



Increases of soil phosphatase and urease activities in potato fields by cropping rotation practices

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Abstract

Soil phosphatase and urease activities which are responsible for P and N mineralization, respectively, could be indicators of soil health for nutrient availability. In this work, we measured the activities of acid phosphatase, alkaline phosphatase, phosphodiesterase, and non-buffered and buffered urease in soils under rainfed and irrigated potato with different crop rotation managements. Compared with continuous potato (*Solanum tuberosum* L.) production, three-year rotation practices increased all soil enzyme activities in rainfed condition by 10 to 86%. Irrigation increased alkaline phosphatase, phosphodiesterase, and both unbuffered and buffered urease activities, but decreased acid phosphatase activity compared with no irrigation. Under rainfed management, microbial biomass C level was highly correlated with phosphatase and urease activities. When measured under buffered conditions, urease activity was highly correlated with rainfed potato yield. To the extent that urease is an indicator of plant N availability, this may reflect the influence of N availability on yield. This study showed that both cropping system and water management influence the activities of several enzymes considered important for plant uptake of N and P.

Key words: Phosphatase, urease, potato, cropping rotation, irrigation, soil enzymes, microbial biomass carbon.

Introduction

Soil enzymes play important roles in nutrient cycling. Therefore, their activities have been used as indices of soil fertility, quality, and health^{1,2}. For example, phosphatase, produced by microorganisms, increases P availability to plants due to conversion of organic P compounds into bioavailable inorganic phosphate^{3,4}. Similarly, urease converts organic N to inorganic N by hydrolysis of urea to ammonia⁵⁻⁷.

Sustainable cropping requires the knowledge of soil health⁸. Agricultural production, especially potato, in the New England Region of the USA has been declining in recent years. Study reveals that potato production has decreased by over 60,000 acres during the past 30 years⁹. Potato in the northeastern U.S. is usually grown in short (2- or 3-yr) rotations (rotations with wheat and barley), with extensive tillage and minimal crop residue return. The overall productivity of potato has not increased for several decades, despite the increased use of pesticides, nutrients and water. Thus, research is needed to examine the factors that constrain the potato production system. For this purpose, we established a field experiment in Presque Isle, Maine, in 2004, to identify the relative importance of selected factors that impact potato yield and quality. As soil enzymes are among these critical factors, in this work, we (1) evaluated the effects of crop rotation and irrigation on acid phosphatase (acPase), alkaline phosphatase (alPase), phosphodiesterase (diPase) and urease activities and

(2) correlated enzyme activities microbial biomass carbon (C) and potato yield.

Materials and Methods

Location and cropping systems: A long-term field experiment was established in 2004 at the USDA-ARS field research site in Presque Isle, Maine (Latitude 46°41'N, Longitude 68°2'W) on a Caribou sandy loam (Fine-loamy, isotic, frigid Typic Haplorthods). Treatments included a factorial combination of five cropping systems and two water management regimes (rainfed and irrigated), arranged in a randomized complete block design with five replications. Potato (cultivar Russet Burbank) was used in all cropping systems designed, and managed as:

- (1). Continuous Potato (PP): a non-rotation control.
- (2). Status Quo (SQ): 2-yr potato-barley (*Hordeum vulgare* L.) rotation. The barley was underseeded with red clover (*Trifolium pratense*), with primary tillage in the fall.
- (3). Disease Suppressive (DS): 3-yr rotation, with mustard (*Sinapis alba* L.) green manure followed by winter rapeseed (*Brassica napus*) (Yr 1), sudangrass (*Sorghumbicolor* L.) green manure (Yr 2) followed by winter rye (*Secale cereale* subsp. *cereale* L.) potato (Yr 3). The green manure crops were flail-mowed and incorporated. In pretrials, these green manure crops had been demonstrated to reduce soil-borne pathogens or weeds.
- (4). Soil Conserving (SC): 3-yr rotation that minimizes tillage and maximizes soil coverage. Barley underseeded with timothy

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(*Phleum pratense* L.) (Yr 1) timothy sod (Yr 2) potato (Yr 3) with mulch after harvest. The only tillage is during the potato year.

- (5). Soil Improving (SI): same as Soil Conserving, with composted manure added to each crop at the rate of 20 Mg ha⁻¹. This was used to rapidly increase soil organic matter and alter soil physical properties.

