

The soil microbial community in a sewage-sludge-amended semi-arid grassland *

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Summary. The value of sewage sludge for improving the fertility and productivity of a degraded semi-arid grassland soil was tested by quantifying and describing the effects of surface application of sewage sludge on soil chemical properties and the soil microbial community. Three surface application rates (22.5, 45, and 90 Mg sludge ha⁻¹) were tested over the course of two growing seasons. Most nutrient levels, including N, P, and K, increased linearly with increasing sludge application rates. Soil pH, however, declined linearly, from 7.8 to 7.4, with increasing sludge application rates. With the exception of Zn, heavy metals, including Cd, did not increase with the small decrease in pH or with increasing sludge application rates. Soil bacterial, fungal, and ammonium oxidizer populations increased linearly with increasing sludge application rates, and *Streptomyces* spp. populations remained relatively unchanged. The diversity of fungal groups declined initially with increasing sewage sludge rates but rebounded to near pretreatment levels under the low and intermediate application rates within 1 year. High fungal populations and low fungal diversity were related to the high nutrient contents provided by sludge amendment. *Mucor* spp. and *Penicillium chrysogenum* dominated the sludge-amended soils, and their densities in the treated soils in the first growing season were almost directly proportional to the sludge application rates. The improvement in soil fertility of a degraded semi-arid grassland due to sludge application was reflected in populations, diversity, and composition of the soil microbial community.

Key words: Bacteria – Fungi – Fungal diversity – *Streptomyces* spp. – Ammonium oxidizers – Semi-arid rangelands

Degraded semi-arid rangelands are characterized by intensified drought effects and soils low in organic matter (Branson 1985). A reduced availability of both soil moisture and organic matter may limit microbial populations and their activities and, thus, the availability of plant nutrients (Clark 1977; Coleman et al. 1983). Soil water availability is generally considered the principal determinate of semi-arid grassland productivity (Dodd and Lauenroth 1979), followed by N and P (Lauenroth and Sims 1976). N and P fertilizers can increase soil fertility and grassland productivity, especially when soil moisture is adequate (Black and Wight 1979; Detling 1979). However, the use of such fertilizers on semi-arid rangelands cannot be justified because they are costly and dependent on precipitation for effectiveness. In contrast to synthetic fertilizers, organic amendments are relatively inexpensive and less dependent on precipitation in that they provide a mulch layer in addition to nutrients. Thus, in areas of irregular yet intense precipitation, a surface-applied organic fertilizer-mulch is likely to be more effective than an inorganic fertilizer in improving the fertility and productivity of degraded semi-arid rangelands. Stabilized sewage sludge may be particularly well suited to this use (Fresquez et al. 1988a).

Although the organic and nutrient value of sewage sludge varies widely with source, treatment, and application rates, a typical sewage sludge provides soil with significant additions of micronutrients, as well as the obvious contribution of organic matter, N, P, and K (Sommers 1977; McCaslin and O'Connor 1982). The N-P-K content of sewage sludge, for example, is

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roughly equivalent to a 4-2-1 commercial mixture (Elliott 1986), and sewage sludge typically has higher nutrient contents than other types of sludges or other traditional organic amendments such as hay and manure (Sommers 1977; Atalay and Blanchar 1984). Also, sewage sludge is considered a slow-release fertilizer because many of its nutrients are carried predominately in organically bound forms that must be mineralized to inorganic forms prior to plant uptake (Sommers 1977). For these reasons, sewage sludge applications, whether incorporated into the soil or applied on the surface, generally result in improved soil chemical and physical conditions (Khaleel et al. 1981). Improvements in the availability of plant nutrients, soil water-holding capacity, and organic-matter contents in sewage-sludge-amended soils usually lead to increases in plant productivity (Elliott 1986). However, one major drawback to the use of sewage sludge on lands used for the purpose of increasing plant productivity is the introduction of potentially phytotoxic heavy metals like Cu, Pb, Zn, and especially Cd (Chaney 1983).

