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# Water Treatment Residuals and Biosolids Co-applications Affect Phosphatases in a Semi-arid Rangeland Soil

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## Water Treatment Residuals and Biosolids Co-applications Affect Phosphatases in a Semi-arid Rangeland Soil

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Abstract: Co-application of biosolids and water treatment residuals (WTR) land has not been extensively studied but may be beneficial by sorbing excess biosolidborne or soil phosphorus (P) onto WTR, reducing the likelihood of off-site movement. Reduction of excess soil P may affect the role of specific P-cleaving enzymes. The research objective was to understand the long-term effects of single co-applications and the short-term impacts of repeated co-applications on soil acid phosphomonoesterase, phosphodiesterase, pyrophosphatase, and phytase enzyme activities. Test plots were  $7.5 \times 15$  m with treatments consisting of three different WTR rates with a single biosolids rate (5, 10, and 21 Mg WTR  $ha^{-1}$ ; 10 Mg biosolids ha<sup>-1</sup>) surface co-applied once in 1991 or reapplied in 2002. Control plots consisted of those that received no WTR-biosolids co-applications and plots that received only 10 Mg biosolids  $ha^{-1}$ . Plots were sampled to a 5-cm depth in 2003 and 2004, and soil phosphatases and phytase enzyme activities were measured. Soil phosphodiesterase activity decreased in WTR-amended plots, and pyrophosphatase activity decreased with increasing WTR application rates. In contrast, acid phosphatase and phytase activity increased with

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Address correspondence to James A. Ippolito, USDA-ARS-NWISRL, 3793 N 3600 E, Kimberly, ID 83341-5076. E-mail: jim.ippolito@ars.usda.gov WTR addition, with WTR application possibly triggering a deficiency response causing microorganisms or plants to secrete these enzymes. Biosolids and WTR co-applications may affect enzymatic strategies for P mineralization in this study site. Reductions in phosphodiesterase activity suggest less P mineralization from biomass sources, including nucleic acids and phospholipids. Increased acid phosphatase and phytase activities indicate that ester-P and inositol-P may be important plant-available P sources in soils amended with WTR.

Keywords: Acid phosphomonoesterase, phosphodiesterase, phytase, pyrophosphatase, water treatment residuals

#### **INTRODUCTION**

Water treatment residuals (WTR) and biosolids are both by-products from municipal treatment processes. Aluminum (Al)–based WTR are considered a waste product from drinking water treatment facilities. Alum  $[Al_2(SO_4)_3 \cdot 14H_2O]$  is the main component used in the treatment process for colloid destabilization, flocculation, and water clarification. Biosolids are a by-product of wastewater treatment. Both products have been extensively studied separately for their effects and benefits for land application as an alternative method of beneficial reuse.

The benefits of WTR soil application include increased organic carbon, improved structure, and increased water-holding capacity (Bugbee and Frink 1985; Elliott et al. 1990; Rengasamy, Oades, and Hancock 1980). The overwhelming concern with WTR land application, however, is the tremendous phosphorus (P) sorption on WTR amorphous metal oxides. When applied alone, WTR could significantly reduce plant-available P via surface adsorption. Several studies have shown plant P-deficiency symptoms or reduced yields associated with WTR application (Bugbee and Frink 1985; Heil and Barbarick 1989; Lucas et al. 1994). Harris-Pierce, Barbarick, and Redente (1993) studied the effects of co-application on aboveground plant biomass of four shortgrass prairie species and found no significant differences in biomass or tissue concentrations in any plant species. Ippolito, Barbarick, and Redente (2002) examined the effects of different combinations of WTR and biosolids on western wheatgrass and blue grama and showed that WTR reduced available P to both plant species. Ippolito, Barbarick, and Redente (2002) stated that unless a supplemental P source is supplied, such as biosolids, excessive WTR application rates should be avoided because of their adverse effect on P availability to plants.