The experiments were conducted on 16 m x 4 m plots, each consisting of 4 rows. A standard agronomic row spacing of 0.95 m (between potato rows) and 35 cm (between potato plants within rows) was used for all experiments. Fertilizer was applied at the annual rate of 2240 kg ha⁻¹ of 10-10-10 (NPK) (fertilizer forms were ammonium nitrate, di-ammonium phosphate and potassium muriate) in bands approximately 5 cm to each side and 5 cm below the seed. This application rate was supposed to meet the growth requirements of potato and other rotation crops. Tubers were covered with soil by making ridges twice during the growing season. Weed management was accomplished by conventional tillage between rows at planting and the application of a mixture of herbicides consisting of metribuzin and metolochlor (Dupont Agricultural Products, Wilmington, DE, USA) before emergence at labeled rates. Tensiometers were placed to a depth of 15 cm in irrigated plots, and irrigation was applied when 25% of the tensiometers showed water level at 0.5 MPa. Irrigation was applied 3-5 times in each growing season, with 1.25 cm of water applied each time.

Soil sample collection: Soil samples were collected at 0-20 cm depth in May 2007 after a complete cycle of three-year crop rotation. Samples were collected from potato phases of the crop rotation from eight places within a plot with a hand probe (7 cm diameter), composited and sieved to 2 mm. A subsample was stored in sealed plastic bags at 4°C under field moist conditions. Soil pH was measured in water (1:2 ratio) (Table 1).

Phosphatase and urease activities: Phosphatase was measured by using established procedures³ with acPase activity determined at pH 6.5, alPase activity at pH 11.0 and diPase activity at pH 8.0. *p*-nitrophenyl phosphate was used as the substrate for both acPase and alPase, while *bis-p*-nitrophenyl phosphate was used as the substrate for diPase. Activity was expressed as mg *p*-nitrophenol (*p*-NP) released per g dry soil in 1-h incubation.

Urease activity was measured under non-buffered conditions pH 9.0, which is recognized as the optimum pH for urease activity⁵. Specifically, 400 mg of moist soil was mixed with 180 µL of deionized water (non-buffered conditions) or 50 mM Tris-HCl buffer (pH 9.0, buffered conditions) in the presence of 4 µL of toluene. The mixture was then incubated at 37°C for 20 h after addition of 20 µL of 0.2 M urea. Released NH₄-N was extracted with 0.8 mL of 100 ppm Ag₂SO₄ in 2.0 M KCl. After centrifuging for 5 min in a

microfuge, the extract (0.6 mL) was diluted with 11.4 mL of 2.0 M KCl. The NH₄-N concentration was analyzed in a Lachat Autoanalyzer (Lachat Instruments, Mequon WI) using a salicylate method (Lachat method#12-107-06-2-B). Controls were analyzed the same way, but urea was added following the addition of Ag₂SO₄/2.0 M KCl. The NH₄-N released by soil urease was calculated as the difference in NH₄-N between the testing and control samples. After 20-h incubation, urease activity was expressed as mg NH₄-N released per kg dry soil.

Microbial biomass C measurement: Soil microbial biomass C was determined based on the chloroform fumigation extraction method described by Horwath and Paul¹⁰. Briefly, field-moist soil (15 g dry weight equivalent) was fumigated with ethanol-free chloroform vapor for 5 days in a vacuum desiccator at room temperature without light. The unfumigated control was also kept in the dark in a desiccator. The fumigated and unfumigated soils were extracted with 75 mL of 0.5 M K₂SO₄ by shaking on a reciprocal shaker at 180 strokes per min for 1 h. After shaking, the soil suspension was centrifuged and filtered using Whatman No. 42 filter paper. Organic C in these filtrates was determined on a TOC analyzer. Soil microbial biomass C was calculated as the difference in organic C between fumigated and unfumigated soils.

Statistical analysis: The data analysis package in Microsoft Excel 2007 was used for statistical analysis. Data from five field replicates was used to calculate averages and standard errors. Single factor analysis of variance (ANOVA) was used to evaluate the effects of cropping system on soil enzyme activities and microbial biomass C for both rainfed and irrigated management. The Correlation Analysis Tool of Microsoft Excel was used to analyze correlation coefficients between enzyme activities, microbial biomass C and potato yield.

