AB	5.0 ± 1.5 AB	Plant	26.7	***
		Compost		11.6 ± 0.3 A	2.5 ± 0.9 B	3.1 ± 1.0 B	3.6 ± 2.0 B	Management	0.1	n.s.
								Interaction	5.3	***
	C:P	No compost		103 ± 17 B	149 ± 32 A	83 ± 6 B	80 ± 6 B	Plant	28.4	***
		Compost		104 ± 8 B	150 ± 11 A	88 ± 17 B	86 ± 13 B	Management	0.3	n.s.

[a] Cereals are a mixture of oat and wheat in RR vs. pure rye in SOCS.
[b] Weeds in RR; pea in SOCS.
[c] Only one plot had weeds, hence the CI cannot be computed.

Rhizosphere RR soils had higher total organic acid concentrations than bulk soils, especially with fava bean (Figs. 3A and B). Fava bean rhizosphere had the highest concentrations of all organic acids except citrate and fumarate for 2012 (vetch was similarly high) and fumarate for 2013 (no differences among plants – Figs. 3A and B, Supplementary material Table S2). Concentrations of organic acids were generally highest and lowest in unfertilized plots for 2012 and 2013, respectively (Figs. 3A and B, Supplementary material Table S2). Fava bean rhizosphere had the highest concentrations of total and individual organic acids in SOCS, except malonate (pea was similarly high), whereas compost addition had no consistent effect (Fig. 3C, Supplementary material Table S2).

3.3. Soil nutrients in RR soils

Organic P enrichment was higher in legume rhizosphere and mixed plots (Table 2), and was positively correlated to phosphatase activity ($r > 0.68$, $p < 0.001$ for both years). In addition, we found a positive correlation between plant and soil C:N ($r = 0.58$, $p < 0.01$), N:P ($r = 0.75$, $p < 0.001$), and C:P ($r = 0.68$, $p < 0.001$) in 2013 (Supplementary material Table S3).

Rhizosphere soils (especially fava bean) were depleted in Ca–P compared to bulk soils, management had no effect on Ca–P, and stronger acidification resulted in more Ca–P depletion ($r > 0.55$, $p < 0.01$ for both years – Table 2). In contrast, we found Al/Fe–P enrichment with stronger acidification (2012: $r = -0.59$, $p < 0.01$; 2013: $r = -0.36$, $p = 0.07$) and more Al/Fe–P in fava bean rhizosphere for 2012 and in mixed plots for 2013 (Table 2).

The net effect of these changes in soil P fractions on labile resin P and total P is not straightforward, with variable results and complex interactions for both years (results not shown). However, a strong positive correlation between the change in resin P and total P (2012: $r = 0.61$, $p < 0.001$; 2013: $r = 0.81$, $p < 0.001$ – Fig. 4) suggests that patterns in resin P are mostly driven by changes in total P rather than changes in other soil P fractions.

4. Discussion

4.1. P cycling via biomass

Because cereals dominated cover crop biomass and P uptake, they will exert the most control on P release during residue decomposition in these systems. High soil P is the likely explanation for comparable P content in cereals and legumes, contrary to our hypothesis and studies made in P-depleted soils (Hasbullah et al., 2011). Although cereals had higher C:P and C:N and thus a potentially lower mineralization potential than most legumes