Plants require P for energy storage and transfer. They absorb either  $H_2PO_4^-$  or  $HPO_4^{2-}$ , with these P species released and supplied by the dissolution/desorption from P-bearing minerals and the mineralization of

soil organic matter. Yet the contribution of soil organic P ( $P_o$ ) compounds to plant P nutrition is not well understood (Hays, Richardson, and Simpson 2000). Soil P transformations are influenced by factors such as plant species, soil type, and environmental conditions (Chen, Condron, and Davis 2004), with plant roots or soil microorganisms first hydrolyzing  $P_o$  substrates (Marschner 1995). Therefore, plant availability of soil organic P may be characterized by estimating the activity of indigenous phosphatase enzymes, which convert organic P to inorganic P. According to Tabatabai (1994), the general name *phosphatase* has been used to describe a broad group of enzymes that catalyze the hydrolysis of both ester and anhydride  $P_o$ .

Phosphomonoesterase enzymes comprise a large group of biocatalysts involved in hydrolysis of ester-linked organophosphorus compounds to orthophosphate (Sadowsky et al. 2006) and are often referred to as phosphatases. Acid phosphatase and alkaline phosphatase are predominant in acidic or basic soils, respectively (Eivazi and Tabatabai 1977; Juma and Tabatabai 1977, 1978). It has been noted that most microbial  $P_0$  will be rapidly degraded within hours or days of release by the phosphatase enzymes (Condron, Turner, and Cade-Menu 2005). Phosphodiesterase is known to degrade nucleic acids and phospholipids (Razzel and Khorana 1959). Indicative of phosphodiesterase activity, Turner, McKelvie, and Haygarth (2002) suggested the dominance of orthophosphate diesters in grassland soil solutions. Pyrophosphatase catalyzes the hydrolysis of pyrophosphate to orthophosphate (Tabatabai 1994), with activity greater in surface soils (Tabatabai and Dick 1979). Phytate is the most abundant identifiable  $P_0$  compound in soil, comprising up to 50% of total  $P_0$ (Anderson 1980). Hays, Richardson, and Simpson (1999) showed that plant roots possess some phytase activity, allowing soil phytate to be a potential source for plant-available P.

In this article, we examine both the long-term effects of a single coapplication of biosolids and WTR and the short-term impacts of a repeated co-application on soil acid phosphomonoesterase, phosphodiesterase, pyrophosphatase, and phytase enzyme activities. Our objective was to improve the understanding of land-use change impacts due to WTR– biosolid co-application on the role and activity of P-cleaving enzymes in soil.

#### MATERIALS AND METHODS

The city of Fort Collins, Col., owns the 40,000-ha Meadow Springs Ranch and utilizes it for beneficial biosolids land application. In August 1991, treatments were established on-site to assess short-term impacts of a single co-application of municipal biosolids and alum-based WTR on rangeland, shortgrass steppe soil, and vegetation. Test plots were 15  $\times$ 

15 m with treatments consisting of three different WTR rates (5, 10, and  $21 \text{ Mg ha}^{-1}$ ) co-applied with a single biosolids rate ( $10 \text{ Mg ha}^{-1}$ ). The biosolids were surface applied with a side-discharge manure spreader, WTR was applied by hand, and all treatments in 1991 were replicated four times (12 plots total) in a randomized complete block design. In October 2002, the original plots were split in half with one half receiving a second co-application at rates identical to the original 1991 rates. Soil sampling occurred in fall 2003 and 2004.

Climate data from both sampling years indicated that 2003 was drier than 2004 (NOAA 2003–2004). The majority of the 2003 precipitation came early in the year followed by a dry summer and fall, leading up to the October sampling. Precipitation in 2004 was distributed throughout the summer with slightly more than 7.6 cm in the 2 months leading up to the October sampling. The research area receives 33 to 38 cm of mean annual precipitation (NRCS 1980).

Biosolids and WTR were obtained from the city of Fort Collins, Col., wastewater and drinking water treatment facilities, respectively. Biosolids and WTR elemental composition were determined by perchloric–nitric–hydrofluoric–hydrochloric acid (HClO<sub>4</sub>-HNO<sub>3</sub>-HF-HCl) digestion (Table 1; Soltanpour et al. 1996) followed by elemental analysis using inductively coupled plasma–atomic emission spectrometry. Biosolids

*Table 1.* Fort Collins, Col., biosolids and WTR chemical composition in 1991 and 2002 as determined by HClO<sub>4</sub>-HNO<sub>3</sub>-HF-HCl digestion (Soltanpour et al. 1996)