Results

Soil phosphatase activity under rainfed management: Soil in continuous potato under rainfed management had phosphatase activities of 691 mg *p*-NP kg⁻¹ dry soil for acPase, 116 mg *p*-NP kg⁻¹ dry soil for alPase, and 122 mg *p*-NP kg⁻¹ dry soil for diPase. All enzyme activities were higher under potato in crop rotations than in monocrop (PP) (Fig. 1). This increase was relatively greater for alPase and diPase than for acPase, although the specific activity of the acPase was 3-5 fold greater than alPase and diPase. For a given enzyme, similar activities were found in soils from the SQ, DS and SC cropping systems (Fig. 1). The most dramatic increase was observed in SI, with acPase, alPase, and diPase activities 28, 80 and 86% higher, respectively, than PP. The greater phosphatase activity in SI than in other crop rotation systems was probably the addition of compost to the SI system, these substantial increases in phosphatase activities are directly attributable to compost addition.

Soil phosphatase activity under irrigated management: Similar to soils under rainfed management, crop rotation increased soil phosphatase activities under irrigated management (Fig. 2). Irrigated soils in PP had phosphatase activities of 606 mg *p*-NP kg⁻¹ dry soil for acPase, 132 mg *p*-NP kg⁻¹ dry soil for alPase, and 128 mg *p*-NP kg⁻¹ dry soil for diPase. The greatest increase in all three enzymes was observed in soils from SI. Activities of soil

Table 1. Soil pH impacted by cropping rotation and and irrigation.

Rotation system	Rainfed fields		Irrigated fields	
	Average ¹	SD	Average	SD
PP	5.83	0.13	5.94	0.32
SQ	5.77	0.36	5.88	0.22
DS	5.72	0.38	6.06	0.18
SC	5.55	0.42	5.75	0.18
SI	5.62	0.14	6.08	0.40

¹: Average of five field replicates.

acPase, alPase, and diPase were 21, 90 and 85% greater, respectively, in SI than in PP. However, DS resulted in relatively greater increases in alPase and diPase under irrigation than under rainfed management (Fig. 2).

Urease activity under rainfed management: Cropping rotation did not significantly impact soil pH in the five rainfed soils (Table 1). The average soil pH in the five rainfed soils was 5.70 with SD of 0.11. Soil urease activity in PP ranged from 88 mg NH₄-N kg⁻¹ under non-buffered conditions to 110 mg NH₄-N kg⁻¹ under buffered conditions as urease has the maximum activity at pH 9.0⁵. Cropping system influenced soil urease activity, with greater difference between PP and SI (Fig. 3).

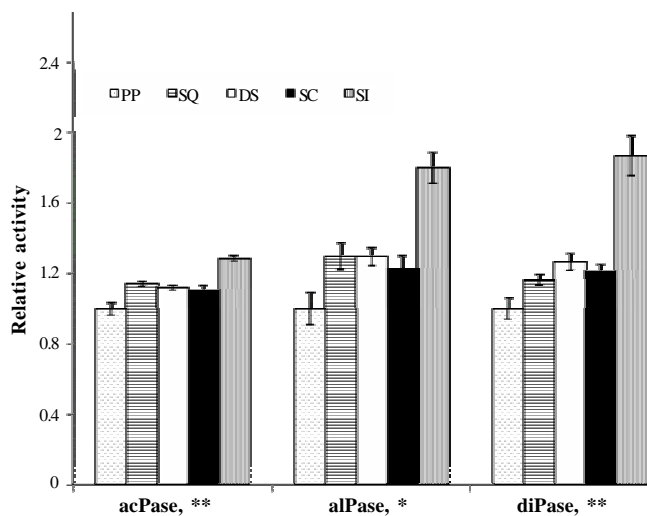


Figure 1. Effect of cropping system on soil phosphatase activities under rainfed management.

The relative activity is based on 0.691 ± 0.026 mg p-NP kg⁻¹ dry soil h⁻¹ for acPase, 0.116 ± 0.010 mg p-NP kg⁻¹ dry soil h⁻¹ for alPase, and 0.122 ± 0.011 mg p-NP kg⁻¹ dry soil h⁻¹ for diPase. Data are presented as the average of five field replicates with standard error bars. The symbol *, **, or *** indicates statistical significant impact of the cropping rotation on a specific enzyme activity at $\alpha=0.05, 0.01$ or 0.001 , respectively.