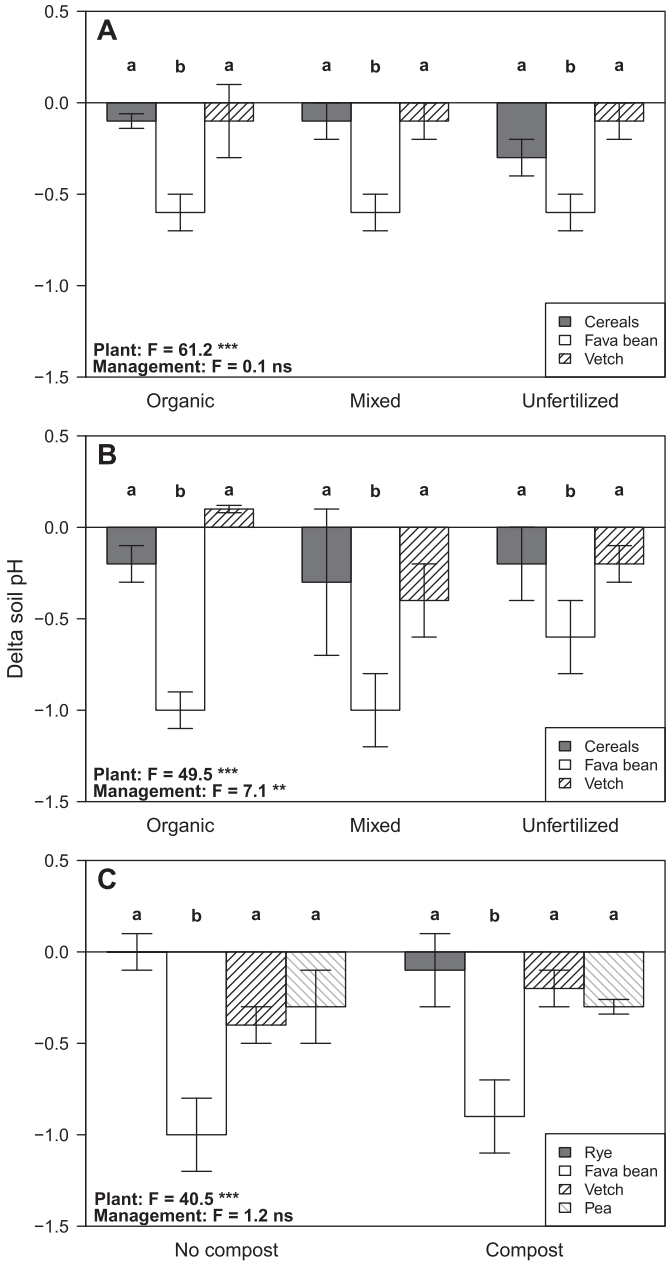

Fig. 1. Delta soil pH (rhizosphere − bulk) at the Russell Ranch Sustainable Agricultural Facility (RR) in 2012 (A) and 2013 (B), and at the Salinas Organic Cropping Systems (SOCS − C). Error bars are 95% confidence intervals of the mean. Significance for ANOVA effects: ns = $p > 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. In RR-2013: mixed < organic = unfertilized. Different lowercase letters represent statistical differences computed with a Tukey HSD test performed after a two-way ANOVA. Letters should be interpreted only within a given management and system (e.g., organic in RR-2012).

