Water Treatment Residuals and Biosolids Long-Term Co-Applications Effects to Semi-Arid Grassland Soils and Vegetation

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Water treatment residuals (WTRs) and biosolids are byproducts from municipal water treatment processes. Both byproducts have been studied separately for land application benefits. There are possible environmental benefits of WTRs and biosolids co-application but these studies are limited. Our objectives were to determine relative long-term (13–15 yr) effects of a single and short-term (2–4 yr) effects of repeated WTR-biosolids co-applications on soil chemistry, microbiology, and plant community structure in a Colorado semiarid grassland. Only relative changes associated between co-applications were studied, as we assumed WTR application would only occur if used as a management practice. Three WTR rates (5, 10, and 21 Mg ha^{-1}) were surface co-applied (no incorporation) with a single biosolids rate (10 Mg ha^{-1}) once in 1991 (long-term plots) and again in 2002 (short-term plots). Soil 0- to 8-, 8- to 15-, and 15- to 30-cm depth pH, electrical conductivity (EC), NO_3–N, NH_4–N, total C, and total N were not affected by WTR application in 2004, 2005, or 2006. Ammonium-bicarbonate diethylenetriaminepentaacetic acid (AB-DTPA)- extractable soil Al was unaffected by WTR application, but extractable P and Mo decreased with increasing WTR rate because of WTR adsorption. Plant tissue P and Mo content decreased with specific plant species and years due to adsorption to WTR; no deficiency symptoms were observed. Plant community composition and cover were largely unaffected by WTR application. Soil microbial community structure was unaffected by WTR co-application rate (total ester-linked fatty acid methyl ester [EL-FAME] concentrations ranged from 33.4 to 54.8 nmol g^{-1} soil), although time since biosolids-WTR application affected a subset of microbial community fatty acids including markers for Gram-positive and Gram-negative bacteria. Overall, WTR-biosolids co-applications did not adversely affect semiarid grassland ecosystem dynamics.

Abbreviations: AB-DTPA, ammonium-bicarbonate diethylenetriaminepentaacetic acid; EC, electrical conductivity; EL-FAME, ester-linked fatty acid methyl ester; PCA, principal component analysis; WTR, water treatment residual.

FOREST, RANGE & WILDLAND SOILS

Water treatment residuals and biosolids are both byproducts from municipal treatment processes. Aluminum-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 in the water purification treatment process used to destabilize and flocculate colloids, creating WTR. Biosolids are a byproduct of wastewater treatment. Both byproducts have been studied separately for their effects and benefits for land application. Because WTR have the propensity to strongly adsorb P (Ippolito et al., 2003; Makris et al., 2004), land co-application may be advantageous to municipalities as a means of reusing high P-bearing biosolids in an environmentally sound manner (Ippolito et al., 1999). However, soil co-application studies of WTR and biosolids are limited.

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Harris-Pierce et al. (1993) studied the short-term effects of WTR-biosolids co-application on soils and aboveground plant biomass of four shortgrass prairie species. Aboveground biomass and canopy cover of individual plant species were not affected by increasing WTR rate (5, 10, and 21 Mg ha^{-1}) co-applied with a single biosolids rate (10 Mg ha^{-1}). The authors noted a decrease in fringed sage (*Artemesia frigida*) and blue grama (*Bouteloua gracilis*) P and Mo plant tissue content with increasing WTR rate. Harris-Pierce et al. (1993) also noted little change in soil elemental content with soil depth associated with increasing co-applications.

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.] A. Love) and blue grama and showed that WTR reduced plant-available P to both species. No visual P deficiencies were observed, and the authors suggested that co-application can aid municipalities dealing with excessive biosolids-borne P. Ippolito and Barbarick (2006) added WTR to a high P-bearing biosolids-amended soil, which resulted in significant P decreases with increasing WTR rates. Although these studies support the concept of WTR beneficial reuse concomitant with biosolids usage, the long-term benefits of the WTR-biosolids co-applications were not researched.

Agyin-Birikorang et al. (2007) added WTR to heavily manured soils, noting that Al-based WTR immobilized P and remained stable 7.5 yr following initial land application. In a similar study, Bayley et al. (2008a) focused on long-term WTR-biosolids co-application effects on P cycling in semiarid rangelands. Using pathway analysis, the authors showed that even after 13 yr following initial co-application, WTR still acted as the major stable P sink.

The Bayley et al. (2008a) study focused primarily on P transformations associated with short-term and long-term WTR-biosolids co-applications. Longer-term studies on soils, plant diversity and productivity are necessary to accurately assess the lasting environmental impacts of co-applying these materials on rangeland ecosystem stability. For example, 12 yr after initial application, Sullivan et al. (2006a) noted differences in semiarid rangeland plant and soil microbial communities as affected by biosolids treatments alone. Reasons for persistent effects were indicative of a successional shift from a community of low nutrient availability and tight nutrient cycling, to one with more readily available resources and decreased need for symbiotic arbuscular mycorrhizal fungi associations.

In the current study we focused our efforts on describing relative changes in soil chemistry, soil microbiology, and plant community composition as affected by WTR in long-term single or short-term repeated WTR-biosolids co-applications to a Colorado semiarid grassland. Control plots which had received either 0 Mg ha^{-1} of WTR or biosolids, or 10 Mg ha^{-1} biosolids only (identical rate as used in our study), were not established as part of this study. However, others have compared soil, plant, and microbial community structural changes in the WTR-biosolids co-amended soils to biosolids-only amended soils and non-amended control soils from plots of an adjacent study (Bayley, 2006; Bayley et al., 2008a).

Bayley et al. (2008a) noted that the lowest WTR-biosolids co-application rate (5 Mg WTR ha^{-1} + 10 Mg biosolids ha^{-1}) was comparable with a 10 Mg biosolids ha^{-1} application rate (from an adjacent study) in terms of P fractionation dynamics. The authors also found that the majority of P fractionation data collected from co-applied plots were greater than a true control (i.e., received no application of WTR or biosolids, again from an adjacent study). Bayley (2006) observed few differences between either the lowest single co-application rate and single biosolids application, or lowest repeated co-application rate and repeated biosolids application in terms of soil pH, EC, inorganic C, total C and N, Olsen and Mehlich-III extractable P. However, most soil constituent differences were observed between the single or repeated co-application and control soil. On an adjacent set of research plots, Sullivan et al. (2006a) found that biosolids application at 10 Mg ha^{-1} increased several soil chemical constituents and significantly affected the soil microbial and plant community composition as compared with a control (0 Mg ha^{-1}). These findings tell us that biosolids, not WTR, were responsible for differences between the lowest WTR (5 Mg ha^{-1}) application rate co-applied with a constant biosolids rate (10 Mg ha^{-1}) compared with control soil (0 Mg ha^{-1} of any treatment), and support our contention to focus efforts on describing relative short- and long-term changes in the WTR-biosolids co-amended ecosystem with regards only to WTR application.