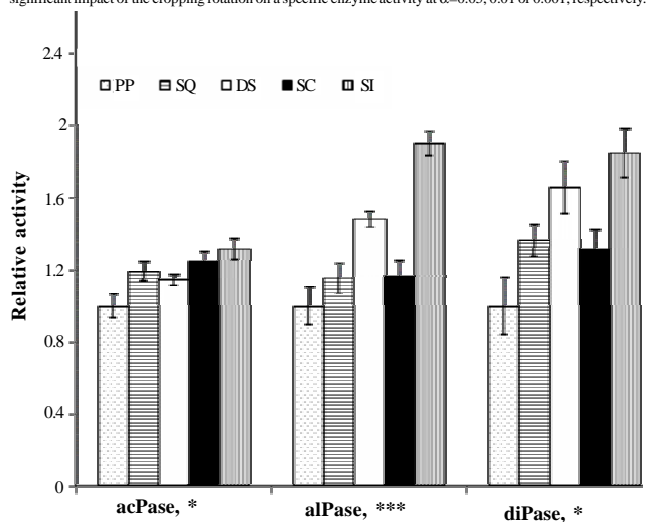


Figure 2. Effect of cropping system on soil phosphatase activities under irrigated management.

The relative activity is based on 0.606 ± 0.041 mg p-NP kg⁻¹ dry soil h⁻¹ for acPase, 0.132 ± 0.014 mg p-NP kg⁻¹ dry soil h⁻¹ for alPase, and 0.128 ± 0.020 mg p-NP kg⁻¹ dry soil h⁻¹ for diPase. Data are presented as the average of five field replicates with standard error bars. The symbol * or *** indicates statistical significant impact of the cropping rotation on a specific enzyme activity at $\alpha=0.05$ or 0.001 , respectively.

Urease activities under irrigated management: Irrigation consistently increased soil pH in the five rotation fields although the SD value of the field replicates for each soil was greater than the increase (Table 1). The average soil pH in the five irrigated soils was 5.94 with SD of 0.14. Higher soil urease activity in PP was observed with irrigation than with rainfed management, ranging from 100 mg NH₄-N kg⁻¹ under non-buffered conditions to 138 mg NH₄-N kg⁻¹ under buffered conditions. Also, while the highest relative urease activity was observed in SI under rainfed management (Fig. 3), the same was not observed under irrigated management (Fig. 4). Buffered soil urease activity was not significantly impacted by cropping system when irrigated (Fig. 4).

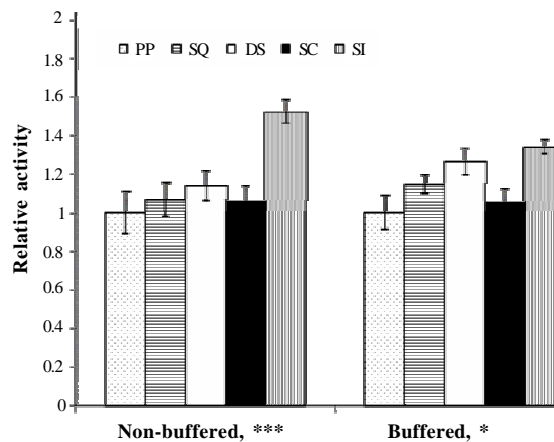


Figure 3. Effect of cropping system on soil urease activity under rainfed management.

The relative activity is based on 88 ± 10 mg NH₄-N kg⁻¹ dry soil released in 20 h for the activity measured under non-buffered conditions, and 110 ± 8 mg NH₄-N kg⁻¹ dry soil released in 20 h for the activity measured under buffered (pH 9.0) conditions. Data are presented as the average of five field replicates with standard error bars. The symbol * or *** indicates statistical significant impact of the cropping rotation on a specific enzyme activity at $\alpha=0.05$ or 0.001 , respectively.

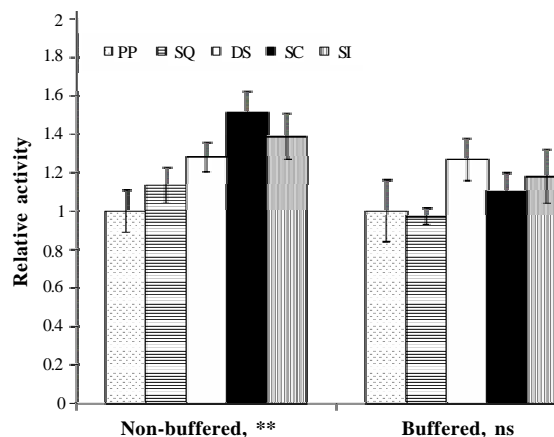


Figure 4. Effect of cropping system on soil urease activity under irrigated management.