Property	1991 biosolids	2002 biosolids	1991 WTR	2002 WTR
$K (mg kg^{-1})$	1896	420	4178	1785
$P(mgkg^{-1})$	16141	11350	550	545
$Fe (mg kg^{-1})$	4948	19050	19500	14576
$Cu (mg kg^{-1})$	547	162	44	36
$Zn (mg kg^{-1})$	772	254	30	33
Ni $(mg kg^{-1})$	19	5	10	6
Mo $(mgkg^{-1})$	16	1.9	1.4	0.4
$Cd (mg kg^{-1})$	5	0.6	0.1	0.1
$Cr (mg kg^{-1})$	40	6	17	8
Pb $(mg kg^{-1})$	119	7	2	< 0.05
$Ca (mgkg^{-1})$	28361	$ND^a$	3438	12474
Al $(mgkg^{-1})$	8618	12650	63300	59016
Organic N $(mg kg^{-1})$	41161	41750	3885	3485
$NO_3-N (mg kg^{-1})$	98	3	64	118
$NH_4-N (mg kg^{-1})$	3640	5442	51	9
рН	7.3	7.3	6.8	7.1
$EC (dS m^{-1})$	5.0	20.2	0.5	1.8

<sup>*a*</sup>ND, not determined.

NO<sub>3</sub>-N and NH<sub>4</sub>-N were determined following methods outlined by Mulvaney (1996), and pH (Thomas 1996) and electrical conductivity (EC; Rhoades 1996) were determined using a saturated paste extract.

The research site soil is classified as an Altvan loam, Aridic Argiustoll, 0–3% slopes. The Altvan series consists of deep, well-drained soils that formed in mixed alluvial deposits (NRCS 1980). Background soil characteristics have been described elsewhere (Sullivan et al. 2006). Soil samples were collected in October 2003 and 2004. Ten soil cores from the 0- to 5-cm depth were obtained from each plot and composited. Soils were sampled to a shallow depth because this zone was most likely impacted to a greater extent by surface co-application with no incorporation. In 2004, soil samples were collected from an adjacent study to gather control samples consisting of four plots that received no biosolids or WTR, four plots that received a single 10 Mg ha<sup>-1</sup> biosolids in 1991, and four plots that received a repeated  $10 \text{ Mg ha}^{-1}$  biosolids in 1991 and again in 2002. Soil sampling and compositing identical to the co-applied plots were performed. All soils samples were placed in a cooler and transported to Colorado State University, where they were air dried and passed through a 2-mm sieve prior to analysis.

### **Enzyme Assays**

Acid phosphomonoesterase, phosphodiesterase, and pyrophosphatase enzyme assays were conducted according to procedures outlined by Tabatabai (1994). A procedure for determining phytase enzyme activity was adapted from Engelen et al. (2001) and outlined as follows. Each soil sample was analyzed in duplicate along with two blanks. One gram of soil was placed in a 50-mL Erlenmeyer flask with 4 mL of buffer [30 g sodium acetate (NaOAc) + 10 g calcium chloride (CaCl<sub>2</sub>) + 0.1 g Tween  $20 L^{-1}$ ; pH 5.5] and 0.2 mL of toluene. The flasks were incubated for 5 min at 37 °C, followed by addition of 10 mL of phytic acid substrate (8.4 g phytic acid L<sup>-1</sup> adjusted to pH 5.5; P3168, Sigma Chemical Co., St. Louis, Mo.) and vortexing. Samples were incubated again at 37 °C for 65 min. Immediately after incubation, 4 mL of color stop mixture was added [2.5% ammonium molybdate (H<sub>24</sub>Mo<sub>7</sub>N<sub>6</sub>O<sub>24</sub>.4H<sub>2</sub>O) by weight + 0.06\% ammonium metavanadate (NH<sub>4</sub>VO<sub>3</sub>) by weight + 2.6 M HNO<sub>3</sub>  $L^{-1}$ ] and then swirled to completely mix. Samples were then filtered through Whatman no.2 filter paper, and phosphate content was determined colorimetrically at 415 nm. Standards were prepared containing 0, 5, 10, 15, 20, 25, and  $30 \text{ mg P L}^{-1}$ . Four mL of standard solution were placed into a 50-mL Erlenmeyer flask and incubated for 5 min at 37 °C, followed by addition of 10 mL of deionized water, vortexing, and incubation at 37 °C for 65 min. Four mL of color stop was added; solutions were swirled, filtered, and analyzed for P as previously described. Statistical tests were performed using the Proc GLM model in SAS version 9.1 (2002) with differences within each P fraction examined using ANOVA at a significance level ( $\alpha$ ) of 0.10.

