

Water Treatment Residuals and Biosolids Coapplications Affect Semiarid Rangeland Phosphorus Cycling

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Land coapplication of water treatment residuals (WTR) with biosolids has not been extensively researched, but the limited studies performed suggest that WTR sorb excess biosolids-borne P. To understand the long-term effects of a single coapplication and the short-term impacts of a repeated coapplication on soil P inorganic and organic transformations, 7.5- by 15-m plots with treatments of three different WTR rates with a single biosolids rate (5, 10, and 21 Mg WTR ha⁻¹ and 10 Mg biosolids ha⁻¹) surface coapplied once in 1991 or surface reapplied in 2002 were utilized. Soils from the 0- to 5-cm depth were collected in 2003 and 2004 and were sequentially fractionated for inorganic and organic P (P_o). Inorganic P fractionation determined (i) soluble and loosely bound, (ii) Al-bound, (iii) Fe-bound, (iv) occluded, and (v) Ca-bound P, while organic P fractionation determined (i) labile, (ii) biomass, (iii) moderately labile, (iv) fulvic acid, (v) humic acid, and (vi) nonlabile associated P_o. Pathway analysis showed that humic, fulvic, and nonlabile P_o did not play a role in P transformations. Biomass P_o and moderately labile P_o contributed to the transitory labile P_o pool. Labile P_o was a P source for Fe-bound and WTR-bound inorganic phases, with the Fe-bound phase transitory to the occluded P sink. The Al-bound phase additionally contributed to the occluded P sink. The Ca-bound phase weathered and released P to both the Fe-bound and WTR-bound P phases. Overall, the WTR fraction, even 13 yr after the initial application, acted as the major stable P sink.

Abbreviations: β , standardized pathway coefficients; P_o, organic phosphorus; WTR, water treatment residuals.

Water treatment residuals (WTR) and biosolids are both by-products from municipal treatment processes. Aluminum-based WTR are considered a waste product from drinking water treatment facilities. Alum [Al₂(SO₄)₃·14H₂O] 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 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 C, improved structure, and increased water-holding capacity (Bugbee and Frink, 1985; Elliott et al., 1990; Rengasamy et al., 1980). The overwhelming concern with WTR land application is the tremendous P sorption on WTR amorphous metal (hydr)oxides. When applied alone, WTR

could significantly reduce plant-available P via surface adsorption, leading to plant P deficiency symptoms or reduced yields (Bugbee and Frink, 1985; Heil and Barbarick, 1989; Lucas et al., 1994; Ippolito et al., 2002). Water treatment residuals, however, can be utilized in areas prone to off-site P movement.

Novak and Watts (2004) studied WTR use in coastal sandy soils where long-term manure application created an environment with excessive P concentrations. They found that WTR significantly increased the soil P adsorptive capacity. Dayton and Basta (2005a) found that WTR sorptive capacity can be estimated to optimize its use in high P risk situations. A strong correlation existed between measured WTR Al oxide concentration and maximum P sorption. Ippolito et al. (2003) found that WTR sorbed 1.25% P by weight due to a large amount of microporosity and thus a high WTR surface area. Makris et al. (2004) found similar results, noting that WTR sorbed nearly 10,000 mg P kg⁻¹ WTR within 10 d and lost only 0.2 to 0.8% after 80 d, asserting that micropore-bound P will remain sorbed in circumneutral pH aqueous media. The Ippolito et al. (2003) and Makris et al. (2004) studies found micropore P extremely resistant to desorption, leading to long-term stability and suggesting the acceptance of WTR for beneficial reuse when applied to or with high-P-bearing materials.

Soil coapplication studies of WTR and biosolids are limited. Ippolito and Barbarick (2006) added 0 to 10 g of WTR to 10 g of high-P-bearing biosolids-amended soil and then extracted P using five different methods. Bray-I and Mehlich-III extracts, measures