The relative activity is based on 100 ± 11 mg NH₄-N kg⁻¹ dry soil released in 20 h for the activity measured under non-buffered conditions, and 138 ± 22 mg NH₄-N kg⁻¹ dry soil released in 20 h for the activity measured under buffered (pH 9.0) conditions. Data are presented as the average of five field replicates with standard error bars. The symbol ** or ns indicates statistical significant impact of the cropping rotation on a specific enzyme activity at $\alpha=0.01$ or no significant impact at $\alpha=0.05$, respectively.

Soil microbial biomass carbon: Soil microbial biomass C under rainfed management increased from 69 mg kg⁻¹ in the PP system to 155 mg kg⁻¹ in the SI system (Fig. 5). Irrigation increased microbial biomass C compared to rainfed management in PP (Fig. 5). However, when analyzed across all cropping systems, soil microbial biomass C did not differ among irrigated cropping systems (Fig. 5).

Discussion

Crop rotation effect on enzyme activities: Both phosphatase and urease activities occurred in soil from all five cropping systems. Among the three types of phosphatase activities measured, acPase activity was greatest, ranging from 500 to 1000 mg p-NP kg⁻¹ dry soil released in a 1-h incubation. Activities of alPase and diPase were comparable in a range from 70 to 340 mg p-NP kg⁻¹ dry soil. These results are consistent with previous observations on other soils^{3,11,12}. Specific phosphatase activity levels observed in our

study were also in the same range as those reported for soil from a century-long continuous winter wheat system in Oklahoma¹² but acid phosphatase activity was lower than the values (600-1000 mg p-NP kg⁻¹ dry soil h⁻¹) observed in a managed grassland in UK¹¹. Inorganic P fertilizer applied probably reduced acid phosphatase activity in cropland compared with grassland.

The incubation time reported for measuring urease activity varies from 30 min to 24 h^{6,7,13,14}. We used a 24-h incubation to measure urease activity and found it to range from 88 to 200 mg NH₄-N kg⁻¹ of dry soil. Assuming a linear relationship between measured activity and length of incubation, this would be similar to 8.6-30.6 mg NH₄-N kg⁻¹ of dry soil reported for 2-h incubation by Kandeler *et al.*¹³. Urease activity in the rhizosphere of potato plants under different soil management practices in Kentucky ranged from 42 to 92 mg NH₄-N kg⁻¹ dry soil 24 h⁻¹⁷. Whereas urease activity could be measured under either buffered or non-buffered conditions⁵, our data measured under non-buffered conditions had higher levels of statistical significance (Figs 3 and 4), even though the activity measured under buffered conditions was greater. Stronger correlations were found between urease activity and other parameters when measurements were made using non-buffered conditions for the rainfed soils, but not for the irrigated soils (Table 2). As there were no statistically significant difference in pH values between these rotation soils, the difference in these urease activities measured under non-buffered conditions should not be an artifact of pH effect, rather a true indicator of this enzyme activity present in these soils.

Potatoes require large amounts of soil P for tuber formation¹⁵, primarily because of the potato plant's low efficiency for acquiring soil P¹⁶. To increase P uptake, some plants can directly modify their rhizosphere to increase their access to previously unavailable soil P pools by developing more extensive root systems, exuding organic acids and phosphatases, and through association of roots with mycorrhizae. Furthermore, it is reasonable to hypothesize that these more P-efficient plants may improve soil P availability for subsequent crops by further enriching the soil with organic acids and phosphatase in root exudates and associated microorganisms.

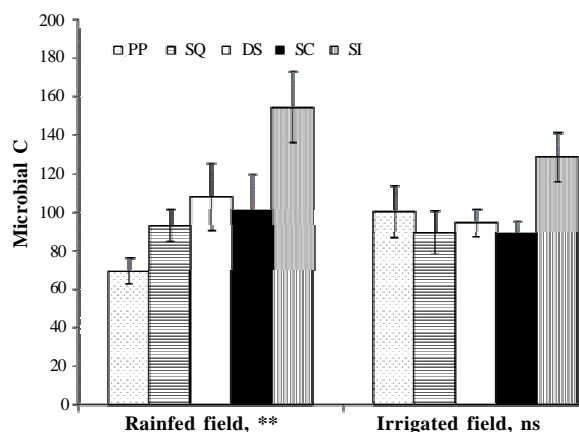


Figure 5. Soil microbial biomass C levels under rainfed and irrigated management.