Historically, range reclamation research has focused on improving range productivity via fertilization (Power and Alessi 1971; Dodd and Lauenroth 1979; Wight and Black 1979) and physical land treatments designed to reduce erosion and increase infiltration (Aldon 1966; Aldon and Garcia 1972). The success of these attempts has traditionally been monitored in terms of above-ground plant biomass production. Dried, anaerobically digested sewage sludge has been used extensively in mine-spoil reclamation (Fresquez and Lindemann 1983), and could potentially accomplish the goals of rangeland reclamation through physical (reduction of rain-splash and sheet-flood erosion and subsequent increases in infiltration and water retention), chemical (addition of soil nutrients), and biological actions (addition of available C required by soil microbial communities to process nutrients). Moreover, improvements in soil fertility and productivity are likely to be more readily demonstrated by below-ground ecosystem responses than by the traditional above-ground approach (Elkins et al. 1984; Frequez et al. 1986; Parker et al. 1987).

The objective of the present study was to determine the responses of microbial populations, and the diversity and composition of fungal groups in degraded semi-arid grassland soils as affected by the addition of anaerobically digested sewage sludge.

Materials and methods

Study site. In June 1985, dried, anaerobically digested sewage sludge obtained from the treatment facility in Albuquerque, New Mexico, was surface-applied to a degraded semi-arid grassland site

70 km northwest of Albuquerque. The vegetative community on the site was classified as a *Gutierrezia sarothrae* / *Bouteloua gracilis* *Hilaria jamesii* (snakeweed/blue grama-galleta) type, a prevalent and representative degraded grassland in the Rio Puerco Study Area (Francis 1986). Sewage sludge was applied at rates of 0, 22.5, 45, and 90 Mg ha⁻¹ to each of four 3- \times -20-m plots, in a randomized complete block design of 16 plots. The area was fenced to prevent grazing.

Soil analyses. Soil samples were taken from non-rhizosphere soil from each of the 16 plots in June 1985 (pretreatment), August 1985, and August 1986. Five systematically located subsamples from each plot were taken from the 0–15 cm depth with a 5-cm-diameter bucket auger. Subsamples were composited in sealable, sterile plastic containers, placed in an ice-chest, and transported back to the laboratory where they were passed through a 2-mm sieve.

All 16 composite samples from each of the three sampling periods were analysed for numerous chemical and physical properties. Soil pH, electrical conductivity, and water-soluble cations (Na, Ca, Mg, and K) were determined from saturated paste extracts (USDA 1954). The DTPA (diethylenetriaminepentaacetic acid) method was used to extract levels of Zn, Fe, Mn, Cu, Cd, Pb, and Al, while B was extracted with hot water (Association of Official Analytical Chemists 1980). Heavy metals and soluble cations were determined by atomic absorption; available P was determined by Olson's procedure using NaHCO₃ as an extractant; NH₄⁺-N was determined colorimetrically, using a 1:5 soil: KCl extract; and NO₃⁻-N was determined via Cd reduction, using a 1:5 soil: water extract (EPA 1974). Total Kjeldahl N was determined by the micro-Kjeldahl procedure (USDA 1972). The percentage of organic matter was determined by the Walkley-Black procedure (Allison 1965), and soil texture was determined by the hydrometer method (USDA 1972). A comparison of the chemical and physical properties of the pretreatment soil and the applied sewage sludge is given in Table 1.

Microbial analyses. Subsamples for microbial analysis were obtained from composite soil samples taken in August 1985 and 1986. All microbial enumeration analyses were conducted within 2 weeks of sample collection. Soil samples were stored at 4 °C during any intervening period.

Microbial populations were estimated by the dilution and plating technique described by Wollum (1982). Dilutions were plated in triplicate on nutrient agar for aerobic heterotrophic bacteria, starch-nitrate-casein agar (Küster and Williams 1964) for *Streptomyces* spp. and rose-bengal-streptomycin agar (Martin 1950) for fungi. The plates were incubated for 7 days at 25 °C for bacteria and fungi, and 10 days for streptomycetes. Ammonium oxidizer (*Nitrosomonas* spp.) populations were estimated by the most-probable-number method (Alexander and Clark 1965), using an ammonium carbonate medium and five replicates per dilution. The culture tubes were read after 6 weeks of incubation at 25 °C. All microbial populations are reported per gram of oven-dry soil.