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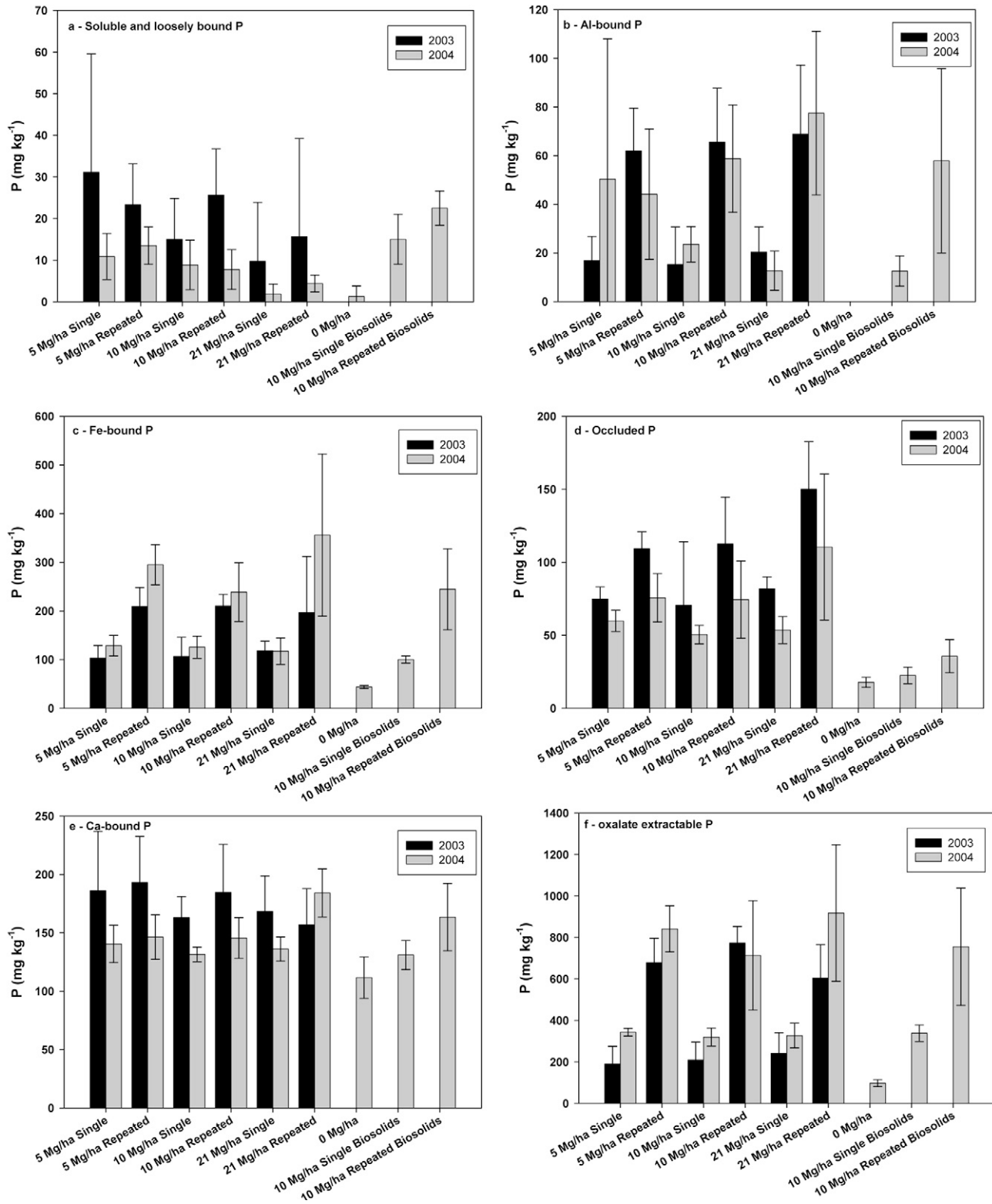


Fig. 1. Soil (a) soluble and loosely bound P, (b) Al-bound P, (c) Fe-bound P, (d) occluded P, (e) Ca-bound P, and (f) oxalate-extractable P from plots of 10 Mg ha⁻¹ biosolids coapplied with 5, 10, or 21 Mg ha⁻¹ water treatment residuals, 0- to 5-cm depth, 2003 and 2004. Single and repeated coapplications occurred in 1991 and 2002, respectively. The background control (0 Mg ha⁻¹), single, and repeated 10 Mg biosolids ha⁻¹ soils were measured only in 2004. Error bars represent one standard error of the mean.

of P plant availability, showed significant P decreases with increasing WTR rate and reduced the overall risk of off-site P movement to low according to the Colorado Phosphorus Index Risk Assessment (Sharkoff et al., 2003). They also found significant decreases in water-extractable P with increasing WTR applica-

tion and suggested using a water extract to predict P availability in WTR-amended soils. Harris-Pierce et al. (1993) studied the effects of coapplication on aboveground plant biomass of four shortgrass prairie species and found no significant differences in biomass or tissue concentrations in any plant species. In a follow-up study,

Ippolito et al. (2002) examined the effects of different combinations of WTR and biosolids on western wheatgrass [*Pascopyrum smithii* (Rydb.) Barkworth & D. R. Dewey] and blue grama [*Bouteloua gracilis* (Kunth) Lag. ex Griffiths] and showed that WTR reduced the plant-available P to both species. Neither study, however, observed any plant P deficiency symptoms and Ippolito et al. (2002) suggested that coapplication can aid municipalities dealing with excessive biosolids-borne P. Although these studies support the concept of WTR beneficial reuse, the long-term stability of the WTR-P complex was not researched.

Biosolids land application programs often target an agronomic rate based on soil test N concentration and crop N requirements. This application method tends to oversupply P. Thus, biosolids coapplication with WTR mechanistically is a sound practice in terms of sorbing excess biosolids-borne P. Our study objectives were to understand both the long-term effects of a single coapplication of biosolids and WTR and the short-term impacts of a repeated coapplication on soil P biogeochemistry. We used sequential extraction techniques to determine the dominant inorganic and organic P pools and an oxalate extract to determine P associated with the amorphous WTR fraction. Transformations between organic and inorganic pools were determined using pathway analysis. Our hypothesis was that single and repeated WTR-biosolids coapplications would show similar P fractionation patterns, and based on previous findings (Makris et al., 2004; Ippolito and Barbarick, 2006), we further hypothesized the amorphous WTR fraction to dominate all soil inorganic P fractions.

MATERIALS AND METHODS

In August 1991, 15- by 15-m test plots were established at the 10,500-ha Meadow Springs Ranch (40°53'46" N, 104°52'28" W) owned by the city of Fort Collins, CO, with treatments consisting of three different WTR rates (5, 10, and 21 Mg ha⁻¹) coapplied with a single biosolids rate (10 Mg ha⁻¹). Both materials were surface applied with no incorporation. Biosolids were applied with a side-discharge spreader, WTR by hand, and all treatments were replicated four times in a randomized complete block design. In October 2002, the original plots were split in half. One half received a second surface (no incorporation) application using the same treatments at the original rates.

Biosolids and WTR were obtained from the city of Fort Collins wastewater and drinking water treatment facilities, respectively. Biosolids and WTR elemental composition were determined by HClO₄-HNO₃-HF-HCl digestion (Table 1; Soltanpour et al., 1996) followed by elemental analysis using inductively coupled plasma-atomic

Table 1. Background soil analysis and 1991 and 2002 biosolids and water treatment residual (WTR) analysis, Fort Collins, CO, Meadow Springs Ranch. All values are expressed on a dry-weight basis.