MATERIALS AND METHODS

Experimental Design, Biosolids and WTR Analyses, Site Characteristics

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, USA. Treatments consisted of three different Al-based WTR rates (5, 10, and 21 Mg ha^{-1}) co-applied with a single biosolids rate (10 Mg ha^{-1}), chosen to evaluate the viability of this land application method. 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. The entire test plot area was fenced to exclude cattle. In October 2002, the original plots were split in half. One-half received a second surface (no incorporation) co-application using the same biosolids and WTR treatments as the original rates. This resulted in a split–plot block design with rate as the main factor and co-application time (1991 or 2002) as the split factor. As with Sullivan et al. (2006a), long-term and short-term plots are defined as those which received WTR-biosolids co-applications either once in 1991 (long-term) or twice, both in 1991 and 2002 (short-term), respectively.

Biosolids and WTR were obtained from the city of Fort Collins, CO wastewater and drinking water treatment facilities, respectively. Soil was collected to a depth of 30 cm from areas outside the plots and were used to represent background soil conditions at the study site. Biosolids, WTR, and soil elemental composition were determined by $HClO_4$–HNO_3–HF-HCl digestion (Table 1; Soltanpour et al., 1996) followed by elemental analysis using inductively coupled plasma–atomic emission spectrometry (ICP–AES; Thermo Solutions IRIS Advantage high resolution axial ICP–AES; Thermo Jarrell Ash, Franklin, MA). Nitrate-N and NH_4–N were determined following methods outlined by Mulvaney (1996), and pH (Thomas, 1996) and EC (Rhoades, 1996) were determined using a saturated paste extract. Biosolids total N content was determined by a concentrated H_2SO_4 digestion (Bremner, 1996) and organic N content via subtraction of inorganic N species from total N. Regulated biosolids elemental constituents fell below the EPA 40 CRF Part 503 Table 1 limits (USEPA, 1993).

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 area receives 330 to 380 mm of mean annual precipitation (NRCS, 1980). The research site soil is classified as an Altvan loam (fine-loamy over sandy or san-

Table 1. Fort Collins, Colorado, USA Meadow Springs Ranch background soil, and 1991 and 2002 biosolids and water treatment residual (WTR) total elemental analysis as determined by $HClO_4$–HNO_3–HF-HCl digestion. All values are expressed on a dry weight basis.

Property	Background Soil	1991 Biosolids	2002 Biosolids	1991 WTR	2002 WTR
Al, mg kg^{-1}	8626	8618	12650	63300	59020
P, mg kg^{-1}	353	16100	12440	550	545
Cu, mg kg^{-1}	9.6	550	475	44	36
Zn, mg kg^{-1}	37	770	652	30	33
Mo, mg kg^{-1}	0.1	16	19	1.4	0.4
Ba, mg k^{-11}	163	474	66	101	NA
Ni, mg kg^{-1}	7	20	17	10	6
Cd, mg kg^{-1}	0.7	5.0	2.6	0.1	0.1
Cr, mg kg^{-1}	12	40	21	17	8
Pb, mg kg^{-1}	8.6	120	39	2	< 0.05
Ca, mg kg^{-1}	2538	28360	NA†	3438	12470
Fe, mg kg^{-1}	10030	4948	19050	19500	14500
K, mg kg^{-1}	2770	1900	460	4180	1780
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
EC, dS m^{-1}	0.2	5.0	20	0.5	1.8

† NA = not analyzed.

dy-skeletal, mixed, superactive, Mesic Aridic Argiustoll) with 0 to 3% slopes. The Altvan series consists of deep, well-drained soils that formed in mixed alluvial deposits (NRCS, 1980).

Soil Chemical Characterization

One composite soil sample, comprised of three cores, was obtained from each plot using a mechanical probe to a depth of 30 cm in late June of 2004, 2005, and 2006. All samples were separated into 0- to 8-, 8- to 15-, and 15- to 30-cm depths, returned to the laboratory and air-dried. Depth increments were identical to that collected by Harris-Pierce et al. (1993) in the same research plots. All soils were ground to pass a 2-mm sieve and analyzed for AB-DTPA (Barbarick and Workman, 1987) extractable Al, P, Mo, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn using ICP–AES, pH and EC using a saturated paste, and NO_3–N and NH_4–N using a 2-M KCl extract. Total C and N were also determined on ball-mill ground soil using a LECO-1000 CHN auto-analyzer (Nelson and Sommers, 1996). We utilized 10% duplicate samples and accepted no more than 5% error between samples for all analyses. Sample concentrations below detection limits were noted as non-detectable.

Plant Community Characterization

During mid-June of 2004, 2005, and 2006 aboveground plant cover by species was determined in each plot using seven 15-m transects with measurements obtained every 1.0 m. In 2005 and 2006, the two dominant plant species at the site, western wheatgrass (*Pascopyrum smithii* (Rydb.) A. Löve) and squirreltail (*Elymus elymoides* (Raf.) Swezey), were harvested from each plot for tissue nutrient analyses. Samples were placed in coolers with ice packs to keep plants cool during transport to the laboratory. Plants were immediately rinsed with double distilled water (DDW) to remove dust, dried to constant mass at 55°C, ground, weighed and then analyzed for total C and N by LECO-1000 CHN auto-analyzer (Nelson and Sommers, 1996). A subsample was digested in concentrated HNO_3 digest (Huang and Schulte, 1985) and analyzed for Al, P, Mo, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn using ICP–AES. Duplicates and errors accepted were similar to that used in soil chemical characterization.

Soil Microbial Community Structure

Soils for microbial community structure were collected in late July 2006 from the 0- to 8-cm depth in all plots. Samples were only collected from the 0- to 8-cm depth because this depth was affected by co-application to a greater extent than subsoil based on soil chemistry findings from 2004 and 2005. Samples were placed in a cooler and transported to Colorado State University. Fresh soil was kept cool, passed through a 2-mm sieve, and a portion placed in a −80°C freezer for subsequent microbial community analysis. A separate portion was weighed and placed in an oven at 105°C for 24 h to determine gravimetric moisture content.

Microbial community structure was characterized by EL-FAME analysis on fresh soil subsamples stored in a deep freezer at −80°C. Lipids were extracted from 4 g of deep-frozen soil in a 1:2:0.8 mixture of chloroform/methanol/phosphate buffer (pH = 7.4) as described by Bossio and Scow (1998). The mild alkaline transesterification method of Schutter and Dick (2000) was employed to extract fatty acids from lipid samples. In brief, lipids were extracted with 0.2 M KOH during a 37°C, hour-long incubation with periodic mixing, followed by addition of 1.0 M acetic acid to neutralize the pH of the tube contents. The EL-FAMEs were partitioned into an organic phase by addition of hexane, which was removed from the aqueous phase after centrifugation at 480 × *g* for 10 min. An internal standard (20 μg of fatty acid 19:0) was added to each EL-FAME sample before the hexane solvent was completely evaporated off with N so that EL-FAMEs could be quantified relative to the internal standard.

