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Parallel shifts in plant and soil microbial communities in response to biosolids in a semi-arid grassland

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Abstract

Approximately 70,150 dry Mg of biosolids from over 450 wastewater treatment facilities are applied to the semi-arid rangelands of Colorado every year. Research on semi-arid grassland responses to biosolids has become vital to better understand ecosystem dynamics and develop effective biosolids management strategies. The objectives of this study were to determine the long-term (~12 years) effects of a single biosolids application, and the short-term (~2 years) effects of a repeated application, on plant and microbial community structure in a semi-arid grassland soil. Specific attention was paid to arbuscular mycorrhizal fungi (AMF) and linkages between shifts in plant and soil microbial community structures. Biosolids were surface applied to experimental plots once in 1991 (long-term plots) and again to short-term plots in 2002 at rates of 0, 2.5, 5, 10, 21, or 30 Mg ha^{-1}. Vegetation (species richness and above-ground biomass), soil chemistry (pH, EC, total C, total N, and extractable P, NO_3–N, and NH_4–N), and soil microbial community structure [ester-linked fatty acid methyl esters (EL-FAMEs)], were characterized to assess impacts of biosolids on the ecosystem. Soil chemistry was significantly affected and shifts in both soil microbial and plant community structure were observed with treatment. In both years, the EL-FAME biomarker for AMF decreased with increasing application rate of biosolids; principal components analysis of EL-FAME data yielded shifts in the structure of the microbial communities with treatment primarily related to the relative abundance of the AMF specific biomarker. Significant ($p \leq 0.05$) correlations existed among biomarkers for Gram-negative and Gram-positive bacteria, AMF and specific soil chemical parameters and individual plant species' biomass. The AMF biomarker was positively correlated with biomass of the dominant native grass species blue grama (*Bouteloua gracilis* [Willd. ex Kunth] Lagasca ex Griffiths) and was negatively correlated with western wheatgrass (*Agropyron smithii* Rydb.) biomass. This study demonstrated that applications of biosolids at relatively low rates can have significant long-term effects on soil chemistry, soil microbial community structure, and plant community species richness and structure in the semi-arid grasslands of northern Colorado. Reduced AMF and parallel shifts in the soil microbial community structure and the plant community structure require further investigation to determine precisely the sequence of influence and resulting ecosystem dynamics.

Keywords: Biosolids; Grassland soil; Soil microbial community; Arbuscular mycorrhizae; FAME

1. Introduction

Decades of scientific research and practical experience provide support for land application of biosolids as a beneficial means for restoring or re-vegetating disturbed lands and improving forage for livestock and wildlife (US Environmental Protection Agency, 1991). Land application of biosolids results in a range of soil improvements, which may then directly or indirectly affect the structure, diversity, or richness of plant and animal communities at a given site (US Environmental Protection Agency, 1999).

In semi-arid grasslands, low to intermediate application rates often result in stimulation of above-ground biomass, improvement of soil structure and fertility, and ecosystem restoration (Fresquez et al., 1991; Cuevas et al., 2000; Mata-González et al., 2002). However, changes in microbial community structure in response to biosolids are not well known, and research into the long-term effects of biosolids

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application is needed. Results from a few studies demonstrate varied responses of microbial communities to biosolids, and changes in the soil microbial communities have differed depending on the length of the study, biosolids application method and rate, as well as microbial community characterization methods (Dennis and Frequez, 1989; Pascual et al., 1999; Lawlor et al., 2000; Barbarick et al., 2004; García-Gil et al., 2004). In a previous study which examined the long-term impacts (~12 years) of a single biosolids application and short-term impacts (~2 years) of a repeated application, we found that soils receiving a repeated application of 21 or 30 Mg biosolids ha^{-1} had greater bacterial biovolumes and C and N mineralization activities than nonamended control plots, whereas in long-term plots, mineralization activities were stimulated only with the 30 Mg ha^{-1} rate (Sullivan et al., 2005). While increased microbial activity may be due to nutrient inputs via biosolids (short-term effects) or altered plant tissue chemistry (long-term effects), they may also be due to changes in soil microbial community structure.

As symbionts of plant roots, arbuscular mycorrhizal fungi (AMF) are critical components of soil microbial communities which influence above-ground productivity and plant community development (Sanders et al., 1996; van der Heijden et al., 1998), confer improved water relations (Allen and Allen, 1986; Neumann and George, 2004), increase nutrient uptake and stress tolerance (Lapointe and Molard, 1997), and assist in stable aggregate formation and enhanced C and P dynamics in the rhizosphere (Coleman and Crossley, 1996; Sanders et al., 1996; Jeffries et al., 2003). Plant–AMF associations may be sensitive to biosolids in the environment, either due directly to heavy metals or nutrient content or indirectly through the effects of biosolids on plant communities, but data is sparse on the exact effects of biosolids on the AMF fraction of the soil microbial community in semi-arid rangelands. The symbiotic role of AMF in aiding plant nutrient acquisition and uptake places these organisms at the crucial interface between the biotic and abiotic ecosystem components (Rillig, 2004), and a better understanding of factors affecting this symbiosis is key to understanding overall ecosystem dynamics (Bever et al., 2001). With over 70,150 dry Mg of biosolids being applied to the semi-arid rangelands of Colorado every year, research on semi-arid rangeland responses to biosolids both above- and below-ground, with specific reference to AMF, has become crucial (Goldstein, 2000; Barbarick et al., 2004).