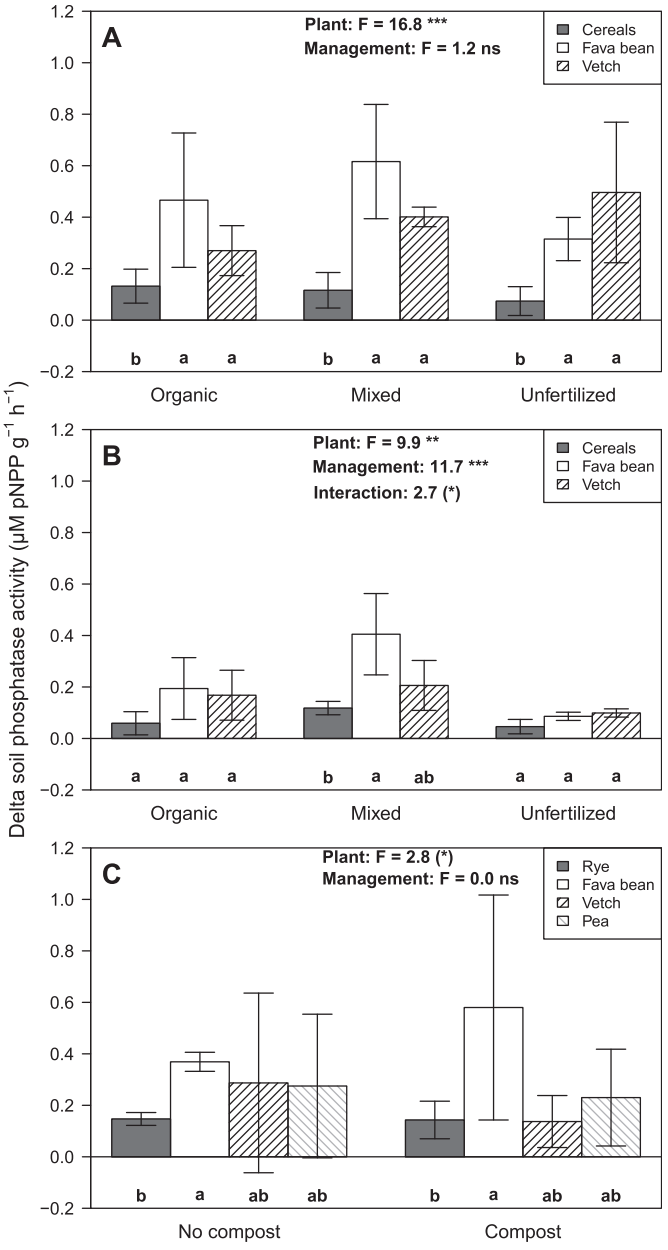

Fig. 2. Delta soil phosphatase activity (rhizosphere − bulk) at the Russell Ranch Sustainable Agricultural Facility (RR) in 2012 (A) and 2013 (B), and at the Salinas Organic Cropping Systems (SOCS − C). Error bars are 95% confidence intervals of the mean. Significance for ANOVA effects: ns = $p > 0.1$, (*) = $p < 0.1$, ** = $p < 0.01$, *** = $p < 0.001$. In RR-2013: mixed > organic = unfertilized. Different lowercase letters represent statistical differences computed with a Tukey HSD test performed after a two-way ANOVA. Letters should be interpreted only within a given management and system (e.g., organic in RR-2012).

(Eichler-Lobermann et al., 2008; Brennan et al., 2013), C:P values below 200:1 suggest a relatively fast mineralization and P release for cereal residues (Nachimuthu et al., 2009). As residue biomass plays a larger role than P content in affecting microbial dynamics – e.g., microbial respiration and microbial P (Alamgir et al., 2012) -, cereals will play a central role in controlling cover crop effects on P cycling in these soils.

Increasing soil P by adding manure, compost, or mineral fertilizer generally increased residue P and mineralization potential – similar to Pearse et al. (2006). This suggests a positive feedback where high soil P generates P-rich residues that stimulate decomposition and soil P cycling. However, high residue P content may also lower microbial P turnover, increase soil P_{org} and limit soil P availability (Alamgir et al., 2012), highlighting how P enrichment may instead decrease soil P cycling rates.

4.2. Rhizosphere pH, phosphatase and organic acids

Rhizosphere pH, phosphatase and organic acids were significantly affected by fava bean compared to other plants, which generally did not differ substantially from bulk soils. This could