Data represent the average of five field replicates with standard error bars. The symbol ** or ns indicates statistical significant impact of the cropping rotation on a specific enzyme activity at $\alpha=0.01$ or no significant impact at $\alpha=0.05$, respectively.

Table 2. Correlation coefficients among soil biochemical parameters and yield.

(A). Rainfed Management.

	Microbial C	acPase	alPase	diPase	Urease-u	Urease-b	Yield
Microbial C	1						
acPase ¹	0.960** ⁶	1					
alPase ²	0.980**	0.989**	1				
diPase ³	0.984**	0.945*	0.981**	1			
Urease-u ⁴	0.969**	0.917*	0.966**	0.992**	1		
Urease-b ⁵	0.879*	0.865 [^]	0.876 [^]	0.841 [^]	0.845 [^]	1	
Yield	0.852 [^]	0.855 [^]	0.870 [^]	0.831 [^]	0.848 [^]	0.972**	1

(B). Irrigated Management

	Microbial C	acPase	alPase	diPase	Urease-u	Urease-b	Yield
Microbial C	1						
acPase ¹	0.400	1					
alPase ²	0.801	0.689	1				
diPase ³	0.580	0.753	0.946*	1			
Urease-u ⁴	0.147	0.821 [^]	0.487	0.562	1		
Urease-b ⁵	0.329	0.365	0.715	0.762	0.556	1	
Yield	0.467	0.036	0.651	0.661	-0.203	0.574	1

1. acPase, acid phosphatase; 2. alPase, alkaline phosphatase; 3. diPase, phosphodiesterase; 4. Urease-u, urease activity measured under non-buffered conditions; 5. Urease-b, urease activity measured under buffered conditions (pH 9.0). 6. Symbol [^], *, and ** represents statistical significance at P=0.1, 0.05 and 0.01, respectively.

Previously, Tarafadar and Claassen¹⁷ have reported that organic P compounds serve as a P source for higher plants due to mineralization by phosphatases that are produced by plant roots and microorganisms. The moderate increase in phosphatase activities in the SQ, DS and SC systems (Fig. 1) could be partially attributed to the secretion of phosphatases from other crops rotated with potatoes in these systems. The greatest increase in phosphatase activity in the SI system (Fig. 1) was probably a result of compost addition. The percentage increase in alPase (80%) and diPase (87%) activities was greater than that of acPase (28%) in this system (Fig. 1).

As with phosphatase, urease is also produced by both plants and microorganisms, urease activity measured in these soils may likely represent the combined influence of both sources⁷. Under rainfed management, cropping system influenced soil urease activity (Fig. 3). Kandeler *et al.*¹³ reported soil urease activity to be lower in soils following potato compared to rotation crops of sugar beet, winter wheat, spring barley and alfalfa. It is possible that increased soil urease activity in the SQ, DS and SC systems compared with the PP system was a result of greater secretion of enzyme by other crops than potato in the rotation.

Irrigation impacts on soil enzyme activities: We calculated water management impacts on phosphatase, urease, and microbial biomass C by subtracting the value measured in a given rainfed cropping system from that measured in the corresponding irrigated cropping system. Lower levels of acPase activity were observed under irrigated than rainfed management (Fig. 6) However, irrigation increased alPase, diPase and urease activities (Fig. 6). To our knowledge, there are no published reports on the effects of irrigation on soil phosphatase and urease. However, Speir and Cowling¹⁸ did observe soil acid phosphatase activity to be negatively affected by soil moisture content over a 12-month period. Our observation that irrigation decreased microbial biomass C in the Soil Improving system (Fig. 6) may indicate that increased soil water retention associated with compost application may have led to soil water content that exceeded the optimal for soil microbial biomass. The differential responses to irrigation for soil enzymes and microbial biomass suggest that different mechanisms may influence their content and composition. For example, Kandeler *et al.*¹³ report that urease activity was mainly located in the 63-2 and 2-0.1 μm clay-size fractions whereas the coarse and fine sand particles accumulated disproportionately higher amounts of xylanase. The predominance of xylanase and urease in different particle size fractions depends apparently not only on the location of soil microorganisms and their substrate but also on the mechanisms of enzymes to adsorb and bind onto mineral and organic particles. There are contrasting reports on the location of acid phosphatase in soil. Rojo *et al.*¹⁹ observed that this activity is mainly associated with larger soil fractions (2000-100 μm) containing plant debris and less humified organic matter, but Marx *et al.*²⁰ report the highest accumulation of acid phosphatase activity in the 2-0.1 μm soil fraction. As compost addition in our Soil Improving system would be characterized by adding relatively large, less humified organic matter, the potentially direct impact of compost application on enzyme activity must be considered. In addition, the interactions of compost addition (i.e. increased soil water retention) and irrigation on decomposition, hydrolysis, and other processes should also be acknowledged.