The objectives of this study were to determine to what degree the soil environment and structures of plant and soil microbial communities were affected by short- and long-term applications of biosolids to a semi-arid grassland in northern Colorado. Moreover, we sought to determine if shifts in microbial community structure and relative abundance of AMF biomarker, in response to biosolids, were correlated with shifts in plant community structure, which would indicate a dynamic, reciprocal relationship between above and below-ground communities. The hypotheses of this research were three-fold with regard to increasing biosolids application rate: (1) plant community structure would be altered and productivity would increase in response to biosolids; (2) soil microbial community structure would be altered in response to biosolids; (3) AMF would respond positively to plant biomass increases and would consequently increase with increasing biosolids application rate.

2. Materials and methods

2.1. Field site

This study was conducted in 2003 and 2004 on long-term experimental research plots within the Meadow Springs Ranch. The ranch (1750 m elevation) is owned by and is located ~32 km north of the City of Fort Collins, CO (40 53′46″N, 104 52′28″W). It is a semi-arid, shortgrass steppe rangeland community dominated by perennial grasses, including blue grama and western wheatgrass. The Meadow Springs Ranch typically receives 25% of the 380-mm mean annual precipitation in the form of winter snow (Soil Conservation Service and Forest Service, USDA, 1980). However, from July 2002 to June 2003 total precipitation for the year exceeded 2128 mm, with roughly 1700 mm in the form of snow. Precipitation from July 2003 to June 2004 dropped to 822 mm with roughly 686 mm in the form of snow (National Oceanic and Atmospheric Administration, 2002–2004). The mean frost-free period is 125 d with a mean annual temperature of 9.4 °C. The surface soil texture is sandy loam bordering on gravelly and is classified as a deep, well-drained Aridic Argiustoll (Soil Conservation Service and Forest Service, USDA, 1980).

2.2. Experimental design

Plots (15×15 m) were originally established in 1991 (Pierce et al., 1998; Harris-Pierce, 1994) and arranged in a randomized complete block design with four replicate blocks. In 1991, a single application (0, 2.5, 5, 10, 21 or 30 Mg ha^{-1}) of biosolids was surface applied to each plot. In the fall of 2002, the plots were divided in two, and a second application was applied to the eastern half of each of the original plots, resulting in a split-plot block design with rate as the main factor and reapplication (yes or no) as the split factor. The rate applied to each plot (7.5×15 m) in 2002 corresponded to the rate applied to the whole plot in 1991. We defined short-term plots as those that received biosolids application in both 1991 and 2002, and long-term plots as those that only received biosolids application in 1991. The application rates were chosen as realistic application rates for semi-arid rangelands, and with large expanses of such native grasslands, many years often occur between reapplication at a single location. Biosolids originated from the City of Fort Collins wastewater

Table 1
Chemical properties on a dry-weight basis of biosolids applied to Meadow Springs Ranch experimental plots in 2002

Constituent	2002 Biosolids
pH	7.3
EC	20.2 dS m^{-1}
Organic N	41,750 mg kg^{-1}
NH_4–N	5440 mg kg^{-1}
NO_3–N	2.9 mg kg^{-1}
P	11,350 mg kg^{-1}
K	420 mg kg^{-1}
Fe	19,050 mg kg^{-1}
Al	12,650 mg kg^{-1}
Cu	162 mg kg^{-1}
Zn	254 mg kg^{-1}
Ni	5.0 mg kg^{-1}
Mo	1.9 mg kg^{-1}
Cd	0.58 mg kg^{-1}
Cr	6.15 mg kg^{-1}
Pb	7.1 mg kg^{-1}
As	5.42 mg kg^{-1}
Se	0.22 mg kg^{-1}
Hg	0.19 mg kg^{-1}
Ag	<0.01 mg kg^{-1}

treatment plant and were surface applied without incorporation. The chemical properties of the biosolids surface-applied to Meadow Springs Ranch experimental plots in 1991 have been previously described by Barbarick et al. (2004), and the chemical properties of the biosolids applied in 2002 to the short-term study plots are found in Table 1. Elemental composition was determined by $HClO_4$–HNO_3–HF–HCl digestion (Soltanpour et al., 1996) followed by elemental analysis with inductively coupled plasma atomic emission spectrometry (ICP-AES). Total N content of biosolids was determined by a concentrated H_2SO_4 digestion (Bremner, 1996). Regulated chemical constituents fall below the EPA 40 CFR Part 503 limits (US EPA, 1993).

2.3. Soil sampling procedures

In July 2003 and June 2004, vegetation and soil samples were collected from each pot within 10 randomly located 0.5 m^2 quadrants. Soil samples (1–3 cores per quadrant, depending on sampling method as described below.) were obtained 24 h before vegetation sampling began to assure as little perturbation to the soil microbial community as possible while maintaining measurements in the same sampling locations. Vegetation was clipped by species within each quadrant, dried to a constant mass and weighed.