Samples were then analyzed by gas chromatography (GC) analysis with an Agilent 6890 gas chromatograph (Agilent Technologies, Inc., Palo Alto, CA) at the University of Delaware. The GC capillary column had an Ultra 2 Agilent #1909 1B-102 crosslinked 5% phenyl methyl silicone, 25 m long with an internal diameter of 0.2 mm and film thickness of 0.33 μm. Flame ionization detection (FID) was achieved at a temperature of 250°C using a carrier gas of hydrogen at a flow rate of 0.8 mL min^{-1}. Samples were run using the Microbial ID (Newark, DE) Eukary methods and peak naming table; all functions of the GC were under the control of the computer and this method. To clean the column between samples, oven temperature was ramped from 170 to 300°C at a rate of 5°C min^{-1}, and the maximum temperature was held for 12 min.

Standard nomenclature was used to describe EL-FAMEs. Numbering of carbons begins at the aliphatic (ω) end of the fatty acid molecule. The number preceding the colon represents the total number of carbon atoms, while the number following the colon represents the total number of double bonds. The configuration of the double bond is designated by either *c* for *cis* or *t* for *trans*. The prefixes "i" and "a" represent iso- and anteiso branched EL-FAMEs, respectively. The following biomarkers were assigned to Gram-positive bacteria: i14:0, a15:0, i15:0, a16:0, i16:0, a17:0, i17:0, i17:1G (Zak et al., 1996; Bossio and Scow, 1998), Gram-negative bacteria: 16:1ω7c, 17:0 cy, and 19:0 cy (Paul and Clark, 1996; Zak et al., 1996), fungi: 18:1ω9c, 18:2ω6c and 18:3ω6c (Vestal and White, 1989; Paul and Clark, 1996; Zak et al., 1996; Bossio and Scow, 1998; Bååth, 2003; Högberg et al., 2007), and arbuscular mycorrhizal (AM) fungi: 16:1ω5c (Paul and Clark, 1996; Schutter and Dick, 2000).

Statistical Approach

Statistical analysis was performed on all soil chemical and plant data (per individual depth for soils data) using a split-plot in time design in the Proc GLM model, SAS software version 9.1 (SAS Institute, 2002) to evaluate the effect of co-applications. We tested our hypotheses using an $\alpha = 0.05$ and calculated a Fisher's Protected Least Significant Difference (LSD; Steel and Torrie, 1980) when significance was observed within treatments or between timing of application. If a significant interaction existed between treatment and time, significance for the interaction is only presented. Plant community cover data was analyzed using square root transformed data for variables that were not normally distributed.

Analysis of variance tests ($\alpha = 0.05$) determined if WTR-biosolids co-application time or rate significantly affected total concentrations of soil EL-FAMEs (as an index of microbial biomass), relative amounts of bacterial EL-FAMEs (summed for all Gram-positive and Gram-negative bacteria), relative amounts of fungal EL-FAMEs, and relative amounts of AM fungi. Microbial data were analyzed as a split-plot block design in SAS version 9.1. Microbial EL-FAME data were analyzed by principal components analysis (PCA) using the PC-ORD statistical package (MjM Software, Gleneden Beach, Oregon). Data were normalized as relative mole %, followed by arcsine-square root transformation, before the PCA procedure (correlation matrix method) to meet assumptions of normality.

RESULTS AND DISCUSSION

Soil Chemical Characterization

Water treatment residuals contained appreciable quantities of Al (Table 1), but AB-DTPA extractable Al content, with depth, was mostly unaffected by application rate or time of application (Table 2). Ippolito et al. (1999) subjected WTR and soil from the

Meadow Springs Ranch to AB-DTPA, noting that AB-DTPA extracted approximately 10 times more Al from WTR than soil. The authors hypothesized, however, that relatively insoluble Al-P soil precipitates were forming with increasing WTR rate, which could explain the lack of significance in AB-DTPA extractable Al in the current study. Haustein et al. (2000) applied WTR up to 18 Mg ha^{-1} and determined soluble Al content in runoff. The authors showed that the WTR-treated plots did not statistically differ from the control at 1 d, 1 mo, or 4 mo following application. Soil pH ranged from 5.4 to 5.7 in the Haustein et al. (2000) study, and it was suggested that the pH was not low enough for Al to be soluble. A pH of <4.7 is necessary for the Al^{3+} ion to dominate a soil system (Bohn et al., 1985); the soil pH in our study, across all depths, ranged from 5.5 to 6.9.

Increasing WTR application rate caused a decrease in AB-DTPA extractable P in the soil surface in 2004 and 2006, and a significant treatment × time interaction was observed in the soil surface in 2005 (Table 3). We at least expected to observe significant AB-DTPA extractable P decreases with increasing WTR rate because WTR have the propensity to adsorb large quantities (>10,000 mg kg^{-1}) of P (Ippolito et al., 2003; Makris et al., 2004) due to the amorphous nature of WTR. It is interesting to note that the decrease in extractable P content was still evident in the long-term plots, implying long-term P adsorption, stability, and implications for improved environmental quality. Bayley et al. (2008a) focused on the long-term WTR-biosolids co-application plots used in the current study. Using pathway analysis, the authors showed that even after 13 yr following initial co-application that WTR still acted as the major stable P sink. Novak and Watts (2005) showed that adding 6% WTR (w w^{-1}) to soils containing excess water-extractable P reduced soluble P content by between 45 and 91%. The authors suggested that WTR can be effective at reducing potential off-site P movement. Prevention of off-site soluble P movement would reduce the likelihood of P induced waterway eutrophication.

The short-term plots contained greater P concentrations in the 8- to 15-cm depth as compared with the long-term plots in both 2005 and 2006 (Table 3). Eghball et al. (1996) suggested that vertical soluble P transport can be significant in coarse-textured soils like soils found in the Altvan series (fine-loamy over sandy or sandy-skeletal), but in our system downward transport of biosolids particles must have occurred. Elliott et al. (2002) studied incorporated co-application of biosolids and WTR to a coarse-textured soil. The authors noted a significant decrease in P leaching when WTR was co-applied with biosolids as compared with biosolids alone, relating the decrease to a reduction in the soil phosphorus saturation index (PSI). The PSI is a measure of the molar ratio of total sorbable P to the amorphous Al and Fe components capable of P fixation (Elliott et al., 2002). Bayley (2006) studied the 0- to 5-cm depth Meadow Springs Ranch soils within and outside our study area, showing that plots receiving only 10 Mg biosolids ha^{-1} had a PSI of 0.22 while plots receiving co-applications had substantially lower PSI values; the 21 Mg ha^{-1} WTR rate lowered the PSI below that of the control (0 Mg biosolids and 0 Mg WTR ha^{-1}), supporting the contention that biosolids particles, and not soluble P which would most likely have interacted with WTR, was transported downward.