Property	Background soil	Biosolids		WTR	
		1991	2002	1991	2002
K, mg kg ⁻¹	2770	1900	420	4180	1780
P, mg kg ⁻¹	353	16100	11400	550	545
Fe, mg kg ⁻¹	10030	4948	19050	19500	145
Cu, mg kg ⁻¹	9.6	550	160	44	36
Zn, mg kg ⁻¹	37	770	250	30	33
Ni, mg kg ⁻¹	7	20	5	10	6
Mo, mg kg ⁻¹	0.1	16	1.9	1.4	0.4
Cd, mg kg ⁻¹	0.7	5.0	0.6	0.1	0.1
Cr, mg kg ⁻¹	12	40	6	17	8
Pb, mg kg ⁻¹	8.6	120	7	2	<0.05
Ca, mg kg ⁻¹	2538	28360	ND†	3438	12470
Al, mg kg ⁻¹	8626	8618	12650	63300	59020
Organic N, mg kg ⁻¹	1545	41160	41750	3885	3485
NO ₃ -N, mg kg ⁻¹	1.2	98	3	64	120
NH ₄ -N, mg kg ⁻¹	3.9	3600	5400	51	9.0
pH	5.5	7.3	7.3	6.8	7.1
Electrical conductivity, dS m ⁻¹	0.2	5.0	20	0.5	1.8

† Not determined

emission spectrometry. Nitrate N and NH₄-N were determined following methods outlined by Mulvaney (1996), and pH (Thomas, 1996) and electrical conductivity (Rhoades, 1996) were determined using a saturated paste extract.

Climate data from both sampling years indicated that 2003 was drier than 2004 (Table 2; National Oceanic and Atmospheric Administration, 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 >9.1 cm in the 2 mo leading up to the October sampling. The research area receives 330 to 380 mm of mean annual precipitation (NRCS, 1980).

The cattle-grazed Meadow Springs Ranch is a semiarid, shortgrass steppe rangeland community dominated by perennial grasses including blue grama and western wheatgrass. The research site soil is classified as an Altvan loam (fine-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Aridic Argiustoll), 0 to 3% slopes. The Altvan series consists of deep, well-drained soils that formed in mixed alluvial deposits (NRCS, 1980). Soil samples were collected in October 2003 and

Table 2. monthly precipitation, minimum and maximum temperature data for Fort Collins, CO, Meadow Springs Ranch, 2003 and 2004.

Month	2003			2004		
	Precipitation	Min. temperature	Max. temperature	Precipitation	Min. temperature	Max. temperature
	cm	°C		cm	°C	
Jan.	0.13	10.5	-7.5	0.28	6.5	-9.4
Feb.	0.79	4.2	-10.3	0.28	5.4	-9.3
Mar.	2.57	12.0	-3.2	0.38	16.6	-2.5
Apr.	0.58	17.5	0.5	2.57	15.9	1.0
May	4.95	17.7	3.3	2.72	23.2	6.2
June	3.58	23.5	9.2	4.42	24.0	9.4
July	0.30	33.3	14.2	1.78	28.9	12.3
Aug.	2.69	30.1	13.2	3.45	26.7	10.3
Sept.	0.48	23.3	5.6	5.74	23.9	7.1
Oct.	0.08	21.7	1.8	1.85	17.0	1.8
Nov.	0.56	8.6	-5.7	0.51	9.5	-5.7
Dec.	0.36	7.2	-8.9	0.00	7.6	-8.2
Total	17.1			24.0		