In 2003, soil samples were obtained with a cordless drill, fitted with a 2.54 cm diameter wood auger bit (15 cm long). Three cores were taken on a diagonal across each of the 10 sampling quadrants within each plot; these cores (30 total) were mixed together to form one composite sample per plot. In 2004, due to moist soil conditions, a hand spade was used to extract one soil sample (0–15 cm) per quadrant in each plot, and these samples (10 total) were composited per plot. Before sampling the next treatment plot, sampling utensils were rinsed with a 5% bleach solution to wash off excess soil, followed by ethanol to prevent cross-contamination among samples. Composite samples were stored on ice in the dark until transport to the lab where soils could be sieved through a 2 mm mesh and partitioned for various analyses. After sieving, gravimetric water content was determined for each sample, at least 50 g of each sample were stored at −80 °C for lipid extraction, and the remaining portion was stored for approximately 24 h at 5 °C until other analyses could commence. In 2004, samples were allowed to air-dry one full day in opened Ziploc® bags, after collection, before analyses began due to excessive moisture conditions.

In early July of 2003 and 2004, three random soil samples were collected from each experimental plot using a hydraulic Giddings probe to a depth of 30 cm. Soil cores were divided into two depth increments (0–15 and 15–30 cm), and cores were composited per plot by depth increment. Samples were transported back to the lab in Ziplock® bags and a 10 g sub-sample of each was partitioned for determination of gravimetric water content before further processing. Samples were then air-dried and passed through a 2-mm sieve. Samples analyzed for total C and N were further ground to a fine powder.

2.4. Soil chemical and physical analyses

Soil samples collected by the hydraulic probe were analyzed for pH, electrical conductivity (EC), total C, total N, KCl-extractable NH_4–N and NO_3–N, and ammonium bicarbonate-diethylenetriaminepentaacetic acid (AB-DTPA)-extractable P. Soil pH was determined by the saturated paste method of Thomas (1996), and EC was measured by the method of Rhoades (1996). Total C and N were measured using a LECO CHN-1000 automated analyzer (LECO, St Joseph, MI) according to the protocols of Nelson and Sommers (1996). Soil NH_4–N and NO_3–N were extracted in 2 M KCl according to Mulvaney (1996) and analyzed on a Perstorp Enviroflow flow injector (Perstorp Analytical, Inc., Silver Spring, MD). The method of Barbarick and Workman (1987) was used for soil AB-DTPA-extractable P, followed by determination of constituent concentrations on an inductively coupled plasma-atomic emission spectrophotometer (Thermo Jarrell Ash Corp., Franklin, MA). Soil particle-size analysis was performed according to the methods of Gee and Bauder (1986).

2.5. Soil microbial community analysis

Microbial community structure was characterized by ester-linked fatty acid methyl ester (EL-FAME) analysis. Lipids were extracted from 4 g of soil (stored at −80 °C) in a 1:2:0.8 extractant mixture of chloroform:methanol:phosphate buffer using a modified method of White et al. (1979). The mild alkaline transesterification method of Schutter and Dick (2000) was employed to extract fatty acids from lipid

Table 2
Main effects of biosolids application rate on selected soil chemical parameters in the top 15 cm of soil as measured in 2003

Rate (Mg ha^{-1})	pH	P (mg kg^{-1})	Total C (g kg^{-1})	EC		Total N		NO_3–N		NH_4–N	
				Short-term (dS m^{-1})	Long-term (dS m^{-1})	Short-term (g kg^{-1})	Long-term (g kg^{-1})	Short-term (mg kg^{-1})	Long-term (mg kg^{-1})	Short-term (mg kg^{-1})	Long-term (mg kg^{-1})
30	5.85c	165a	18.4a	1.92a	0.36a	2.1a	1.6a	16.6a	3.18a	108a	2.93a
21	5.91bc	109b	17.3a	1.51a	0.45a	2.3a	1.6a	14.1a	3.93a	93.2a	3.28a
10	5.95bc	63.4c	14.2b	0.74b	0.28a	1.3b	1.3a	6.65b	1.53b	42.6b	2.08ab
5	6.01abc	56.0c	14.6b	0.38b	0.24a	1.2b	1.3a	2.93c	1.33b	11.2bc	2.00ab
2.5	6.34a	37.4cd	13.3b	0.35b	0.29a	1.2b	1.0a	1.48c	0.88b	4.23c	1.60b
0	6.26ab	25.9d	13.6b	0.31b	0.31a	1.3b	1.3a	0.90c	1.03b	1.03c	1.23b
LSD	0.36	26.8	2.3	0.75	NS	0.4	NS	3.51	0.90	33.8	1.28
$p_r > F$	0.04	<0.0001	0.0007	0.0009	0.19	<0.0001	0.21	<0.0001	<0.0001	<0.0001	0.03

Soil EC, total N, NO_3–N, and NH_4–N exhibited significant interaction effects of reapplication×rate in 2003. Within columns, means followed by different letters are significantly different at $\alpha = 0.05$. NS: not significant.

samples. An internal standard (20 μg of 19:0) was added to each EL-FAME sample before the hexane solvent was completely evaporated off with nitrogen. Samples were analyzed by gas chromatography (GC) analysis with an Agilent 6890 gas chromatograph (Agilent Technologies, Inc., Palo Alto, CA) by the University of Delaware. The GC capillary column was 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 ramped from 170 to 300 °C at a rate of 5 °C min^{-1}, with a hold at the maximum temperature for 12 min. Biomarkers of specific functional groups were assigned according to Schutter and Dick (2000).