Short-term co-applications also increased AB-DTPA extractable Mo concentrations as compared with the long-term plots in the 0- to 8- and 8- to 15-cm depths in 2005 and 2006 (Table 4). Biosolids particles themselves were most likely transported downward, as with the case of our 2005 and 2006 P observations. However, in 2005 a significant treatment effect was observed in the 0- to 8- and 8- to 15-cm depths, with increasing WTR rate causing a decrease in AB-DTPA extractable Mo. Ippolito et al. (2002) suggested that WTR adsorption of Mo was the responsible reduction mechanism. In support of our Mo observation and that of Ippolito et al. (2002), Tisdale et al. (1985) suggested that soils (or in our case WTR) containing appreciable quantities of Fe or Al, especially non-crystalline Fe and Al forms, also tend to have low Mo availability. At a soil pH of ~5.5, about 80% of the soluble Al species would be in the form of $Al(OH)_2^+$ (Bohn et al., 1985). Adriano (2001) showed the MoO_4^{2-} species can form associations and be bound to $Al(OH)_2^+$ via ligand exchange.

Table 2. Ammonium bicarbonate-diethylenetriaminepentaacetic acid (AB-DTPA) extractable Al in the long-term (single co-application, 1991) and short-term (repeated co-application, 2002) 0- to 8-, 8- to 15-, and 15- to 30-cm soil depths as affected by increasing water treatment residuals rate co-applied with a single biosolids rate (10 Mg ha^{-1}) at the Meadow Springs Ranch semiarid rangeland site in 2004, 2005, and 2006. Values inside parentheses represent 1 standard error of the mean (n = 4).

Year	Depth	Long/short-term	Water treatment residuals rate, Mg ha^{-1} 5	10	21	Treatment effect, *LSD*	Time effect, *LSD*	Treatment × time interaction
	cm		———mg Al kg^{-1}———					
2004	0–8	Long-term	0.27 (0.27)	0.14 (0.14)	0.15 (0.14)	NS‡	NS	NS
		Short-term	1.04 (1.04)	1.29 (1.29)	0.52 (0.52)			
	8–15	Long-term	ND†	0.77 (0.44)	0.38 (0.38)	NS	NS	NS
		Short-term	0.64 (0.64)	0.38 (0.38)	2.94 (2.94)			
	15–30	Long-term	ND	2.95 (1.94)	0.51 (0.51)	NS	NS	NS
		Short-term	1.54 (1.09)	0.13 (0.13)	0.77 (0.61)			
2005	0–8	Long-term	3.06 (0.95)	1.26 (0.91)	0.81 (0.77)	NS	NS	NS
		Short-term	2.31 (0.86)	1.56 (0.93)	0.95 (0.62)			
	8–15	Long-term	1.24 (0.65)	0.58 (0.32)	3.01 (1.09)			*
		Short-term	1.10 (0.47)	1.55 (0.90)	0.30 (0.30)			
	15–30	Long-term	1.10 (0.92)	ND	2.13 (0.78)	NS	NS	NS
		Short-term	1.51 (0.66)	1.58 (0.96)	1.17 (0.84)			
2006	0–8	Long-term	3.24 (1.90)	3.66 (2.22)	0.93 (0.75)	NS	NS	NS
		Short-term	0.36 (0.36)	2.40 (1.38)	3.45 (2.06)			
	8–15	Long-term	0.93 (0.93)	0.06 (0.06)	0.84 (0.84)	NS	NS	NS
		Short-term	0.93 (0.93)	1.08 (0.80)	0.82 (0.49)			
	15–30	Long-term	0.34 (0.20)	2.44 (0.86)	1.12 (0.69)	NS	NS	NS
		Short-term	1.24 (0.96)	2.82 (1.87)	0.46 (0.39)			

† ND = non-detectable.

* Significance at 5% probability level.

‡ NS = not significant.

Table 3. Ammonium bicarbonate-diethylenetriaminepentaacetic acid (AB-DTPA) extractable P in the long-term (single co-application, 1991) and short-term (repeated co-application, 2002) 0- to 8-, 8- to 15-, and 15- to 30-cm soil depths as affected by increasing water treatment residuals rate co-applied with a single biosolids rate (10 Mg ha^{-1}) at the Meadow Springs Ranch semiarid rangeland site in 2004, 2005, and 2006. Values inside parentheses represent 1 standard error of the mean (*n* = 4). Italicized values represent the least significant difference.

Year	Depth	Long/short-term	Water treatment residuals rate, Mg ha^{-1}			Treatment effect, *LSD*	Time effect, *LSD*	Treatment × time interaction
	cm		5	10	21			
			mg P kg^{-1}					
2004	0–8	Long-term	48.1 (5.3)	37.0 (3.5)	31.0 (3.8)	*†, *11.3*	NS	NS
		Short-term	50.4 (6.2)	45.4 (7.5)	32.8 (1.2)			
	8–15	Long-term	14.2 (2.8)	10.5 (1.2)	11.2 (1.8)	NS	NS	NS
		Short-term	13.3 (1.5)	14.2 (1.5)	12.5 (1.1)			
	15–30	Long-term	8.6 (1.1)	6.8 (1.1)	7.5 (1.2)	NS	NS	NS
		Short-term	7.9 (2.2)	7.5 (0.9)	8.4 (1.1)			
2005	0–8	Long-term	27.8 (4.8)	26.0 (3.9)	21.8 (1.4)			*
		Short-term	43.1 (3.2)	30.0 (3.1)	26.2 (0.9)			
	8–15	Long-term	10.4 (1.2)	9.1 (1.1)	9.8 (1.2)	NS	*, *0.9*	NS
		Short-term	13.2 (0.8)	9.5 (0.6)	10.5 (0.1)			
	15–30	Long-term	7.9 (1.2)	5.1 (0.7)	7.0 (0.5)	NS	NS	NS
		Short-term	6.5 (0.6)	6.6 (0.4)	5.7 (0.7)			
2006	0–8	Long-term	35.7 (1.9)	28.3 (1.3)	21.5 (1.9)	*, *3.9*	NS	NS
		Short-term	34.8 (2.9)	30.9 (2.4)	28.6 (1.5)			
	8–15	Long-term	8.8 (1.1)	6.7 (0.3)	7.9 (0.5)	NS	*, *1.1*	NS
		Short-term	12.0 (2.3)	9.9 (1.7)	9.8 (0.8)			
	15–30	Long-term	5.3 (0.4)	4.2 (0.3)	5.0 (0.2)	NS	NS	NS
		Short-term	7.3 (1.3)	4.6 (0.5)	5.6 (0.2)			

* Significance at 5% probability level.

†NS = not significant.

Table 4. Ammonium bicarbonate-diethylenetriaminepentaacetic acid (AB-DTPA) extractable Mo in the long-term (single co-application, 1991) and short-term (repeated co-application, 2002) 0- to 8-, 8- to 15-, and 15- to 30-cm soil depths as affected by increasing water treatment residuals rate co-applied with a single biosolids rate (10 Mg ha^{-1}) at the Meadow Springs Ranch semiarid rangeland site in 2004, 2005, and 2006. Values inside parentheses represent 1 standard error of the mean (*n* = 4). Italicized values represent the least significant difference.