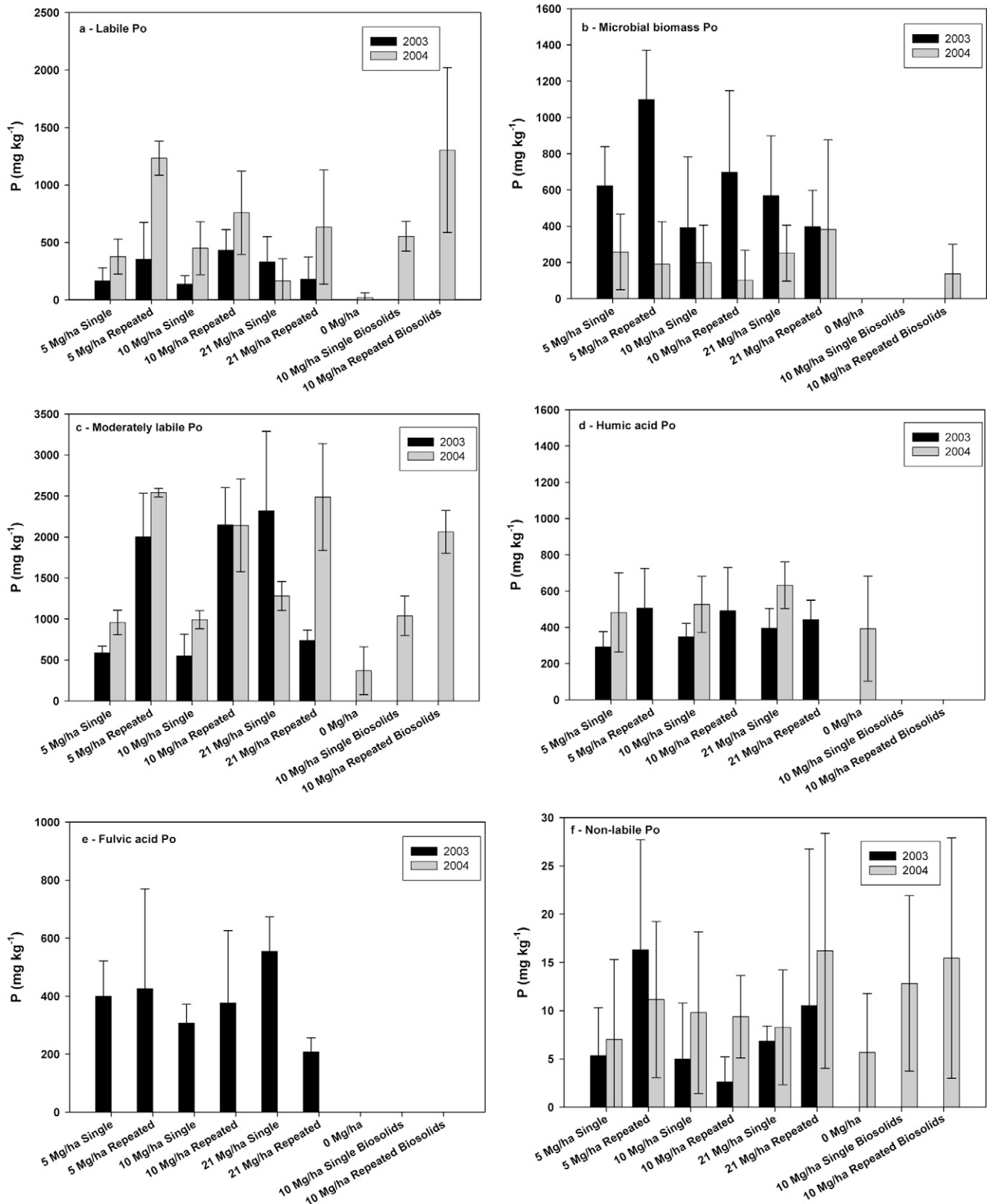


Fig. 2. Soil (a) labile organic P (P_o), (b) microbial biomass P_o , (c) moderately labile P_o , (d) humic acid P_o , (e) fulvic acid P_o , and (f) nonlabile P_o from plots of 10 Mg ha⁻¹ biosolids coapplied with 5, 10, or 21 Mg ha⁻¹ water treatment residuals, 0- to 5-cm depth, 2003 and 2004. Single and repeated coapplications occurred in 1991 and 2002, respectively. The background control (0 Mg ha⁻¹), single, and repeated 10 Mg biosolids ha⁻¹ soils were measured only in 2004. Error bars represent one standard error of the mean.

2004. Ten soil cores from the 0- to 5-cm depth were obtained from each subplot and composited. In 2004, soil samples were collected from an adjacent study to gather control samples from four plots that received no biosolids or WTR, four plots that received 10 Mg ha⁻¹ biosolids in

1991, and four plots that received 10 Mg ha⁻¹ biosolids in 1991 and again in 2002. Soil sampling and compositing was identical to the coapplied plots. All soil samples were placed in a cooler and transported to

Colorado State University, air dried, and passed through a 2-mm sieve before analysis.

Inorganic Soil Phosphorus Fractionation

Inorganic P sequential fractionation was performed according to methods outlined by Kuo (1996). The inorganic P extraction procedure identified soluble and loosely (1 mol L⁻¹ NH₄Cl), Al (hydr)oxide surface bound (0.5 mol L⁻¹ NH₄F), Fe (hydr)oxide surface bound (0.1 mol L⁻¹ NaOH), occluded (0.3 mol L⁻¹ Na₃C₃H₆O₇ + 1 mol L⁻¹ NaHCO₃ + Na₂S₂O₄; within the matrices of retaining components or minerals [Evans and Syers, 1971]), and the Ca-bound P (0.25 mol L⁻¹ H₂SO₄) fraction. Soils were washed and centrifuged twice with saturated NaCl between each step to ensure complete removal of P associated with a particular phase, with the NaCl solution added to the previous filtrate. All extracts were filtered through a 0.2- μ m membrane before colorimetric P determination (882-nm wavelength), following a modified ascorbic acid procedure (Rodriguez et al., 1994). Modifications were made to the occluded P fraction due to insufficient color development following a procedure outlined by Weaver (1974).

Oxalate Extractable Phosphorus

The oxalate [(NH₄)₂C₂O₄] extractable fraction identifies P adsorbed to amorphous, noncrystalline, or poorly ordered Al and Fe oxides, unlike the assumed mostly crystalline Al (hydr)oxide surface bound P extracted during the inorganic P sequential fractionation. The oxalate-extractable fraction should essentially be associated with the WTR, and the procedure outlined by Loeppert and Inskeep (1996) was used. Following extraction, all solutions were centrifuged, decanted, passed through a 0.2- μ m membrane, and analyzed for P using inductively coupled plasma-atomic emission spectrometry.

Organic Soil Phosphorus Fractionation

Organic P (P_o) sequential fractionation was performed according to methods outlined by Zhang and Kovar (2002), separating pools into labile organic ([0.5 mol L⁻¹ NaHCO₃ + K₂S₂O₈] - 0.5 mol L⁻¹ NaHCO₃), microbial biomass ([CHCl₃ + 0.5 mol L⁻¹ NaHCO₃ + K₂S₂O₈] - [0.5 mol L⁻¹ NaHCO₃ + K₂S₂O₈]), moderately labile ([1.0 mol L⁻¹ HCl + K₂S₂O₈] - 1.0 mol L⁻¹ HCl), fulvic acid ([0.5 mol L⁻¹ NaOH, acidified to pH 0.2] + K₂S₂O₈), humic acid ([0.5 mol L⁻¹ NaOH + K₂S₂O₈] - fulvic acid), and nonlabile (ashed at 550°C + 1.0 mol L⁻¹ H₂SO₄) associated P. All extracts were filtered through a 0.2- μ m membrane before P determination as described above.