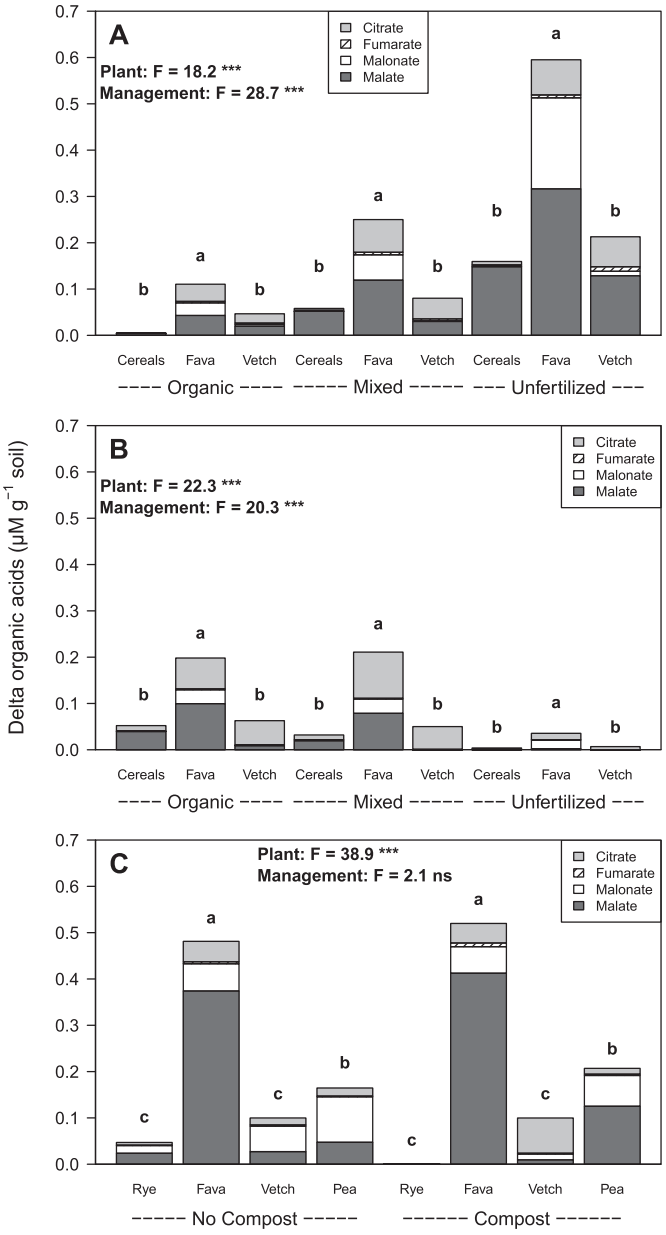

Fig. 3. Delta organic acids (rhizosphere − bulk) at the Russell Ranch Sustainable Agricultural Facility (RR) in 2012 (A) and 2013 (B), and at the Salinas Organic Cropping Systems (SOCS − C). Error bars are not presented for clarity. Significance for ANOVA effects computed on total organic acids (μM g^{-1} soil): ns = $p > 0.05$, *** = $p < 0.001$. In RR-2012: unfertilized > mixed > organic. In RR-2013: organic = mixed > unfertilized. Different lowercase letters represent statistical differences computed with a Tukey HSD test performed after a two-way ANOVA. Letters should be interpreted only within a given management and system (e.g., organic in RR-2012). Statistical significance for individual organic acids can be found in.Supplementary material Table S2.

result from fava bean exploiting a larger soil volume via its denser rooting system compared to other legumes (Nuruzzaman et al., 2005). Stronger effects on rhizosphere properties may also result from changes in microbial activity and species composition in fava bean rhizosphere compared to other legumes and cereals (Wang et al., 2012). Finally, high soil P and near-neutral soil pH may explain why fava bean differed from other legumes in this study, whereas it had similar effects to other legumes in pot studies conducted on P-depleted soils (Nuruzzaman et al., 2006; Rose et al., 2010). In contrast, phosphatase activity was high in the rhizosphere of all legumes, and the positive correlation with plant %N and N:P confirms the central role of N-fixation in increasing the activity of N-rich phosphatases in the rhizosphere of legumes (Houlton et al., 2008).

Higher soil P via additions of compost, manure or fertilizers only affected organic acids, consistently with Pearse et al. (2006) and Nuruzzaman et al. (2006) who found that organic acids were affected by P fertilization and soil P availability but not pH or phosphatase. However, given that all concentrations of organic acids were substantially below the threshold of 5 μM g^{-1} soil where organic acids can mobilize soil P effectively (Richardson et al., 2011), this will unlikely have a major effect on soil P cycling. Thus, rhizosphere properties were relatively unaffected by soil P in this study, contrary to our hypothesis that rhizosphere properties would be most affected at low soil P.

4.3. Changes in rhizosphere P fractions

Rhizosphere P fractions changed but not in ways consistent with substantial P mobilization: the reduction of Ca–P with lower pH is partially canceled by a concomitant increase in Al/Fe–P, higher phosphatase activity did not deplete organic P, and organic acids did not deplete Al/Fe–P. This is in contrast to many studies conducted on weathered low-P soils – e.g., Kamh et al. (1999) and Rose et al. (2010) – where changes in soil P were consistent with changes in rhizosphere properties, e.g., depletion of P_{org} with higher phosphatase activity. On the other hand, in less-weathered soils similar to those in this study, Hedley et al. (1982) found higher rhizosphere P_{org} despite higher phosphatase activity and stronger effects of pH on P fractions than organic acids and phosphatase. Furthermore, Kuo et al. (2005) and Takeda et al. (2009) found no indication of long-term P mobilization driven by cover crops in temperate farming systems. This suggests that P fractions in less-weathered soils may not respond as strongly to changes in rhizosphere pH, organic acids and phosphatase as they do in older, P-depleted soils.

Furthermore, concomitant increases in rhizosphere resin P, total P and P_{org} in addition to correlations between plant and rhizosphere C, N, and P suggest that deposition and turnover of root and microbial cells are largely responsible for increases in rhizosphere P rather than P mobilization (Hedley et al., 1982). Phosphorus mobilization could still occur and be masked by P deposition, implying that P mobilization fluxes would be relatively small and have a low impact on soil P cycling.

4.4. Overall effect of cover crops on soil P cycling

Our results suggest a small role for legume-driven soil P mobilization in less-weathered soils, despite significant changes to rhizosphere properties with fava bean. Relatively low legume biomass in legume-cereal mixtures (Brennan and Boyd, 2012), smaller root allocation in legumes vs. cereals (Pearse et al., 2006) and lower root length per unit of root biomass for legumes (Nuruzzaman et al., 2005) will further attenuate any legume effect on soil P dynamics. Therefore, our results are consistent with those of Kuo et al. (2005) and Takeda et al. (2009) who found no indication of P mobilization due to cover crops in less-weathered soils.

In contrast, P uptake via cover crops was substantial, although the dominance of cereals in mixtures may delay residue decomposition, increase soil P_{org} and reduce soil P availability compared to other plants that stimulate efficient soil P recycling – e.g., *Phacelia tanacetifolia* (Eichler-Loebermann et al., 2008). However, as residue C:P was lower than 200:1 for cereals, net microbial immobilization should be minimized during decomposition (Nachimuthu et al., 2009). As a result, cereals will have a larger role in increasing P cycling rates than legumes in these less-weathered soils.