2.6. Statistical analyses

All univariate data were analyzed by analysis of variance, specifically the general linear model (GLM) procedure in SAS (SAS Institute, 2002), and mean comparisons were performed using a Fisher-protected LSD. Correlations of univariate data including individual plant species biomass, soil chemical parameters, and individual EL-FAME biomarkers were performed using the PROC CORR command within SAS. All SAS procedures were conducted with $\alpha = 0.05$ significance level. Multivariate statistical procedures were performed on microbial community analyses. Specifically, EL-FAME data were analyzed by principal components analysis (PCA) after normalizing the data as relative mol%, followed by arcsine-square root transformation; plant community data were analyzed by PCA after normalizing the data as relative biomass%, followed by logarithmic transformation. The PC-ORD statistical package (MjM Software, Gleneden Beach, OR, 1999) was used for all multivariate analyses.

3. Results

3.1. Soil chemistry

In both sampling years of this study, biosolids application had significant effects on the soil chemical properties assayed (0–15 cm depth). A more detailed discussion of soil chemistry, including heavy metals, is presented in Sullivan et al. (2005), but extractable levels of heavy metals did not approach EPA drinking water limits in any biosolids amended plots. Effects of reapplication, and the interactions between treatment rate and short- or long-term plots, were not significant for P, pH, or total carbon (Table 2). Treatment rate and reapplication interactions

Table 3
Main effects of biosolids application rate on selected soil chemical parameters in the top 15 cm of soil as measured in 2004

Rate (Mg ha^{-1})	pH	P (mg kg^{-1})	Total C (g kg^{-1})	Total N (g kg^{-1})	EC		NO_3–N		NH_4–N	
					Short-term (dS m^{-1})	Long-term (dS m^{-1})	Short-term (mg kg^{-1})	Long-term (mg kg^{-1})	Short-term (mg kg^{-1})	Long-term (mg kg^{-1})
30	5.56c	29.3c	19.3a	2.08a	2.34a	0.31a	14.7a	2.73a	19.8a	1.15a
21	5.55c	39.3b	14.3bc	1.46cd	0.97b	0.26a	8.01b	2.31ab	6.95b	0.98a
10	5.88b	40.2b	16.4ab	1.63ab	0.72bc	0.29a	6.11bc	2.39ab	4.32b	1.01a
5	6.14ab	50.9a	13.5bcd	1.30bcd	0.39cd	0.50a	2.23cd	1.11bc	1.12b	0.82a
2.5	6.10ab	39.2b	10.4cd	0.99cd	0.36cd	0.28a	2.13cd	1.19bc	1.10b	0.76a
0	6.24a	56.3a	9.06d	0.89d	0.27d	0.33a	0.89d	0.78c	0.64b	1.03a
LSD	0.30	8.93	4.59	0.50	0.42	NS	5.17	1.36	8.01	NS
$p_r > F$	<0.0001	<0.0001	0.0028	0.0016	<0.0001	0.34	0.0004	0.0341	0.0009	0.81

Soil EC, NO_3–N, and NH_4–N exhibited significant interaction effects of reapplication×rate in 2004. Within columns, means followed by different letters are significantly different at $\alpha = 0.05$. NS: not significant.

were significant for EC, total N, NO_3–N, and NH_4–N levels. Levels of P and total carbon increased significantly with increasing biosolids application rate, whereas pH decreased. Levels of EC, total N, NO_3–N, and NH_4–N all increased significantly with increasing application rate on the short-term plots to which biosolids were reapplied in 2002. Similar results were observed in 2004 for soil pH, total C, EC, NO_3–N and NH_4–N (Table 3). In 2004, soil total N increased significantly with increasing biosolids application rate, but the interaction between rate and reapplication was not significant as it had been in 2003. In contrast to 2003, soil extractable P levels decreased with increasing application rate.

3.2. Microbial community structure

Principal components analysis revealed shifts in the soil microbial EL-FAME structure in response biosolids in 2003 (Fig. 1). In the PCA of combined long- and short-term plots, separation of community EL-FAME profiles by application rate was found on principal component 1 (PC 1), where soils of the control plots and plots receiving 2.5 Mg ha^{-1} biosolids grouped very closely to one another to the left of PC 1 (Fig. 1). Communities from soils of the highest treatment levels (30 and 21 Mg ha^{-1}) grouped along the positive regions of PC 1, resulting in a clear separation between the control and 2.5 Mg ha^{-1} treated soils and the soils of the two highest rate treatments. Microbial EL-FAMEs with the highest positive eigenvalues for PC 1 in 2003 were *a*15:0 (Gram-positive), 16:1ω7*c* (Gram-negative), *i*17:0 3OH (Gram-negative). The AM fungal biomarker, 16:1ω5*c*, was negatively associated with PC 1 of Fig. 1.

In 2004, PCA revealed very similar patterns in soil microbial community structure as were observed in 2003. Analysis of all plots revealed separations of community EL-FAME profiles from soils amended with 0 or 2.5 Mg biosolids ha^{-1} from the two highest application rate treatments (data not shown). Separation of soil microbial community structure patterns in 2004 were attributed to several fatty acid biomarkers, including ones that were not as significant in 2003; fatty acids with the greatest eigenvalues included those for 2003, as well as *cy*17:0 (anaerobes, Gram-negatives) and 18:1ω9*c* (*Pseudomonas*, Gram-negative bacteria).