Phosphorus Cycling and Statistical Analysis

To develop a conceptual P biogeochemical cycle within the coapplied plots (control or biosolids-only data not included), the differences between all 2003 and 2004 inorganic and organic sequential P fractionation data were analyzed by first using Pearson correlation matrices at a probability level of $P = 0.10$. Significant correlation coefficients were then used as initial coefficients of linear structure equations subjected to optimization in pathway analysis (Zheng et al., 2002). Pathway analysis evaluates corresponding changes in soil P fractions and requires the development of a conceptual model. The PROC CALIS procedure (SAS Institute, 2002) determined the relative contributions to endogenous (dependent) variables by several exogenous (independent) variables or other endogenous variables, essentially deriving pathway coefficients (Zheng et al., 2002). This procedure also tests the fit of the model. The following model criteria (Hatcher, 1994) were followed: absolute values of entries in the normalized residual matrix should not exceed 2.00; the

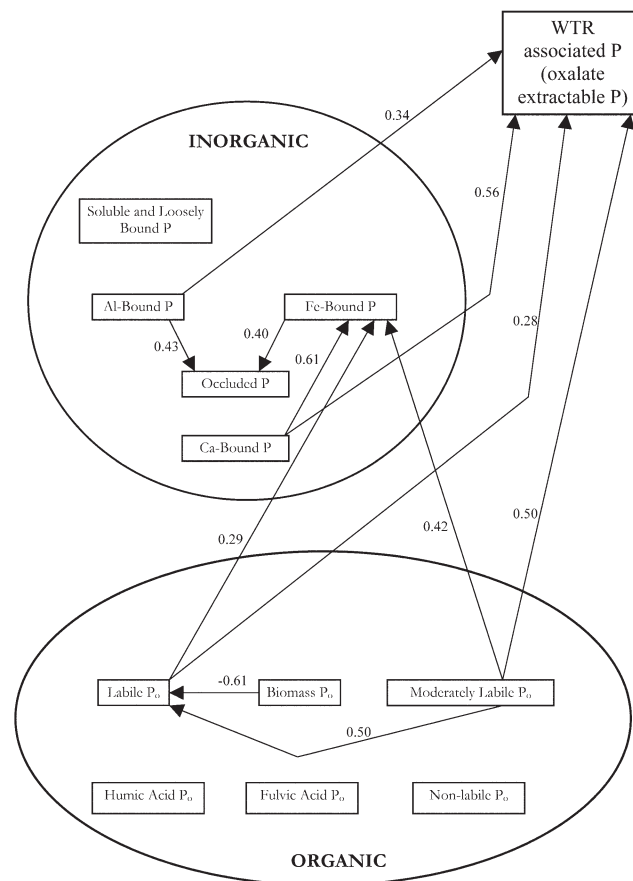


Fig. 3. Significant relationships between P fractions in the 0- to 5-cm depth under biosolids-water treatment residuals (WTR) coapplication. Pathway coefficients are standardized.

P value associated with the model χ^2 test should exceed 0.05, and the closer to 1.00 the better; the comparative fit index and the non-normed fit index should both exceed 0.9, and the closer to 1.00 the better; the R^2 value for each endogenous variable should be relatively large compared with what typically is obtained in research with these variables; and the absolute value of the t statistics for each path coefficient should exceed 1.96 with standardized path coefficients nontrivial in magnitude (i.e., absolute values should exceed 0.05). Standardized pathway coefficients (β) were used to compare which independent variables had the largest effect on dependent variables. All other statistical tests were performed using Proc GLM in SAS Version 9.1 (SAS Institute, 2002), with differences within each P fraction examined by ANOVA at $P = 0.10$.

RESULTS AND DISCUSSION

Inorganic Soil Phosphorus Fractionation

Individual inorganic P fractions are presented in Fig. 1a through 1e. Increasing WTR applications caused a reduction in the 2004 soluble and loosely bound inorganic fraction. Trends were similar between the single and repeated coapplications, supporting our hypothesis that single and repeated WTR-biosolids coapplications would show similar P fractionation patterns. Cox et al. (1997) noted reduced P availability resulting from a decrease in loosely bound P, probably due to P retention on WTR Al compounds. In further support, the 2004 single or repeated WTR applications contained lower soluble and loosely bound P than the single or repeated biosolids-only treatments.

Table 3. Mean change and Pearson's correlation coefficients (only coefficients significant at $P \leq 0.10$ are shown) for difference in inorganic P and organic P (P_o) fractions (from 2003 to 2004) in the 0- to 5-cm soil depth following single and repeated applications of water treatment residuals (WTR) and biosolids.