Year	Depth	Long/Short-term	Water treatment residuals rate,Mg ha^{-1}			Treatment effect, LSD	Time effect, LSD	Treatment × time interaction
	cm		5	10	21			
			mg Mo kg^{-1}					
2004	0–8	Long-term	0.068 (0.009)	0.056 (0.006)	0.052 (0.006)	NS‡	*, *0.038*	NS
		Short-term	0.107 (0.018)	0.161 (0.029)	0.089 (0.018)			
	8–15	Long-term	ND†	ND	ND			
		Short-term	ND	ND	ND			
	15–30	Long-term	ND	ND	ND			
		Short-term	ND	ND	ND			
2005	0–8	Long-term	0.071 (0.010)	0.062 (0.012)	0.056 (0.002)	*, *0.041*	*, *0.048*	NS
		Short-term	0.203 (0.019)	0.139 (0.038)	0.099 (0.015)			
	8–15	Long-term	0.009 (0.003)	0.004 (0.003)	0.005 (0.005)	*, *0.008*	*, *0.007*	NS
		Short-term	0.028 (0.006)	0.008 (0.002)	0.011 (0.002)			
	15–30	Long-term	0.003 (0.002)	ND	ND			
		Short-term	ND	0.003 (0.002)	ND			
2006	0–8	Long-term	0.084 (0.003)	0.084 (0.012)	0.063 (0.010)	NS	*, *0.051*	NS
		Short-term	0.223 (0.043)	0.141 (0.020)	0.168 (0.024)			
	8–15	Long-term	0.015 (0.002)	0.015 (0.004)	0.016 (0.002)	NS	*, *0.008*	NS
		Short-term	0.029 (0.005)	0.017 (0.003)	0.028 (0.002)			
	15–30	Long-term	0.010 (0.002)	0.007 (0.002)	0.007 (0.001)	NS	NS	NS
		Short-term	0.012 (0.005)	0.009 (0.002)	0.012 (0.005)			

* Significance at 5% probability level.

† ND = non-detectable.

‡ NS = not significant.

Water treatment residuals act similarly to non-crystalline Fe and Al mineral species because Al-based WTR have a mineral composition similar to amorphous $Al(OH)_3$, and at soil pH values present in our system should adsorb Mo via ligand exchange.

Soil EC, NO_3–N, NH_4–N, total C, total N, and AB-DTPA extractable Cd, Cu, Fe, Mn, Ni, and Zn were not affected by WTR application rate in any depth in 2004, 2005, or 2006 (data not shown). However, repeated biosolids application significantly increased these constituents in the 0- to 8-cm depth; the 8- to 15- and 15- to 30-cm depths were affected to a lesser degree. Increases were expected in the soil surface because the repeated biosolids application added more of these constituents to the soil (Table 1).

Plant Community

The co-application of WTR with biosolids in this study resulted in few discernable effects in the plant community (Fig. 1 and 2). Few effects were seen either in the plots receiving treatments in 1991 (long-term plots) or plots that received repeated applications in 1991 and 2002 (short-term plots). In 2004, there was more total plant cover (and less bare ground) in plots receiving 10 Mg ha^{-1} of WTR relative to plots receiving 5 or 21 Mg ha^{-1}. The increase in cover in the 10 Mg ha^{-1} plots during 2004 was related to increased perennial grass cover (Fig. 2). In 2005 more plant species were observed in plots receiving 21 Mg ha^{-1} relative to plots receiving lesser amounts (Fig. 1). Repeated treatments in 2002 resulted in less cover of perennial forbs in 2005. The repeated application of WTR and biosolids in 2002 slightly increased plant cover overall in 2005, but slightly decreased it in 2006 (Fig. 1). This was likely attributed to shifts in the plant community composition that occurred between 2005 and 2006.

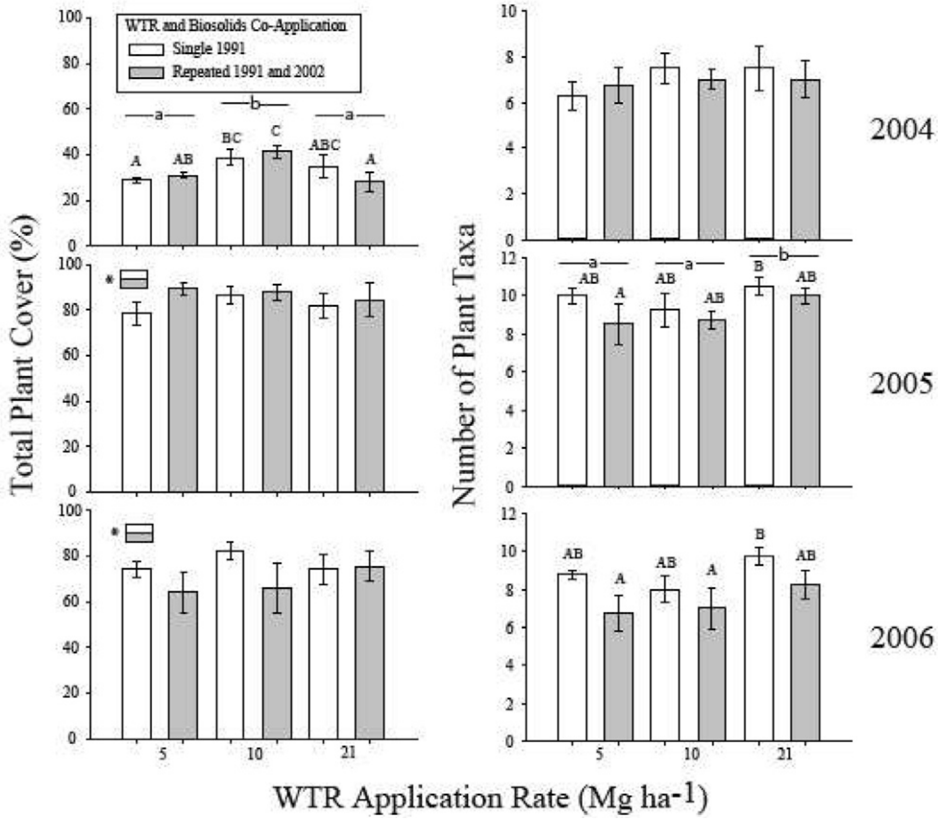

Fig. 1. Total plant cover and number of plant taxa (species) from 2004 through 2006 in replicated (*n* = 4) semiarid grassland plots co-amended with biosolids (10 Mg ha^{-1}) and various rates of water treatment residuals (WTR). Treatments were applied to plots in either 1991 or in 1991 and 2002. Capital letters indicate significant differences across WTR application rates and reapplication treatment within a year using a Fisher's LSD test (α = 0.05). Lowercase letters indicate significant difference between WTR rates within a year (Fisher's LSD, α = 0.05, *n* = 8). Asterisks (*) within a graph panel indicate a significant effect of the 2002 repeated application (Fisher's LSD, α = 0.05, *n* = 12). Error bars represent one standard error of the mean.