#### RESULTS

The 2003 single or repeated co-applications did not affect acid phosphomonoesterase-cleaved P concentration, although a significant treatment by time interaction was observed in 2004 (Figure 1; Table 2). Acid phosphomonoesterase activity was significantly greater in the 2004 co-applied plots when compared to the control and biosolids-alone plots. Soils not receiving WTR had little to no measurable phosphomonoesterase activity.

In 2003, increases in the single WTR rates caused a decrease in phosphodiesterase-cleaved P content (Figure 2; Table 2). In contrast, no differences were observed for the 2003 repeated co-applications or for the 2004 single or repeated co-applications. Opposite of phosphomonoesterase, 2004 soils that received WTR showed significant reductions in



*Figure 1.* Phosphomonoesterase-cleaved P from plots of  $10 \text{ Mg ha}^{-1}$  biosolids coapplied with 5, 10, or 21 Mg ha<sup>-1</sup> WTR, 0–5 cm deep, 2003 and 2004. Single and repeated co-applications occurred in 1991 and 2002, respectively. The background control (0 Mg ha<sup>-1</sup>) and single and repeated 10 Mg biosolids ha<sup>-1</sup> soils were measured only in 2004. Error bars represent one standard error of the mean.

<b>Table 2.</b> Analysis of variance $(P > F)$ with significance determined at $\alpha = 0.10$
(italicized values) for the 2003 and 2004 single and repeated co-applications of
biosolids and WTR, and 2004 co-application data compared to either single/
repeated biosolids application or control soils $(n = 4)$

Analysis of variance	Phosphomonoesterase	Phosphodiesterase	Pyrophosphatase	Phytase
2003	P > F	P > F	P > F	P > F
$TRT \times time$	0.11	0.74	0.41	0.76
Swtr vs Rwtr	0.12	0.20	0.81	0.30
$\mathbf{S}_{\mathrm{wtr}}$	0.54	0.05	0.11	0.79
R <sub>wtr</sub>	0.54	0.72	0.32	0.37
2004				
$TRT \times time$	0.01	0.18	0.96	0.89
Swtr vs Rwtr	0.41	0.38	0.74	0.10
$S_{wtr}$		0.16	0.28	0.53
R <sub>wtr</sub>		0.18	0.03	0.25
Swtr vs Sbio	<0.01	0.23	0.15	0.08
Rwtr vs Rbio	<0.01	0.36	0.13	< 0.01
Swtr vs UC	< 0.01	0.06	0.64	0.22
R <sub>wtr</sub> vs UC	<0.01	0.05	0.24	< 0.01

*Note.* TRT =WTR treatment,  $S_{wtr}$  = single (1991) WTR application,  $R_{wtr}$  = repeated (2002) WTR application,  $S_{bio}$  = single (1991) biosolids application,  $R_{bio}$  = repeated (2002) biosolids application, UC = untreated control.

phosphodiesterase activity compared to the control soils. Enzyme activity under biosolid-only application was extremely variable.

Increasing WTR application rates, although not significant, generally led to a decrease in pyrophosphatase activity (Figure 3; Table 2). Most co-applications were no different than single or repeated biosolids application or the control. In 2004, however, increasing repeated co-application rates led to a decrease in pyrophosphatase activity. This observation suggests that pyrophosphatase activity was influenced by the increasing WTR addition.

In general, phytase activity decreased from 2003 to 2004 (Figure 4; Table 2). In 2004, phytase activity was significantly greater in the repeated co-applications than in the single co-applications, the repeated biosolids-only plots, and the control plots. Single 2004 co-applications were also greater than the single biosolids applications. Results indicate that WTR application had a positive effect on phytase activity.