WTR treatment	mg kg ⁻¹										Pearson's correlation coefficients				
	Soluble and loosely bound P	Al-bound P	Fe-bound P	Occluded P	Ca-bound P	WTR-bound P	Labile P _o	Biomass P _o	Moderately labile P _o	Fulvic acid P _o	Humic acid P _o	Nonlabile P _o			
5, single	-20.2	33.5	25.4	-15.0	-45.6	154.2	212.0	-365.3	371.8	-399.3	189.9	1.7			
10, single	-6.2	8.2	18.6	-20.1	-31.5	110.4	313.0	-194.5	442.8	-307.3	178.0	4.8			
21, single	-7.9	-7.6	-1.0	-28.3	-32.1	86.1	-13.5	-145.7	541.8	-207.7	189.0	-2.3			
5, repeated	-9.8	-17.8	85.5	-33.8	-46.8	162.2	879.2	-907.7	539.0	-426.2	576.1	-5.2			
10, repeated	-17.9	-6.8	28.2	-38.2	-39.0	-59.6	323.6	-595.6	-8.9	-376.3	263.2	6.7			
21, repeated	-11.3	8.6	159.3	-39.8	27.3	313.9	301.1	-186.1	167.6	-554.6	669.8	9.4			
Nonlabile P _o	-0.43	-	-	-	-	-	-	-	-	-	-	1.00			
Humic acid P _o	-	-	0.50	-	-	0.41	0.44	-	-	-	-	1.00			
Fulvic acid P _o	-	-	-	-	-	-	-	-	-	-	-	1.00			
Moderately labile P _o	-	0.47	0.49	0.64	-	0.73	0.39	-	1.00	-	-	-			
Biomass P _o	-	0.37	-	0.38	0.40	0.31	-0.52	1.00	-	-	-	-			
Labile P _o	-	-	-	-	-	0.36	1.00	-	-	-	-	-			
WTR-bound P	-	0.51	0.69	0.46	0.44	1.00	-	-	-	-	-	-			
Ca-bound P	-	-	0.52	-	1.00	-	-	-	-	-	-	-			
Occluded P	-	0.53	0.51	1.00	-	-	-	-	-	-	-	-			
Fe-bound P	-	-	1.00	-	-	-	-	-	-	-	-	-			
Al-bound P	-	1.00	-	-	-	-	-	-	-	-	-	-			
Soluble and loosely bound P	1.00	-	-	-	-	-	-	-	-	-	-	-			

Repeated coapplications led to an increase over single coapplications in Al-bound, Fe-bound, and occluded P content (Fig. 1b, 1c, and 1d). Cox et al. (1997) noted increased Al-bound and Fe-bound P in the soil surface following WTR surface application. In our study, the observed P increase in the Al and Fe fractions could be attributed to the repeated biosolids application. Maguire et al. (2000) found that biosolids additions led to increases in both Al- and Fe-bound P when compared with untreated control soils. In comparison, occluded P levels can be attributed to WTR. While background occluded P concentrations were present and some increases were associated with biosolids addition alone, there were overall increases observed with WTR addition. Interestingly, the Al-bound, Fe-bound, and occluded P fractions showed no significant treatment effect nor did they change significantly from 2003 to 2004, again supporting our original hypothesis that P fractionation trends would be similar between single and repeated coapplications. This suggests that while there may be slow shifts to and from these pools, these three fractions are relatively stable in our system.

Overall, the inorganic Ca-bound P fraction generally decreased from 2003 to 2004 (Fig. 1e), and could be attributed to increased rainfall in 2004 which caused dissolution of Ca-P soil mineral phases. Calcium was present in the biosolids and thus was added to the soil with each biosolids addition. In addition, soil from the control plots contained a moderate level of Ca-bound P. Using scanning electron microscopy with energy dispersive spectroscopy, Ippolito and Barbarick (unpublished data, 2007) found evidence of Ca phases (i.e., Ca-P, CaCO₃) in these soils even though soil pH values ranged from 5.2 to 5.9.

Oxalate-Extractable Phosphorus

The noncrystalline Al and Fe fraction is one of the most important inorganic fractions in WTR studies because the majority of WTR-borne Al and Fe are in the amorphous, noncrystalline form. Amorphous WTR forms have the propensity to sorb a tremendous amount of P (Ippolito et al., 2003; Makris et al., 2004). In general, the oxalate-extractable P fraction increased from 2003 to 2004 (Fig. 1f). The 2004 single or repeated coapplication oxalate-extractable Al content (2520 and 6390 mg kg⁻¹ on average, respectively) significantly increased compared with the control (770 mg kg⁻¹) and single or repeated biosolids-only applications (990 and 1510 mg kg⁻¹, respectively), while the 2004 single coapplication Fe content (850 mg kg⁻¹ on average) was greater than the control (680 mg kg⁻¹) or biosolids-only application (730 mg kg⁻¹). Although Fig. 1f indicates that biosolids alone are contributing to P bound in the amorphous phase, WTR application caused a marked increase in the amorphous Al and to a lesser extent Fe phases present. Increased precipitation in 2004 possibly led to an increase in mineralization, immobilization, and weathering, and thus greater P flow in the system. This, in turn, should have led to a greater chance for P sorption to occur onto WTR if WTR were encountered by P, further emphasizing the importance of WTR as a P sink. In a comparison between agricultural and forest soils, Simard et al. (1995) suggested that amorphous Fe and Al (inorganic NaOH-extractable P) were important sinks for added and mineralized P in agricultural soils. Zheng

et al. (2002) noted the inorganic NaOH-extractable P fraction acted as a sink for inorganic P, probably through the formation of amorphous and crystalline Al and Fe phosphate formation.

Oxalate-extractable P was similar within 2003 single, 2003 repeated, 2004 single, and 2004 repeated coapplications, supporting our original hypothesis that trends would be similar between single and repeated coapplications. Repeated coapplication had greater P in both 2003 and 2004 compared with the single coapplication, suggesting a P shift from either the organic, inorganic, or both fractions to the oxalate-extractable fraction. These findings are consistent with other studies (Dayton and Basta, 2005a; Ippolito et al., 2003; Makris et al., 2004) that have observed significant P sorption to amorphous Al in WTR. In a laboratory study, Dayton and Basta (2005b) blended WTR with biosolids and noted reductions in CaCl₂-extractable P, illustrating the adsorptive capacity of WTR. In our study, no observable trends were present, which indicated a WTR treatment effect, and most concentrations were similar to those soils that received only biosolids. Both biosolids-alone applications (single or repeated) had significantly greater P concentrations compared with soil that received no treatments, indicating that biosolids were the main contributor of P in the coapplied soils.