All of these effects appeared to be transient as they were not observed in subsequent years. These observations are in contrast to a similar study at this site described by Sullivan et al. (2006b) where biosolids only were applied at rates of up to 30 Mg ha^{-1}; 10 Mg ha^{-1} resulted in increased plant community biomass and reduced species richness as compared with no biosolids applied. The main difference between this study and that of Sullivan et al. (2006b) was the co-application of WTR in this study.

Plant Tissue Chemistry

The 2005 and 2006 western wheatgrass and bottlebrush Al, P, and Mo concentrations are presented in Table 5. Western wheatgrass Al content decreased with increasing WTR rate in 2005. Ippolito et al. (1999) showed that WTR rates up to 250 g kg^{-1} decreased western wheatgrass and blue grama (*Bouteloua gracilis* H.B.K. Lag) shoot Al concentration. It has been hypothesized (Millard et al., 1990) that $AlPO_4$ precipitates at the root surface and acts as a barrier to reduce Al transport into the root, and subsequent to the shoot. In addition to Al precipitation, chelation, immobilization in non-sensitive cell sites, or other metabolic exclusion mechanisms could be a means of plant Al resistance (Fageria et al., 1988; Taylor, 1991).

Western wheatgrass P content was unaffected by increasing WTR rate, but the 2006 squirreltail bottlebrush P concentration decreased with increasing WTR rate. Although nonsignificant, the short- and long-term 2005 bottlebrush P content followed a similar trend. No deficiency symptoms were observed. Ippolito et al. (1999, 2002) noted a decrease in blue grama and western wheatgrass shoot P concentration associated with increasing WTR application rate. The decrease in plant P content was due to P adsorption on to WTR, and Bayley et al. (2008a) showed that WTR acts as the major P sink in this system. Others (Oladeji et al., 2007; Lucas et al., 1994; Elliott and Singer, 1988; Bugbee and Frink, 1985) have found reduced P content of plants grown in WTR-amended soils. Mahdy et al. (2007), however, found that WTR addition at rates up to 30 g kg^{-1} to calcareous soils significantly increased corn P shoot and root concentrations.

Each plant species, in both 2005 and 2006, showed a significant reduction in Mo content in the short-term versus long-term plots. Opposite of the previous findings associated with repeated biosolids application, the decrease in plant Mo content must be associated with WTR application because biosolids added Mo to

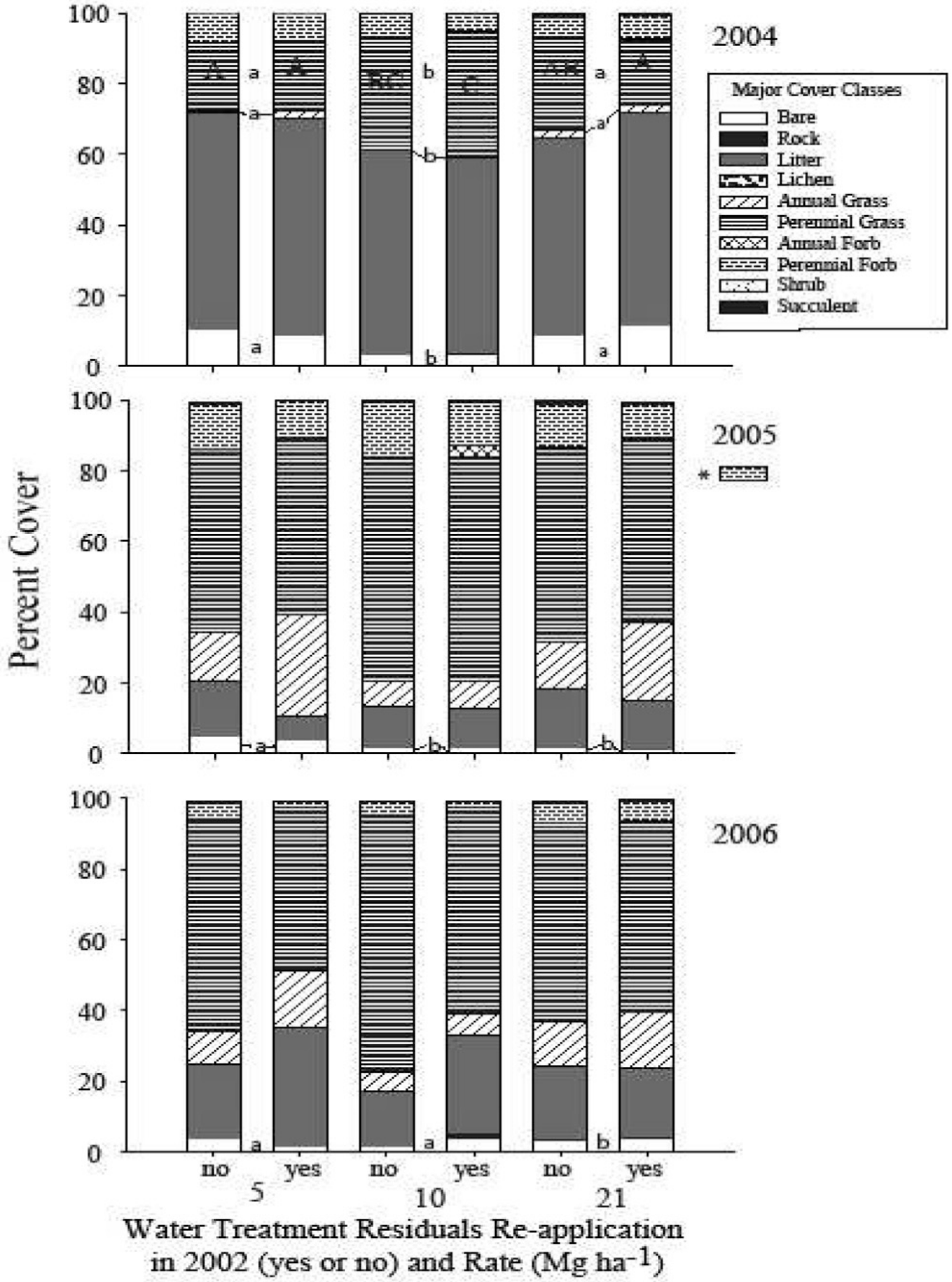

Fig. 2. Percentage of cover of various plant life forms and other cover classes from 2004 through 2006 in replicated (*n* = 4) semiarid grassland plots co-amended with biosolids (10 Mg ha^{-1}) and various rates of water treatment residuals (WTR). Treatments were applied to plots in either 1991 or in 1991 and 2002. Capital letters indicate significant differences across WTR application rates and reapplication treatment within a year using a Fisher's LSD test (α = 0.05, *n* = 4). Lowercase letters indicate significant difference between WTR rates for the corresponding cover class within a year (Fisher's LSD, α = 0.05, *n* = 8). Asterisks (*) within a graph panel indicate a significant effect of the 2002 repeated application on the indicated cover class, in this case, perennial forbs in 2005 (Fisher's LSD, α = 0.05, *n* = 12).