#### DISCUSSION

The presence of acid phosphomonoesterase activity was primarily a WTR phenomenon, and although the pH of the soils used in the current study



*Figure 2.* Phosphodiesterase-cleaved P from plots of  $10 \text{ Mg ha}^{-1}$  biosolids coapplied with 5, 10, or 21 Mg ha<sup>-1</sup> WTR, 0–5 cm deep, 2003 and 2004. Single and repeated co-applications occurred in 1991 and 2002, respectively. The background control ( $0 \text{ Mg ha}^{-1}$ ) and single and repeated 10 Mg biosolids ha<sup>-1</sup> soils were measured only in 2004. Error bars represent one standard error of the mean.

ranged from 5.2 to 5.9, little activity was present in control or biosolidsamended soils. Chen, Condron, and Davis (2004) observed increased acid phosphomonoesterase activities in soils with pH values less than 7.0, as have others (Dick, Cheng, and Wang 2000; Turner and Haygarth 2005). Acid phosphomonoesterase, however, is known to be secreted by both plants and microorganisms (Tarafdar and Marschner 1994) under soil P-deficiency conditions, and therefore it was possible that any WTR addition may have triggered a deficiency response causing enzyme secretion. Margesin and Schinner (1994) associated high phosphomonoesterase activity with severe deficiency in available P. Deficiency conditions could have led to increased enzyme production, and in turn increased P solubility by releasing bound Po and by Po mineralization via increased hydrolytic cleavage (George et al. 2002). Jansson (1981) also showed that in acidified environments where aluminum concentrations are often increased, the production of acid phosphomonoesterase is enhanced. In support of this contention, soils under WTR application contained significantly greater Al concentrations as compared to control soils (Bayley 2006). Turner and Haygarth (2005) correlated high acid phosphomonoesterase activity and low phosphodiesterase activity to acidic soils. They suggested that a soil pH effect on the two



*Figure 3.* Pyrophosphatase-cleaved P from plots of  $10 \text{ Mg ha}^{-1}$  biosolids coapplied with 5, 10, or 21 Mg ha<sup>-1</sup> WTR, 0–5 cm deep, 2003 and 2004. Single and repeated co-applications occurred in 1991 and 2002, respectively. The background control (0 Mg ha<sup>-1</sup>) and single and repeated 10 Mg biosolids ha<sup>-1</sup> soils were measured only in 2004. Error bars represent one standard error of the mean.

phosphatases may be linked to dominant microorganisms present, plant exudation of phosphomonoesterase, or the rapid inactivation of phosphodiesterase in acidic soils.

Most soils that received WTR showed a reduction in phosphodiesterase activity compared to the background (control) soils. Lower phosphodiesterase activity may be a rate-limiting step in regulating organic P turnover in our system, as suggested by Turner and Haygarth (2005). Phosphodiesterase activity may also be inhibited by the presence of orthophosphate, ethylenediaminetetraacetic acid (EDTA), or citrate (Tabatabai 1994). Interestingly, the majority of fresh organic P inputs (i.e., biosolids) to soil contain phospholipids and nucleic acids, and phosphodiesterase is involved in the degradation of these compounds (Cosgrove 1967). Biosolids addition alone at this site resulted in greater bacterial biovolumes (Sullivan et al. 2006). Colvan, Syers, and O'Donnell (2001) and Parham et al. (2002) showed increased phosphodiesterase activity with farmyard manure addition to grassland or cattle manure application to continuous winter wheat, respectively, suggesting greater biological activity with manure addition. With co-application, WTR could have adsorbed P to a greater extent than biosolids could supply,



*Figure 4.* Phytase-cleaved P from plots of  $10 \text{ Mg ha}^{-1}$  biosolids co-applied with 5, 10, or 21 Mg ha<sup>-1</sup> WTR, 0–5 cm deep, 2003 and 2004. Single and repeated co-applications occurred in 1991 and 2002, respectively. The background control  $(0 \text{ Mg ha}^{-1})$  and single and repeated 10 Mg biosolids ha<sup>-1</sup> soils were measured only in 2004. Error bars represent one standard error of the mean.

causing a reduction in microbiological activity and thus lowering phosphodiesterase activity. Reductions in phosphodiesterase activity suggest less P mineralization from biomass sources, including nucleic acids and phospholipids. Lower microbial activity would also suggest that the increase in acid phosphomonoesterase activity was dominated by plant root enzyme exudation.

Increasing WTR application rates generally led to decreases in pyrophosphatase activity, with the single co-applications having greater enzyme activity as compared to repeated co-applications. Parham et al. (2002), using soil that had received manure application every 4 years for more than a century, showed increasing inorganic pyrophosphatase activity, whereas Deng and Tabatabai (1997) observed no correlation between inorganic pyrophosphatase activity and soil pH.