The oxalate-extractable P, a representative of P sorbed to WTR, contained a greater amount of inorganic P than the other inorganic fractions studied, supporting our initial hypothesis that this fraction would dominate all inorganic fractions. In addition, the WTR acted as a long-term (>12-yr) stable P sink, as observed in the single, 1991 coapplication. Agyin-Birikorang et al. (2007) noted similar results, showing that Al-based WTR immobilized P and remained stable 7.5 yr following the initial land application.

Organic Soil Phosphorus Fractionation

In comparison to the overall inorganic P decrease, the overall organic P fraction increased from 2003 to 2004 by 247 mg kg⁻¹. Differences were also evident between the single and repeated coapplications (Fig. 2a–2f). Zheng et al. (2002) showed that mineral fertilizer applications tended to transform labile inorganic P into more stable P_o. This increase suggests net immobilization and may be attributed to the presence of biosolids, increased 2004 precipitation, which possibly promoted plant growth and thus increased P in the overall organic fraction, or increased soil bacteria activity. Similarly, Sui et al. (1999) was not able to absolutely distinguish between organic P increases from biosolids additions vs. additions from increased plant production as a result of the biosolids. Adjacent to our study site, Sullivan et al. (2006) showed that soils that received a repeated application of 21 or 30 Mg biosolids ha⁻¹ had greater bacterial biovolumes. Other researchers have also shown increased microbial biomass with biosolids application under semiarid conditions (Barbarick et al., 2004; García-Gil et al., 2004; Pascual et al., 1999).

Trends within individual organic fractions were lacking for both single and repeated coapplications. In general, labile P_o increased from 2003 to 2004 and increased from single to repeated coapplication (Fig. 2a). This was probably due to the increased precipitation in 2004 leading to greater plant growth and microbial activity, and to the recent addition of biosolids adding this fraction to the system. In comparison, the 2004 background soil had a very low P_o concentration. Sui et al.

(1999) found increased NaHCO₃-extractable P_o, comparable to labile P_o in our study, as affected by biosolids application in the top 5 cm of soil.

Overall, biomass P_o decreased from 2003 to 2004 during the sampling period (Fig. 2b). Biomass P_o is an easily labile organic fraction that can release P to the inorganic fraction. The decrease during the sampling period could be due to increased mineralization of this fraction with P lost to the inorganic soluble and loosely bound fraction, and subsequently either taken up by plants and bound to the amorphous, non-crystalline fraction (i.e., WTR). Zhang and MacKenzie (1997) suggested that these labile P_o phases can act as both sinks and sources for organic and inorganic P phases, respectively.

Comparable to the labile P_o, the moderately labile and humic acid P_o fractions increased from 2003 to 2004 and from single to repeated coapplications (Fig. 2c and 2d). Moderately labile P_o was also greater than the control but similar to biosolids addition alone, suggesting that biosolids addition under coapplication conditions contributed to the moderately labile organic P fraction. Sui et al. (1999) found increased NaOH-extractable P_o, comparable to moderately labile P_o in our study, as affected by biosolids application in the top 5 cm of soil.

Fulvic acid P_o was present in 2003 but not 2004 (Fig. 2e). This decrease may be associated with the increased 2004 precipitation, increased weathering, and thus increased microbial activity possibly transforming fulvic acid to more resistant humic acids. According to the polyphenol theory (Stevenson, 1994), the breakdown of plant biopolymers into monomeric structural units occurs first. Units can polymerize enzymatically or spontaneously to produce humic molecules of increasing complexity. Highly resistant, nonlabile P_o concentrations were relatively low (<16 mg kg⁻¹) and were not affected by increasing single or repeated WTR application or by year (Fig. 2f).

Phosphorus Biogeochemical Cycling

Trends between 2003 and 2004 for the single or repeated application were similar and therefore the data were combined and analyzed for both correlation and pathway analysis. Mean change for each fraction and treatment and significant Pearson's correlation coefficients for the difference between the 2003 and 2004 data for the 11 P fractions are shown in Table 3. Correlation analysis may offer a logical approach to data analysis when variables are jointly affected because of external influences, yet correlation itself is associated with descriptive techniques (Steel and Torrie, 1980). It does not necessarily indicate cause and effect (Zheng et al., 2002). Significant correlation coefficients may be due to noncausal relationships and vice versa, and thus to analyze cause-and-effect relationships correctly, path analysis is recommended (Johnson and Wichern, 1988). Significant correlation coefficients were used, however, as initial coefficients subjected to pathway analysis.

Significant pathway relationships between the organic, inorganic, and amorphous WTR pools are shown in Fig. 3. Based on the best-fit model, Fe-bound P, occluded P, labile P_o, and WTR-associated P (i.e., oxalate-extractable P) were designated as dependent variables, while Al-bound P, Ca-bound P, biomass P_o, and moderately labile P_o were designated independent variables. Humic, fulvic, and nonlabile P_o did not appear to play a role in P transformations. These fractions are moderately labile

to highly resistant, and thus relatively stable. Zheng et al. (2002) and Beck and Sanchez (1994) showed these fractions to be sinks, not sources, of P. Zhang and MacKenzie (1997) showed that the highly resistant organic fraction was a sink for P supplied by NaOH-extractable P_o , essentially from humic compounds.