In 2003, relative mol% change in individual EL-FAMEs in response to biosolids application rate was statistically significant for biomarkers indicative of Gram-positive bacteria (*i*15:0, *a*15:0, *i*17:0, *a*17:0) and AMF (16:1ω5*c*) (Table 4). The relative mol% of Gram-positive biomarkers increased with increasing application rate, whereas the relative abundance of 16:1ω5*c* decreased with increasing application rate. Gram-negative biomarkers (*cy*17:0, *cy*19:0, 3OH *i*13:0, 2OH 16:1, 3OH *i*17:0) and biomarkers of fungi (18:2ω6, 18:3ω6) were unaffected by biosolids application rate in 2003.

In 2004, similar trends to the previous sampling year were observed, and statistically significant differences were observed for relative mol% of AMF and Gram-positive biomarkers (Table 4). Relative mol% of Gram-positive biomarkers in the short-term plots increased significantly

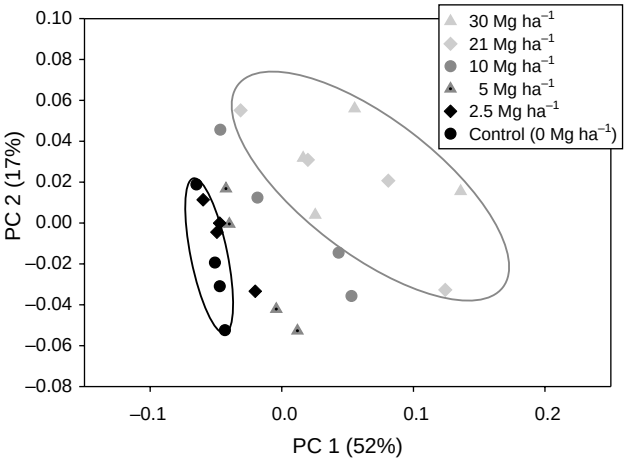

Fig. 1. Principal components analysis of soil microbial community EL-FAME profiles of all biosolids-amended plots, as measured at the Meadow Springs Ranch in 2003. Ellipses are not drawn based on statistical tests, but merely demonstrate communities separated along PC 1.

Table 4
Main effects of application rate on microbial community EL-FAME biomarkers (Gram-positive, Gram-negative, AMF and fungi)

Rate (Mg ha^{-1})	Gram + bacteria (relative mol%)	Gram − bacteria (relative mol%)	AMF (relative mol%)	Fungi (relative mol%)
2003				
30	3.2a	1.3a	0.10e	1.9a
21	3.2a	1.3a	0.20de	1.9a
10	2.7ab	1.2a	0.50cd	2.1a
5	2.4abc	1.3a	0.90c	2.1a
2.5	2.0bc	1.0a	1.40b	1.9a
0	1.8c	1.0a	2.20a	2.2a
LSD	0.08	NS	0.40	NS
$p_r > F$	0.015	0.25	<0.0001	0.79

	Gram + bacteria		Gram − bacteria	AMF	Fungi
	Short-term	Long-term			
2004					
30	14.3a	10.5a	5.40a	0.10b	12.8a
21	15.1a	9.80a	4.10ab	0.00b	11.3a
10	12.4b	10.0a	3.30bc	0.30b	11.2a
5	9.70c	9.30a	2.80bc	0.30b	12.0a
2.5	9.50c	9.30a	2.10c	1.40a	12.4a
0	10.1c	6.90a	3.10bc	1.90a	9.00a
LSD	NS	3.30	1.50	0.60	NS
$p_r > F$	<0.0001	0.309	0.007	<0.0001	0.118

Interaction of rate and reapplication was significant for Gram-positive biomarkers in 2004; biosolids were applied once in 1991 (Long-term plots) and again to only the Short-term plots in 2002. Within columns, means followed by different letters are significantly different at $\alpha = 0.05$. NS: not significant.

with increasing application rate in short-term plots only; no significant differences in Gram-positive biomarkers were detected among application rates in the long-term plots. Gram-negative biomarker relative abundance was significantly greater in plots receiving the highest application rate compared to control plots, and as in 2003, the AMF biomarker decreased nearly 20-fold from the control plots to the 30 Mg ha^{-1} treatment plots.

3.3. Vegetation

Land application of biosolids resulted in long-term changes in plant species richness, with significant reductions in the number of plant species as biosolids application rate increased (Table 5). Plant biomass increased with increasing application rate in 2003 but was unaffected by biosolids in 2004 (Table 5).

In 2003, PCA of the relativized biomass of the above-ground vegetation community revealed shifts in community structure in response to biosolids (Fig. 2). Communities of the control plots grouped tightly to the left on PC 1, and communities of the two highest levels of treatment (21 and 30 Mg ha^{-1}) grouped to the right on PC 1, resulting in a clear separation between treatments. Plant species with

Table 5
Main effects of biosolids application rate on plant community biomass (dry g m^{-2}) and species richness (number of species encountered per plot) in both 2003 and 2004 at the Meadow Springs Ranch

Rate (Mg ha^{-1})	2003		2004	
	Richness	Biomass	Richness	Biomass
30	11.0c	245ab	12.3b	43.2a
21	11.0c	268a	11.5b	45.0a
10	13.3c	241ab	12.5b	38.8a
5	17.4ab	206bc	15.6a	37.0a
2.5	16.4b	197dc	17.8a	44.8a
0	19.6a	163d	16.6a	43.2a
LSD	2.51	42.9	2.41	NS
$p_r > F$	<0.0001	0.0012	0.0002	0.22

Within columns, means followed by different letters are significantly different at $\alpha = 0.01$. NS: not significant.

the greatest eigenvalues for PC 1 included blue grama, buffalo grass (*Buchloe dactyloides* [Nutt.] Engelm.), nylon hedgehog cactus (*Echinocereus viridiflorus* Engelm.), broom snakeweed (*Gutierrezia sarothrae* [Pursh] Britt. and Rusby), plains pricklypear (*Opuntia polyacantha* Haw.), and western wheatgrass. In 2003, blue grama had a strongly negative (to the left) eigenvalue on PC 1, while western wheatgrass had a strongly positive (to the right) eigenvalue on PC 1.