the research plots. In addition, the 2005 squirreltail Mo content decreased with increasing WTR rate. As with plant P, no plant Mo deficiency symptoms were observed. Harris-Pierce et al. (1993) observed a decrease in blue grama Mo shoot concentration with increasing WTR rate. Most likely WTR were adsorbing the molybdate anion as suggested by Ippolito et al. (2002) and supported by Tisdale et al. (1985) and Adriano (2001). In addition to PO_4 and MoO_4, due to its amorphous hydroxyoxide nature WTR have been shown to adsorb other anions such as As(V), As(III), and ClO_4^- (Makris et al., 2006a, 2006b, 2008).

Other western wheatgrass and squirreltail extractable elements (Cu, Mn, and Zn) increased with the short-term co-application as compared with long-term co-application in 2005 and 2006 (data not shown). Total N increased in western wheatgrass in 2005 and 2006, and a significant treatment × time interaction was observed for squirreltail in 2005 (data not shown). As with most AB-DTPA extractable elements, increases in plant concentrations were expected because the repeated biosolids application added more of these elements to the soil.

Soil Microbial Community Structure

Few studies have examined the effect of WTR or WTR-biosolids amendments on soil microorganisms. In 2003, Bayley et al. (2008a) found that microbial biomass P decreased with increasing WTR co-application rates in the re-applied plots. In another study, Bayley et al. (2008b) measured decreased activity of phosphodiesterase enzyme in co-applied WTR-biosolids plots as compared with control soil, which was attributed to a reduction in microbial activity in response to reduced concentrations of soil solution P. To the best of our knowledge, no other study has examined WTR-biosolids co-application effects on microbial community structure.

Lipid and phospholipid fatty acid analyses are powerful methods for detecting changes or differences in microbial community structure (Ramsey et al., 2006). As examples, the specific methodology employed in this study has successfully resolved microbial community differences in agricultural soils due to cropping and tillage management (Drijber et al., 2000) and in forest soils differentially affected by prescribed and wild fires (Hamman et al., 2007; Jiménez Esquilín et al., 2007, 2008). In 2003 and 2004, this method was employed at a separate but nearby study to determine the effects of biosolids application rate (0 to 30 Mg ha^{-1}, applied in 1991 with or without a second application in 2002) on soil microbial communities. Relevant to this study, microbial communities from soils which received 10 Mg ha^{-1} biosolids were enriched in Gram-positive bacterial fatty acid markers but depleted in AM fungal fatty acid marker compared with non-amended soil. Multivariate analysis of EL-FAME data resulted in the separation of communities from

Table 5. Western wheatgrass (*Pascopyrum smithii*) and squirreltail (*Elymus elymoides*) Al, P, and Mo concentrations in the long-term (single co-application, 1991) and short-term (repeated co-application, 2002) plots as affected by increasing water treatment residuals rate co-applied with a single biosolids rate (10 Mg ha^{-1}) at the Meadow Springs Ranch semiarid rangeland site in 2005 and 2006. Values inside parentheses represent 1 standard error of the mean (*n* = 4). The interaction between treatment and time was nonsignificant for all elements. Italicized values represent the least significant difference.

	Western Wheatgrass						Squirreltail					
WTR rate	**Al**		**P**		**Mo**		**Al**		**P**		**Mo**	
Mg ha^{-1}	mg kg^{-1}											
	Long-term	Short-term	Long-term	Short-term	Long-term	Short-term	Long-term	Short-term	Long-term	Short-term	Long-term	Short-term
						2005						
5	9.7 (2.2)	10.9 (3.5)	2782 (175)	3046 (213)	0.270 (0.072)	0.108 (0.037)	5.1 (2.5)	21.8 (18.8)	2674 (92)	2629 (146)	0.738 (0.109)	0.104 (0.059)
10	8.8 (2.0)	11.2 (2.8)	2737 (61)	3014 (180)	0.146 (0.021)	ND‡	2.5 (2.3)	4.8 (4.3)	2502 (34)	2447 (93)	0.611 (0.097)	0.118 (0.012)
21	4.9 (1.6)	5.2 (1.6)	2737 (195)	2670 (235)	0.138 (0.058)	0.083 (0.050)	6.7 (3.7)	22.9 (11.6)	2462 (99)	2372 (47)	0.424 (0.125)	0.073 (0.046)
Trt Effect, *LSD*	*† *5.2*		NS		NS		NS		NS		* *0.136*	
Time Effect, *LSD*	NS		NS		* *0.102*		NS		NS		* *0.200*	
						2006						
5	110 (22)	103 (11)	999 (28)	1181 (51)	0.167 (0.061)	ND	116 (15)	88 (10)	1523 (71)	1486 (88)	0.216 (0.033)	0.022 (0.022)
10	116 (30)	128 (23)	998 (48)	1059 (84)	0.117 (0.025)	0.020 (0.012)	132 (26)	107 (10)	1185 (79)	1283 (115)	0.162 (0.067)	0.021 (0.021)
21	123 (11)	129 (14)	1029 (53)	1118 (109)	0.117 (0.050)	0.009 (0.009)	126 (13)	147 (40)	1245 (73)	1248 (56)	0.116 (0.026)	0.005 (0.005)
Trt Effect, *LSD*	NS		NS		NS		NS		* *214*		NS	
Time Effect, *LSD*	NS		NS		* *0.045*		NS		NS		* *0.119*	

* Significance at 5% probability level.
† NS, not significant.
‡ ND, non-detectable.

control and 10 Mg ha^{-1} plot soils in multidimensional space (Sullivan et al., 2006a).