Biomass P_o and moderately labile P_o transferred P to the labile P_o pool. Phosphorus from these moderately resistant organic fractions, via increased biological weathering and enzymatic activity in 2004, may have been cleaved and released to the various inorganic pools. Addition of biosolids may also have added moderately resistant organic P forms, as suggested by Ajiboye et al. (2004). Biomass P_o decreased from 2003 to 2004, probably due to mineralization, but there was less of a decrease at the higher WTR rates (Table 3). Possibly WTR P sorption limited the amount of microbial activity at higher WTR rates. Opposite, labile P_o increased from 2003 to 2004 but less of an increase was evident at the higher WTR rates. Labile P_o , an intermediate pool, acted as a P source for Fe-bound and WTR-bound inorganic phases, with the Fe-bound phase transitory to the occluded P sink. Thus, although biomass P_o decreased and contributed to the labile P_o pool ($\beta = -0.61$), greater increases in the labile fraction were not evident due to its transitory nature. In addition, if P were released from the organic to inorganic pools, this form would initially be present as a soluble inorganic species. An observed increase in the soluble and loosely bound phase was not evident, however, as this fraction was probably easily utilized by plants and microorganisms, or quickly bound by other inorganic soil phases. Similarly, Campbell et al. (1986) applied monoammonium phosphate in combination with increasing manure applications to a semiarid soil and found no significant change in labile P_o .

Moderately labile P_o increased from 2003 to 2004 yet positively contributed to the labile P_o fraction ($\beta = 0.50$). Zhang and MacKenzie (1997) observed an increase in moderately labile P_o forms, suggesting that this pool is a sink for biological immobilization of soil inorganic P. Our finding suggests that although immobilization occurred in this fraction, some moderately labile forms were degraded and released into the labile organic pool. Schmidt et al. (1996) suggested that, as fertilizer P rates exceed plant removal, excess P is supposed to reduce phosphorylase activity and consequently decrease the mineralization of moderately labile P_o . Biosolids application at 10 Mg ha⁻¹ could have supplied excessive P but the repeated WTR application could have sorbed this P and thus less moderately labile P_o was observed at the higher WTR repeated rates (Table 3). If WTR does adsorb excess P, this system would act more like an unfertilized system. Beck and Sanchez (1994) showed significant decreases in P_o pools with time under unfertilized systems and assumed the changes to be associated with mineralization. The moderately labile P_o fraction also positively correlated with the Fe-bound and WTR-bound P fractions ($\beta = 0.42$ and 0.50, respectively), with both inorganic fractions increasing with time. Thus, the moderately labile P_o pool, directly or indirectly through degradation to labile P_o forms, transferred P to the Fe- or WTR-bound phases. Zheng et al. (2002) and Beck and Sanchez (1994) used pathway analysis to study organic P mineralization to resin P (essentially the soluble and loosely bound form) and suggested that mineralized P_o maintained resin-P levels. Although our findings show no path between organic pools

and the soluble and loosely bound phase, P would first become available as this inorganic pool and then be transformed, sorbed, and incorporated into other pools.

The Al-bound phase, as with the Fe-bound phase, additionally contributed to the occluded P sink. The occluded phase includes P within the matrices of retaining components or minerals (Evans and Syers, 1971), is relatively insoluble and resistant to weathering, and comprises primary and secondary soil minerals or is physically encapsulated. Thus this phase should act as a sink, as in our model. Pathway coefficients for Al- and Fe-bound P to occluded P were positive ($\beta = 0.43$ and 0.40, respectively). Overall, both the Al-bound and occluded phases tended to decrease from 2003 to 2004 (Table 3) and thus the positive coefficient. The Fe-bound phase increased while the occluded phase decreased with time, yet the coefficient was positive. This probably was due to the Fe-bound phase being a transitory pool in the flow of P from Ca-bound P, labile P_o , and moderately labile P_o phases. The Ca-bound phase weathered and released P to both the Fe-bound and WTR-bound P phases.

CONCLUSIONS

Changes in inorganic or organic P fractions, regardless of single or repeated WTR and biosolids coapplications, showed similar trends and supported our original hypothesis. Our hypothesis was that single and repeated WTR-biosolids coapplications would show similar P fractionation patterns, and based on previous research we hypothesized the amorphous WTR fraction to dominate all soil inorganic P fractions. The overall inorganic P decreased slightly from 2003 to 2004; however, the overall organic P fraction increased by 247 mg kg⁻¹ between years, probably due to net immobilization attributed to the presence of biosolids, increased 2004 precipitation promoting increased plant growth, or increased soil bacteria activity. Of all inorganic P pools, the WTR fraction acted as the major inorganic P sink, supporting our hypothesis that this phase would dominate all inorganic phases. Phosphorus from the decomposition of labile and moderately labile P_o pools and from the weathering of the Ca-bound phase contributed to the WTR-bound fraction. Both single and repeated WTR applications increased the concentration of oxalate-extractable P, increasing the amount of amorphous Al present and increasing the surface area for reaction to occur. This finding supports the hypothesis that the WTR phase would dominate all inorganic phases, and as suggested by other researchers, P sorption to amorphous Al as a result of WTR application will provide long-term sorption stability.

Biosolids land application programs often target an agronomic rate based on soil test N concentration and crop N requirements. This application method tends to oversupply P. Our research findings suggest that biosolids coapplication with WTR is both chemically and mechanically a sound practice in terms of sorbing and retaining excess biosolids-borne P both in the short and long term. Thus, coapplication of WTR with biosolids or other high-P-bearing materials should benefit ecosystems where the potential for off-site P movement is present.

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