Table 2
Delta soil P fractions (rhizosphere − bulk) at the Russell Ranch Sustainable Agricultural Facility (RR) in 2012 and 2013. Mean and 95% confidence intervals of the mean are presented. Different letters represent statistical differences computed with a Tukey HSD test performed after a two-way ANOVA. Interaction terms were never significant. n.s. = $p > 0.05$,** = $p < 0.01$,*** = $p < 0.001$.

Year	Variable	Management	Plant			ANOVA			Tukey HSD			
			Cereals	Fava bean	Vetch	Factor	F	Sig	Plant	Sig	Management	Sig
2012		Organic	−3 ± 3	−7 ± 1	−2 ± 17	Plant	4.8	***	Cereals	A		
	Δ Ca−P	Mixed	−5 ± 0	−6 ± 1	−4 ± 3	Management	0.6	n.s.	Fava bean	B		
	(mg P g^{-1} soil)	Unfertilized	−2 ± 1	−11 ± 2	−6 ± 4				Vetch	AB		
		Organic	0 ± 5	5 ± 4	2 ± 5	Plant	16.0	***	Cereals	B		
	Δ Al/Fe−P	Mixed	−1 ± 2	8 ± 1	2 ± 3	Management	0.7	n.s.	Fava bean	A		
	(mg P g^{-1} soil)	Unfertilized	−1 ± 0	6 ± 2	0 ± 2				Vetch	B		
		Organic	6 ± 1	11 ± 5	8 ± 3	Plant	21.4	***	Cereals	C	Organic	ab
	Δ P_{org}	Mixed	4 ± 4	18 ± 0	12 ± 4	Management	7.0	**	Fava bean	A	Mixed	a
	(mg P g^{-1} soil)	Unfertilized	2 ± 0	10 ± 5	7 ± 2				Vetch	B	Unfertilized	b
2013		Organic	−2 ± 24	−20 ± 3	−4 ± 5	Plant	10.0	**	Cereals	A		
	Δ Ca−P	Mixed	−1 ± 4	−14 ± 6	2 ± 2	Management	0.8	n.s.	Fava bean	B		
	(mg P g^{-1} soil)	Unfertilized	−1 ± 3	−12 ± 4	−1 ± 1				Vetch	A		
		Organic	2 ± 4	4 ± 2	5 ± 4	Plant	1.3	n.s.			Organic	ab
	Δ Al/Fe−P	Mixed	3 ± 3	7 ± 5	6 ± 3	Management	6.4	**			Mixed	a
	(mg P g^{-1} soil)	Unfertilized	1 ± 2	1 ± 1	0 ± 1						Unfertilized	b
		Organic	5 ± 2	14 ± 2	14 ± 2	Plant	12.2	***	Cereals	B	Organic	a
	Δ P_{org}	Mixed	5 ± 1	17 ± 7	14 ± 8	Management	6.9	**	Fava bean	A	Mixed	a
	(mg P g^{-1} soil)	Unfertilized	3 ± 3	10 ± 5	4 ± 5				Vetch	A	Unfertilized	b

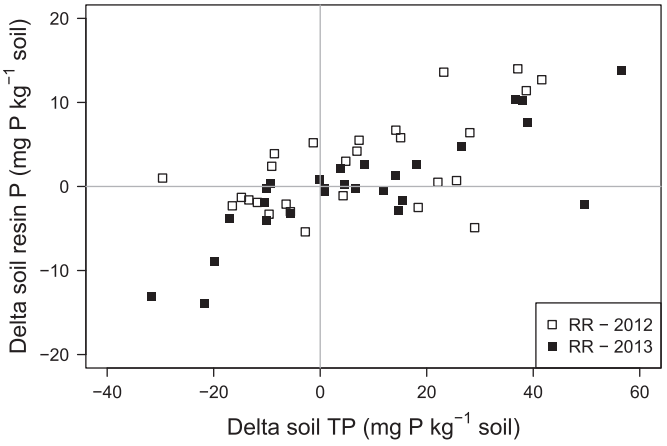

Fig. 4. Relationship between delta resin P (rhizosphere − bulk; mg P kg^{-1} soil) and delta total P (rhizosphere − bulk; mg P kg^{-1} soil) at the Russell Ranch Sustainable Agricultural Facility (RR) in 2012 (open squares) and 2013 (full squares). In 2012: $r = 0.61$, $p < 0.001$. In 2013: $r = 0.81$, $p < 0.001$.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2014.08.013.

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