The decreasing trend in pyrophosphatase activity and significant decreases of activity in the 2004 reapplied plots may have been related to increasing single or repeated WTR rates, with WTR P sorption masking the effects of biosolids addition on pyrophosphatase activity. The WTR used in this study were Al-based, containing large quantities of Al (Table 1). Searle and Hughes (1977) observed no pyrophosphatase activity of mixed cultures

of soil microorganisms in the presence of Al. The authors also noted a promotion in pyrophosphatase activity in the presence of magnesium (Mg), Zn, cobalt (Co), manganese (Mn), and iron (Fe). Biosolids, such as those used in this study (Table 1), are known to contain such nutrients and trace metals. Dick and Tabatabai (1983) showed that at certain concentrations barium (Ba), calcium (Ca), cobalt (Co), Mg, Mn, nickel (Ni), and Zn promoted pyrophosphatase activity, and Fe and copper (Cu) decreased it. Other researchers have shown trace metal precipitation on aluminum (hydoxy)oxide surfaces (Scheckel and Sparks, 2001; Scheidegger, Lamble, and Sparks 1997; Scheidegger et al. 1998; Towle et al. 1997). Saha, Taniguchi, and Sakurai (2002) showed that the presence of prepared hydroxyaluminum greatly increased the adsorption of Zn and cadmium (Cd) at pH values of more than 5. Water treatment residuals are amorphous compounds with high surface area allowing for increased reaction (Ippolito et al. 2003; Makris et al. 2004). Thus, repeated, increasing application rates of WTR could have either reduced pyrophosphatase activity because of an increase in Al present or by adsorption of those trace metals that promote pyrophosphatase activity.

Phytase activity generally decreased from 2003 to 2004, but was greater than in control or biosolids-treated soils in 2004, indicating WTR application positively influenced phytase activity. Increased phytase activities, as with acid phosphomonoesterase, indicate that ester P and inositol P may be important sources of plant-available P in soils amended with WTR. Similar to our phosphomonoesterase results, the increased phytase enzyme activity may be due to WTR application triggering a deficiency response, causing plants or microorganisms to secrete the phytase. Li et al. (1997) used several tropical forage crops that were well adapted to P-deficient conditions and showed that these plants have the ability to not only secrete phytase but acid phosphatases as well. They suggested this to be a plant strategy for enduring P-deficient conditions. Although most plants studied exhibited phytase activity when grown under P-sufficient or P-deficient conditions, Li et al. (1997) also noted pronounced root phytase secretion from several plants grown under P-deficient conditions. They suggested that secretory phytase may provide a mechanism for certain plants to utilize inositol hexaphosphate in soil and may be a widespread adaptive function for some plants to grow in P-deficient soil conditions. Results from their study suggested that plant roots secret phytase independent of microbiological associations under P-deficient conditions.

#### SUMMARY AND CONCLUSIONS

Phosphatase enzymes are responsible for cleaving various organic P forms with a subsequent release of inorganic P to the environment. We studied the activity of four enzymes: phosphomonoesterase, phosphodiesterase, pyrophosphatase, and phytase in response to a single or repeated application of 5, 10, or 21 Mg WTR ha<sup>-1</sup> co-applied with 10 Mg biosolids  $ha^{-1}$ . We observed a reduction in phosphodiesterase activity in co-applied WTR-biosolids plots as compared to background (control) soils. Water treatment residuals are known to sorb excessive quantities of P, with the reduction in soil solution P probably causing a reduction in microbiological activity and thus lowering phosphodiesterase activity. Pyrophosphatase activity decreased with repeated WTR application and as compared to repeated biosolids application. Pyrophosphatase activity may have been reduced as a result of either an increase in soil Al, Al-based WTR addition, or WTR sorbing metals that promote pyrophosphatase activity. Conversely, phosphomonoesterase and phytase activity increased with WTR addition. The WTR addition may have created a P-deficiency-like system, most likely causing plant roots to respond with the release of both phosphomonoesterase and phytase into the soil environment.

In conclusion, co-applications of WTR and biosolids may have altered enzymatic strategies for P mineralization in this study site. Reductions in phosphodiesterase activity suggest less P mineralization from biomass sources, including nucleic acids and phospholipids. In contrast, increased acid phosphatase and phytase activities indicate that ester P and inositol P may be important sources of plant-available P in soils amended with WTR.

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