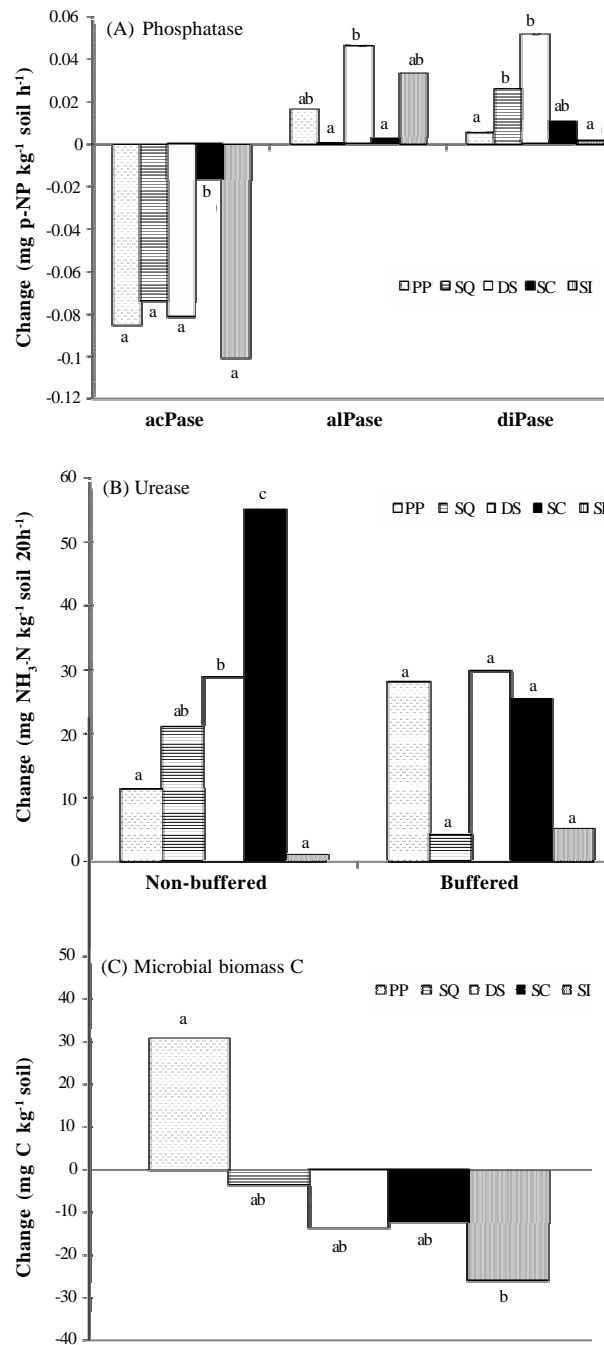


Figure 6. Effect of irrigation on soil biochemical parameters. The change is expressed as the value determined under irrigated management minus that observed under rainfed management. The same letter above or below a column indicates non-significant difference among cropping systems at the $\alpha=0.05$ level.

Correlations among biochemical parameters and yield: As phosphatase and urease activities may be useful indicators of soils potential to supply plant P and N, we analyzed the correlation among enzyme activities, microbial biomass C and yield (Table 2). Under rainfed management, microbial C was highly correlated with phosphatase and urease activities. When measured under buffered conditions, urease activity was highly correlated with rainfed potato yield (Table 2a). To the extent that urease is an indicator of plant N availability, this may reflect the influence of N availability

and potato yield under rainfed condition. It also suggests that urease determination under buffered conditions may be a better indicator of available N. While strong correlations were found under rainfed management, much weaker correlations were observed for irrigated management (Table 2b). Reasons for this observation can only be speculated with our current data. However, regardless of the particular mechanism(s) involved, it is clear that water management had a dramatic influence on soil enzymes and microbial biomass C in this study. Additional research already being conducted in these cropping systems and water management practices may provide further explanation of these relationships.

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