The diversity of fungal groups was determined from soil subsamples obtained from the August 1985 and 1986 soil samples. Subsamples were composited within treatments, generating four composite samples per year. Fungal groups were isolated by placing 1 ml of a 10⁻³ dilution from a 10-g oven-dry weight-equivalent sample in a Petri dish, adding cooled rose-bengal-streptomycin agar, and swirling for an even distribution. Ten plates were inoculated for each composited soil sample and incubated at 25 °C for 7 days. After incubation, we transferred a portion of every colony appearing on the plates to a carrot agar medium (E.E. Staffeldt, 1979, unpublished data). After a 4-day incubation period, the colonies were identified according to the taxonomic keys of Barron (1968), Gilman (1968), Barnett and Hunter (1972), and Domsch et al. (1980).

Table 1. Soil chemical and physical properties of the pretreatment soil (means from 16 plots) and dried sewage sludge

	pH	EC (dS m ⁻¹)	OM (g kg ⁻¹)	Sand (g kg ⁻¹)	Silt (g kg ⁻¹)	Clay (g kg ⁻¹)	Tex- ture	SAR	Water-soluble cations			
									Na (μg g ⁻¹)	Ca (μg g ⁻¹)	Mg (μg g ⁻¹)	K (μg g ⁻¹)
Soil	7.8	0.39	12.53	444	256	300	cl	0.15	4.60	52.10	13.58	5.47
Sludge	6.8	18.01	—	860	68	72	ls	16.83	586.96	58.12	20.67	769.88
Soil N and P				Heavy and trace metals								
	NO ₃ ⁻ -N (μg g ⁻¹)	NH ₄ ⁺ -N (μg g ⁻¹)	TKN (μg g ⁻¹)	P (μg g ⁻¹)	Zn (μg g ⁻¹)	Fe (μg g ⁻¹)	Mn (μg g ⁻¹)	Cu (μg g ⁻¹)	Cd (μg g ⁻¹)	Pb (μg g ⁻¹)	Al (μg g ⁻¹)	B (μg g ⁻¹)
Soil	2.0	3	733	4	0.2	3.5	3.8	1.3	0.01	0.8	0.1	0.02
Sludge	7.0	3681	48600	1599	150.0	259.2	11.8	47.5	0.24	3.3	0.1	0.10

EC, electrical conductivity; OM, organic matter; s, sand; c, clay; l, loam; TKN, total Kjeldahl N; SAR, Sodium adsorption ratio

Diversity was estimated using Shannon's index of species diversity (Zar 1974), which estimates community richness (H') as:

$$H' = \sum_{i=1}^k p_i \log p_i, \quad (1)$$

where $p_i = n_i/N$ or the ratio of isolates in the group "i" to the total number of isolates in the sample and k = the total number of groups. Evenness was estimated by:

$$J = H'/H'_{\max}, \quad (2)$$

where H' = the diversity index value and $H'_{\max} = \log k$ or the theoretical maximum diversity possible (assumes each group has only one isolate).

The similarity in fungal composition among sample populations was estimated using Sørensen's presence community coefficient (SPCC), described by Mueller-Dombois and Ellenberg (1974) as:

$$SPCC = 200C/A+B, \quad (3)$$

where C is the total number of groups common to the two samples, A is the total number of groups in sample A , and B is the total number of groups in sample B . If the two samples contained the same fungal groups, the community coefficient would be 100, whereas if they had no groups in common, the coefficient would be 0.

Statistical analyses. Variations in the chemical properties of the soil, and in the populations of soil microorganisms between unamended (control) and the sewage-sludge-amended soils, were analyzed using a two-way analysis of variance based on treatment and years at the 5% probability level. Orthogonal polynomials were used to characterize the relationship between the sludge application rates and soil chemical and microbiological parameters. Where results were inconsistent between different years (i.e., soil P and the ammonium oxidizers), each year was analyzed separately using a one-way analysis of variance.