A total of 38 FAMEs were extracted from all the soil samples, 25 of which were of microbial origin (<20 C units long; Zelles et al., 1995). We found no statistically significant differences in total microbial EL-FAME concentrations or relative amounts of EL-FAME biomarkers among the plots which received different rates of WTRs along with a 10 Mg ha^{-1} rate of biosolids. Total concentrations of EL-FAMEs ranged from 33.4 to 54.8 nmol g^{-1} soil. The proportion of bacterial EL-FAMEs within soils ranged from 35.3 to 38.2%, fungi ranged from 6.7 to 9.8%, and AM fungi ranged from 1 to 2%. This indicated that soil microbial biomass and community EL-FAME structure were unaffected by increasing application rates of WTR or time of co-application (long- or short-term). Total concentrations of EL-FAMEs ranged from 33.4 to 54.8 nmol g^{-1} soil. The proportion of bacterial EL-FAMEs

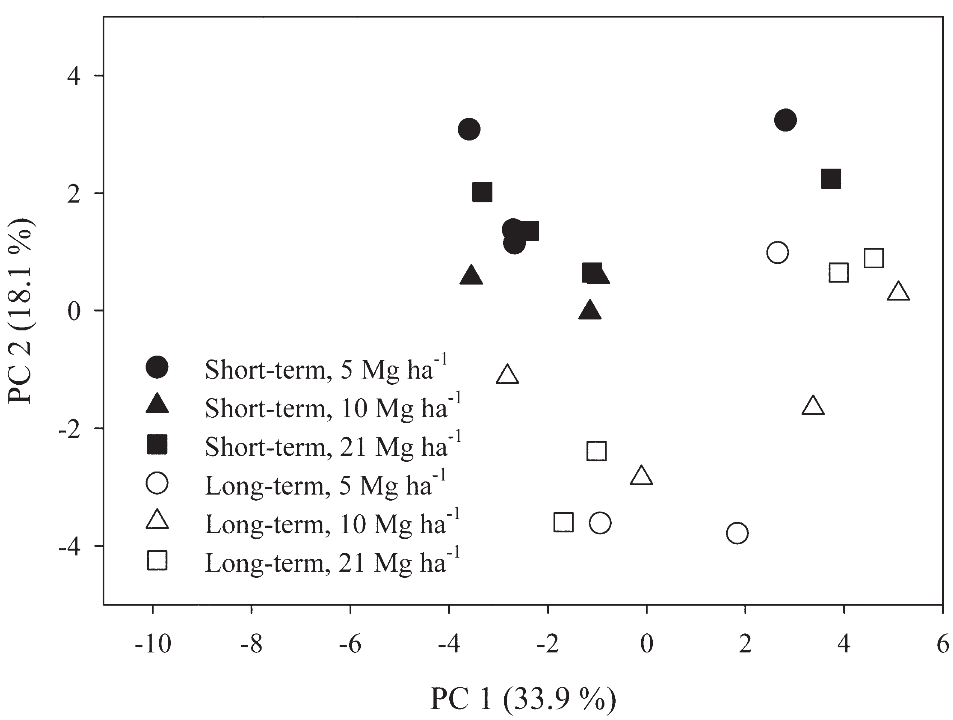

Fig. 3. Principal components analysis of soil microbial community profiles based on 24 ester-linked fatty acid methyl esters (EL-FAMEs) detected in all water treatment residuals-biosolids co-amended plots, as measured at the city of Fort Collins, Colorado owned Meadow Springs Ranch in 2006. Long-term plots were co-amended once in 1991; short-term plots were co-amended in 1991 and in 2002. The percentage of variance explained by each principal component (PC) is shown in parentheses.

within soils ranged from 35.3 to 38.2%, fungi ranged from 6.7 to 9.8%, and AM fungi ranged from 1 to 2%.

Principle components analysis was then conducted on a subset of 24 microbial FAMEs to determine whether differences existed in microbial community composition based on WTR application rate or time since WTR-biosolids co-application; one microbial FAME was found only in one soil sample and therefore this FAME was deleted from the data set before PCA. We found no distinct clustering or separation of microbial communities based on WTR application rates (Fig. 3). Instead, microbial communities were separated along PC 2 according to time since co-application, with communities from the repeated, short-term co-application plots clustering at the top of PC 2, and communities from the long-term, single application plots clustering to bottom of PC 2. Communities from the short-term plots were enriched in EL-FAMEs a15:0 (Gram-positive bacterial marker), i15:1 (a bacterial marker; Zelles, 1997), and 16:0 (ubiquitous EL-FAME). Communities from the long-term plots were enriched in i16:0 and i17:1G (Gram-positive bacterial markers), 16:1ω5c (arbuscular mycorrhizal fungi marker), and 19:0 cy (Gram-negative bacterial marker). Overall, this result contrasts with that found by Sullivan et al. (2006a), where microbial community EL-FAME structure was driven mainly by biosolids application rate, and not time since application.

SUMMARY AND CONCLUSIONS

Land co-application of WTR and biosolids has not been extensively studied. Our study objectives were to determine the long-term (13–15 yr) effects of a single and short-term (2–4 yr) effects of repeated WTR-biosolids co-applications on soil chemistry, soil microbiology, and plant community structure in a semiarid grassland. Three WTR rates (5, 10, and 21 Mg ha^{-1}) were surface co-applied (no incorporation) with a single biosolids rate (10 Mg ha^{-1}) once in 1991 (long-term plots) and again in 2002 (short-term plots).

Ammonium-bicarbonate diethylenetriaminepentaacetic acid extractable Al was relatively unaffected by WTR application, yet AB-DTPA extractable P and Mo concentrations decreased with increasing WTR rate likely due to adsorption on to WTR. The soil pH (5.5–6.9) was not low enough for Al^{3+} to be soluble, yet the soil pH likely favored the presence of $Al(OH)_2^+$ and the subsequent binding of P and Mo to WTR via ligand exchange. The AB-DTPA extractable soil Cd, Cu, Fe, Mn, Ni, and Zn concentrations, and soil pH, EC, NO_3–N, NH_4–N, total C, and total N were not affected by WTR application. However, most of these constituents increased in the soil surface with short-term, repeated biosolids application as expected.

The co-application of WTR with biosolids in this study resulted in few discernable effects in the plant community in the plots receiving treatments in 1991 or repeated applications in 1991 and 2002. Yearly (2004, 2005, or 2006) observable effects appeared to be transient as they were not observed in subsequent years.

In 2005, western wheatgrass Al content decreased with increasing WTR rate potentially due to relatively insoluble $AlPO_4$ precipitates at the root surface. Western wheatgrass P content was unaffected by increasing WTR rate, but in 2006 bottlebrush P concentrations decreased with increasing WTR rate. Decreases in P content were attributed to adsorption by WTR. Each plant species in both 2005 and 2006 showed a significant reduction in Mo content, again due to adsorption by WTR. Plant Cu, Mn, and Zn increased with the short-term co-application as compared with long-term co-application, likely due to repeated biosolids application.

Microbial community structure was characterized by EL-FAME analysis. No statistically significant differences in total EL-FAME concentrations or relative amounts of EL-FAME biomarkers among the plots were observed, indicating that WTR co-applications did not affect soil microbial community biomass or structure in biosolids-amended soil. Differences in community structure could only be resolved based on a subset of 24 EL-FAMEs that were common to each plot. With this subset, communities were differentiated according to time since biosolids-WTR application, but not WTR application rate. The WTR application did not affect the microbial community.

In conclusion, short- and long-term WTR-biosolids soil co-applications caused minimal disruption in soil chemistry, soil microbial diversity, plant nutrient levels, and plant community dynamics. This also suggests that WTR alone would pose almost no threat to plant and soil biology and can be an effective long-term solution to binding of excess soil or biosolids P and Mo with harming the environment in this region.

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