In 2004, PCA of the vegetation at the Meadow Springs biosolids plots yielded similar results (data not shown). Biosolids treatment continued to result in a shift in vegetation community structure, with no statistical differentiation between long-term and short-term plots. Communities of the different treatment levels grouped tightly on PC 1. Plant species with the greatest eigenvalues for PC 1 in 2004 were the same species as in 2003, as well as hairy

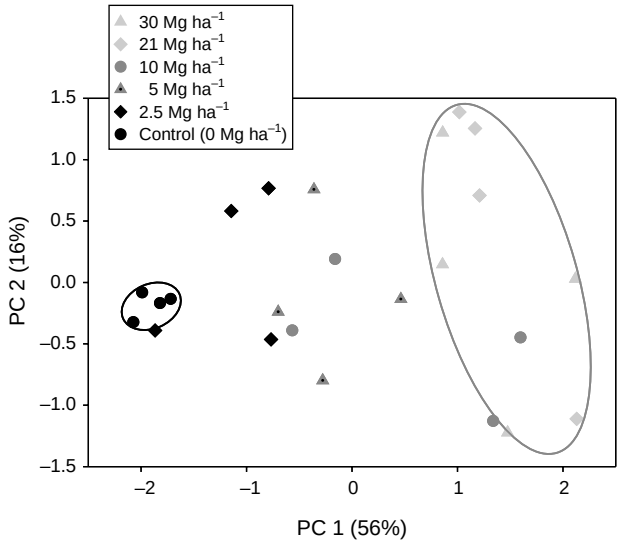

Fig. 2. Principal components analysis of plant biomass by species of all biosolids-amended plots, as measured at the Meadow Springs Ranch in 2003. Ellipses are not drawn based on statistical tests, but merely demonstrate communities separated along PC 1.

Table 6
Main effects of biosolids application rate on biomass (dry g m^{-2}) of individual plant species as measured at the Meadow Springs Ranch in 2003

Rate (Mg ha^{-1})	*Artemisia frigida* Prairie sagewort	*Bouteloua gracilis* Blue grama	*Buchloe dactyloides* Buffalo grass	*Echinocereus viridiflorus* Nylon hedgehog	*Gutierrezia sarothrae* Broom snakeweed
30	0.18a	2.57c	0.05dc	0.05c	0.13c
21	0.11a	2.63c	0.00d	0.37c	0.11c
10	0.01a	5.05ab	0.18cd	1.71c	2.79b
5	0.14a	3.79bc	0.49c	3.97bc	2.37bc
2.5	0.10a	2.96c	1.02b	6.13b	3.09b
0	0.13a	6.91a	1.76a	11.7a	6.49a
LSD	NS	1.86	0.46	4.11	2.66
$p_r > F$	0.28	0.0009	<0.0001	0.0002	0.0013
Rate	*Opuntia polyacantha* Plains pricklypear	*Agropyron smithii* Western wheatgrass	*Vulpia octoflora* Sixweeks fescue	*Hesperostipa comata* Needle-and-thread	*Heterotheca villosa* Hairy goldenaster
30	3.06c	179a	14.0e	28.0b	0.28bc
21	1.33c	142b	12.9e	94.3a	0.01c
10	6.63bc	163ab	39.9d	6.34b	0.23bc
5	4.59c	71.0c	84.8b	21.7b	1.15b
2.5	14.7ab	15.5d	105a	36.3b	2.46a
0	19.8a	22.9d	60.8c	14.5b	2.85a
LSD	9.59	35.7	18.1	48.9	1.13
$p_r > F$	0.0056	<0.0001	<0.0001	0.0206	0.0002

Within columns, means followed by different letters are significantly different at $\alpha = 0.05$.

goldenaster (*Heterotheca villosa* [Pursh] Shinners) and pairie sagewort (*Artemisia frigida* Willd.).

Further analysis of the 10 plant species primarily responsible for the shifts in community clusters on the PCA revealed individual species biomass changes in response to biosolids application rate in both 2003 and 2004 (Table 6). In 2003, blue grama, buffalo grass, nylon hedgehog cactus, broom snakeweed, plains pricklypear, sixweeks fescue, and hairy goldenaster all experienced significant decreases in species biomass from the control plots to the highest level of treatment, while western wheatgrass and needle-and-thread increased significantly at the highest levels of treatment. Western wheatgrass experienced an almost eight-fold increase from the control plots to the 30 Mg ha^{-1} plots. In 2004, only blue grama, nylon hedgehog cactus, broom snakeweed, and hairy goldenaster continued to decline in individual biomass with increasing biosolids application rate, while western wheatgrass and needle-and-thread continued to increase in biomass at the highest levels of treatment (data not shown).