Results and discussion

All the soil chemical properties that changed significantly with the application of sewage sludge are shown in Table 2. With the exception of organic matter and

pH, all other soil chemical properties increased linearly with increasing sludge application rates. Soil organic matter showed a quadratic response to increasing sludge rates, peaking at the middle rate. Important plant nutrients like levels of N (NH₄⁺-N, NO₃⁻-N, total Kjeldahl N) and P, continued to increase in the 2nd year after treatment. Soil P, for example, increased at a higher rate in the second growing season than in the first growing season with increasing sludge rates. Most other soil chemical parameters remained at the same levels or declined after initially rapid 1st-year increases. These soil chemical properties, however, remained significantly elevated relative to control levels.

The small decrease in soil pH values from 7.8 to 7.4 with an increase in sludge application rates was primarily linear, with the decrease becoming smaller at the higher sludge application rates. Albuquerque sewage sludge had a pH value of 6.8, a full pH unit lower than the soil receiving treatment (Table 1). The lowering of the soil pH with sewage sludge application was probably due to leachates from this slightly acidic sludge, and also to subsequent acid-producing microbial reactions in the soil (Miller 1973).

Cation solubility generally increases with decreasing pH in alkaline soils (Bohn et al. 1979). In this respect, some researchers consider sewage sludge to be a natural chelator for certain cations (Elliott 1986). However, the pH at which micronutrients and heavy metals begin to solubilize starts around 7.0 (Kiekens 1983). McCaslin and O'Connor (1982) recently demonstrated that the increase in soluble Fe and Zn following applications of Albuquerque sludge to an alkaline soil was due principally to the sewage sludge directly supplying soluble organometal complexes as opposed to solubilizing existing soil metals. In the present study, Zn was the only micronutrient that showed significant changes following sludge application. Al-

Table 2. Soil chemical properties that showed significant changes following sewage-sludge application (Mg ha^{-1}) measured in August 1985 and 1986 (\pm SE)

Soil properties	August 1985				August 1986			
	0	22.5	45	90	0	22.5	45	90
pH	7.8 (0.05)	7.7 (0.02)	7.6 (0.02)	7.5 (0.06)	7.8 (0.04)	7.6 (0.08)	7.5 (0.05)	7.4 (0.11)
EC (dS m^{-1})	0.37 (0.03)	1.06 (0.11)	1.66 (0.22)	2.23 (0.35)	0.37 (0.03)	0.96 (0.19)	1.26 (0.11)	1.97 (0.49)
SAR	0.40 (0.09)	0.45 (0.04)	0.66 (0.01)	0.89 (0.04)	0.36 (0.06)	0.53 (0.08)	0.48 (0.04)	0.75 (0.14)
OM (g kg^{-1})	11.7 (0.5)	12.9 (0.7)	13.9 (0.8)	12.2 (0.4)	13.4 (0.6)	14.7 (0.6)	14.5 (0.4)	12.1 (0.7)
Na ($\mu\text{g g}^{-1}$)	11.0 (2.3)	22.1 (3.0)	39.1 (2.5)	59.1 (4.6)	10.6 (2.1)	26.9 (7.1)	26.9 (3.2)	54.7 (15.0)
Ca ($\mu\text{g g}^{-1}$)	42.5 (4.0)	131.5 (13.6)	197.4 (27.3)	251.5 (47.9)	46.3 (4.8)	130.7 (24.8)	165.1 (13.4)	259.7 (63.3)
Mg ($\mu\text{g g}^{-1}$)	10.9 (1.0)	29.3 (2.7)	45.8 (7.2)	56.1 (8.0)	12.8 (0.6)	33.3 (7.1)	44.0 (3.8)	71.4 (17.5)
K ($\mu\text{g g}^{-1}$)	6.3 (1.2)	10.2 (1.6)	17.2 (2.0)	19.2 (1.6)	4.3 (1.2)	9.0 (2.7)	12.5 (0.6)	19.9 (6.3)
NO_3^- -N ($\mu\text{g g}^{-1}$)	2.2 (0.2)	21.8 (3.5)	41.8 (11.7)	61.3 (16.7)	0.8 (0.1)	10.0 (2.4)	28.1 (4.2)	53.8 (7.8)
NH_4^+ -N ($\mu\text{g g}^{-1}$)	3.1 (0.6)	9.4 (4.8)	19.6 (5.7)	51.0 (18.3)	3.3 (0.1)	10.3 (1.0)	22.4 (2.8)	39.1 (5.8)
TKN ($\mu\text{g g}^{-1}$)	729 (30)	817 (41)	845 (51)	924 (36)	665 (28)	828 (44)	843 (60)	987 (76)
P ($\mu\text{g g}^{-1}$)	4.9 (0.7)	14.5 (2.9)	19.6 (1.4)	31.1 (3.3)	4.0 (0.4)	20.4 (3.6)	43.9 (3.8)	71.5 (9.9)
Zn ($\mu\text{g g}^{-1}$)	0.29 (0.03)	0.35 (0.02)	0.61 (0.03)	0.76 (0.14)	0.27 (0.05)	0.79 (0.21)	1.01 (0.13)	1.20 (0.50)