3.4. Correlations

In order to evaluate the correspondence between structure of the soil microbial community and vegetative community, a Mantel procedure using the Monte Carlo test was conducted using the PC-ORD program. The Mantel test evaluates the null hypothesis of no correlation between two distance matrices that contain the same set of sample units (Ritchie et al., 2000). In this case, it was used to test the significance of correlation between microbial community EL-FAME and vegetation community structure of sample units which consists of plots receiving different rates of biosolids In both years, the structures of the two communities were significantly correlated ($r = 0.14$, $p = 0.021$ in 2003; $r = 0.14$, $p = 0.050$ in 2004).

Biomass of the dominant plant species as well as selected soil chemical parameters were correlated to varying degrees with the relative abundance of the AMF, Gram-positive and Gram-negative EL-FAME biomarkers in each year. In 2003, the AMF biomarker correlated well ($p \leq 0.05$) with 16 different variables; the greatest AMF correlation coefficients were with extractable levels of soil P ($r = -0.69$, $p < 0.0001$), buffalo grass ($r = 0.68$, $p < 0.0001$) and western wheatgrass ($r = -0.67$, $p < 0.0001$). Gram-positive biomarkers correlated well with 14 variables, with the greatest correlation coefficient observed for EC ($r = 0.54$, $p < 0.0001$). Gram-negative biomarkers correlated most strongly with total C ($r = 0.37$, $p = 0.009$).

In 2004, the AMF marker correlated well only with biomass of blue grama ($r = 0.52$, $p < 0.0001$) and western wheatgrass ($r = -0.49$, $p < 0.0001$). Gram-positive biomarkers correlated well with seven variables, the greatest of which were blue grama ($r = -0.52$, $p = 0.0002$), pH ($r = -0.51$, $p = 0.0002$), and EC ($r = 0.51$, $p = 0.0002$). Gram-negative biomarkers correlated well with seven variables, the greatest of which were EC ($r = -0.49$, $p = 0.0004$) and total soil nitrogen ($r = 0.44$, $p = 0.002$).

4. Discussion

Few other long–term studies or studies conducted on semi–and environments have examined the overall structure

of the soil microbial community in direct response to biosolids application. In studies by Lawlor et al. (2000) and Dennis and Fresquez (1989), shifts in the microbial community structure were detected as a result of biosolids application. Lawlor et al. (2000) found that with FAME and other microbial analyses of biosolids treated plots, overall diversity and biomass changed little, whereas individual species, functions, and specific microbial parameters underwent fluctuations. Similarly, Dennis and Fresquez (1989) found that despite increases in most measured microbial populations with application, fungal diversity initially decreased, and later rebounded to above control levels. In this study, parallel changes in the structure of the soil microbial community and the plant community, as reflected in the correlations between the two communities, could be due to a number of factors influencing feedback. Shifts in individual plant species' leaf area characteristics, carbon allocation strategies (root-to-shoot ratio), relative growth rates, and nutrient concentrations are all traits that determine trophic structure, both above- and below-ground, and can alter feedback between the two systems (Wardle, 2004). During the 12 years since the first biosolids application, feedback between plant and soil microorganisms presumably continue to result in significant shifts in the structure of the microbial populations under different levels of biosolids application rates, as reflected in the separation of soil microbial communities among treated and control plots by PCA analysis.

One group of microorganisms whose relative abundance in soil has appeared to shift in response to biosolids is the AMF, whose biomarker EL-FAME had highly negative eigenvalues on both PC 1 and PC 2 in both years. Further analysis of the individual biomarkers revealed increases in the relative abundance of Gram-positive and Gram-negative EL-FAMEs, whereas the relative abundance of AMF biomarkers decreased in response to biosolids. This trend of increase in bacterial biomarkers as resource quality and quantity increases is consistent with the findings of Porazinska et al. (2003), who measured greater levels of Gram-positive PLFA biomarkers in a Konza prairie soil with elevated nitrogen content and enhanced resource quality as a result of plant species composition and community shifts. The decreasing AMF biomarker and increasing bacterial biomarkers is also consistent with the concept that soil microbial communities of infertile ecosystems (represented here by the control plots) are frequently dominated by fungi and those of more fertile, productive ecosystems (represented here by the highest level of biosolids treatment) are primarily dominated by bacteria (Kourtev et al., 2003; Grayston et al., 2004; Wardle et al., 2004). While we cannot definitively explain why the relative mol% quantities of microbial biomarkers varied between sampling years, the variation may be due to year-to-year differences in soil moisture content, plant community composition, or perhaps to differences in sampling methods. However, the fact that we saw consistent shifts in microbial communities over two sampling years, despite that different sampling methods were employed over the 2-year study, only strengthens our findings regarding long- and short-term responses of microbial communities to single and repeated biosolids applications, respectively.

Among other environmental factors, AMF are sensitive to increased P content of soils (Weissenhorn et al., 1995; Linderman and Davis, 2004; Arnold and Kapustka, 1987) and heavy metals (Leyval et al., 1997), which could be factors in the negative response observed in this study. Extractable soil P levels did increase with increasing biosolids application rate in 2003, but the opposite trend occurred in 2004. Increased nutrient mobility due to wetter soil conditions in 2004, coupled with increased above-ground biomass production and microbial activity (Sullivan et al., 2005) may have led to increased biological immobilization of P and thus lower extractable P levels. In addition, shifts in plant community composition, such as those observed in this study, have resulted in altered AMF abundance and root colonization potential (Gange et al., 1990; Eom et al., 2000; Jeffries et al., 2003; Kourtev et al., 2003).

The EL-FAME method employed in this study is a measure of microbial fatty acids in soil, and considering that AMF are obligate symbionts whose life cycle depends on the host plant roots, it has been suggested that the only truly appropriate location for functional studies of these symbionts is the plant root itself (Jeffries et al., 2003). In a study on the same experimental plots that were the subject of this research project, Barbarick et al. (2004) found that mycorrhizal colonization of blue grama roots increased significantly in the 30 Mg ha^{-1} plots, 23% over the control plots, suggesting that the measures used in this study were inadequate to assess AMF function (i.e. root colonization). However, several studies have found that individual plant species' responses to mycorrhizae vary, depending on environmental circumstances (Eom et al., 2000; Klironomos et al., 2000; Kourtev et al., 2003). Of note is a particular study by Arnold and Kapustka (1987) in which four different plant species (*Geranium* L., *Zea mays* L., *Barbarea vulgaris* R. Br., and *Cirsium arvense* [L.] Scop.) were exposed to biosolids applications. Mycorrhizal colonization did not differ significantly between control and biosolids treated plants for all but one of the test species; *Geranium* L. experienced significantly lower colonization under biosolids treatment than in the control. In addition, a study by Gange et al. (1990), which examined mycorrhizal benefit in an early successional plant community, found mycorrhizal colonization in all tested plant species roots, despite the fact that three species (*Conyza canadensis* [L.] Cronq., *Spergula arvensis* L., and *Stellaria media* [L.] Vill.) were found in a previous study by Harley and Harley (1987) to be non-mycorrhizal. Because different plant species may have different colonization reactions depending on environmental influences, including biosolids application, a measure such as the one used in this study, which does not rely on

the selection of an indicator plant, may be a better ecosystem measure of the response of AMF to biosolids.

A relationship between plant community and AMF biomarker abundance in soils was observed in this study; AMF biomarker in both years was positively correlated with blue grama and negatively correlated with western wheatgrass. The individual species biomass of blue grama was significantly reduced with increasing biosolids application rate, while the reverse was true for western wheatgrass. The botanical and ecological characteristics of these two plant species present a noteworthy contrast in the context of this study. Blue grama is a densely tufted, native, perennial, warm-season grass (Coupland, 1950; Gould, 1979) that exhibits fairly shallow rooting systems, avoids soil salinity (Bowman, et al., 1985), and is rarely tolerant of soil acidity (Wasser, 1982). On the other hand, western wheatgrass is a native, perennial, cool-season grass (Quinnild and Cosby, 1958) capable of aggressive sod-forming due to an abundance of branched rhizomes (Barker and Whitman, 1988), and is highly tolerant of soil salinity (Bultsma and Haas, 1989). Western wheatgrass may have experienced a competitive advantage in this system for several reasons, including: (1) early emergence allowing this species to take advantage of biosolids nutrients earlier in the growing season; (2) salinity tolerance as soil EC was slightly elevated by increasing application rate; and (3) rhizomatous root growth, allowing resource allocation across sites that may have been unavailable to species lacking this growth form. Blue grama is highly dependent on AMF hyphal elongation for resource allocation (Barbarick et al., 2004), but is also sensitive to rising soil salinity and decreasing soil pH, all of which may have put this species at a disadvantage under increasing biosolids application rate. However, both species are considered good forage with high nutrient content for both livestock and wildlife (Dittberner and Olson, 1983), whereas western wheatgrass has the added advantage of stabilizing soil (Day and Ludeke, 1987) and is often considered an indicator of rangelands in good condition (Enevoldsen and Lewis, 1978). The results of this study could simply indicate a shift in community structure that is not detrimental, but more an expedited 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 associations. The increase in plant biomass in response to biosolids, as well as increased microbial mineralization activities under the highest biosolids application rates (Sullivan et al., 2005), suggest that overall, ecosystem functions were enhanced despite the decrease in what is generally seen as a beneficial symbiotic relationship.

The City of Fort Collins is currently applying biosolids at the 2.5 Mg ha^{-1} application rate to the majority of the Meadow Springs Ranch, and this study found few significant impacts of this rate on soil microbial community structure or plant productivity and species composition. With regards to our original hypotheses, both plant and soil microbial community structures were affected by single and repeated applications of biosolids at greater rates to semi-arid rangeland soil. Plant biomass was not consistently affected both years of the study, however. We also hypothesized that AMF would positively respond to biosolids, but it appeared that the shift in plant communities to dominance by western wheatgrass, a facultative mycotroph, resulted in a decline in the biomarker for AMF in soil. To confirm that AM fungi in the soil are being affected by biosolids, we are conducting additional studies to clarify long-term effects of biosolids on mycorrhizal root colonization potential of blue grama and western wheatgrass.

In conclusion, both the soil microbial community and plant community exhibited structural changes, and the effects of a single application at relatively high rates proved to be long-term. Shifts in the soil microbial community structure were directly attributed to increases in relative abundance of bacterial biomarkers, as is often the case in higher fertility soils. On the other hand, higher application rates also resulted in significant declines of the AMF biomarker. This decrease in AMF biomarker was positively correlated with biomass of blue grama, but negatively correlated with western wheatgrass, whose biomass increased with increasing biosolids application rate. Parallel differences in these two communities may be indicative of shifting resource allocation and competitive strategies, resulting in complex microbial–plant interactions and indicating the need for more long-term studies of above- and below-ground communities to determine ecosystem effects of biosolids land-based recycling programs.

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