For abbreviations see Table 1

though the Zn concentration in the soil treated with sewage sludge was significantly higher than the unamended soil, the highest Zn concentrations measured were still far below $40 \mu\text{g g}^{-1}$ DTPA soil Zn that is considered phytotoxic (Chaney 1983). In fact, the normal levels of DTPA Zn in soil range from 0.5 – $20 \mu\text{g g}^{-1}$ (Bohn et al. 1979). Thus, this essential plant micronutrient appears to be present at levels just above that required for optimum plant growth with sewage sludge amendment applied at the highest level. Other trace metals that were noticeably unchanged following sludge application were Fe, Mn, Cu, Cd, Pb, Al, and B. This is an important consideration because concerns over heavy metals, particularly Cd levels, frequently limit sewage sludge applications (Elliott 1986).

Average soil bacterial, fungal, and especially ammonium oxidizer populations increased linearly with increasing sewage sludge application rates. Although, *Streptomyces* spp. populations in the amended treatments remained relatively unchanged in 1985, the amended treatments still contained significantly higher *Streptomyces* spp. populations than the control (Table 3). In August 1986, *Streptomyces* spp. populations showed a quadratic response, with the light and intermediate treatment levels containing the highest popu-

lations of *Streptomyces* spp., and the control and highest treatment level containing the lowest. Fungal populations, in contrast, were significantly higher in the sewage-sludge-amended soils compared with the unamended control in both years. In contrast, the diversity of fungal groups decreased with increased sewage sludge application rates (Table 4). This may have been due to the rapid proliferation of *Mucor* spp. relative to the other fungal groups. By August 1986, some native soil fungi had rebounded to pretreatment levels in the low and intermediate treatment levels as evidenced by an increase in diversity despite sustained high population counts (Table 3).

Generally, fungal diversity indices are lower in communities under soil stress (as opposed to a moderately low fertility status), or soils high in nutrient levels (Guillemat and Montegut 1960; Joffe 1967; Fresquez and Aldon 1984; Fresquez et al. 1986; Fresquez et al. 1988b). When soils are then enriched with nutrients, as in the present study, fungal populations generally increase, and fungal diversity usually decreases. Typically, microbial species that are better adapted to the enriched conditions respond with increasing population sizes (Atlas and Bartha 1981). These relationships generally hold true for plant com-

Table 3. Soil microbial populations in an untreated soil and sewage-sludge-amended soils (Mg ha^{-1}) measured in August 1985 and 1986 (\pm SE)

Microbial populations	August 1985				August 1986			
	0	22.5	45	90	0	22.5	45	90
Aerobic heterotrophic bacteria (10^5 g^{-1})	33 (5.5)	54 (7.6)	72 (7.0)	132 (2.3)	23 (3.7)	44 (7.2)	51 (6.2)	89 (5.4)
Fungal propagules (10^3 g^{-1})	18 (1.7)	43 (6.0)	50 (4.3)	48 (11.1)	16 (1.7)	39 (6.7)	42 (8.1)	38 (5.4)
<i>Streptomyces</i> spp. (10^5 g^{-1})	76 (11.4)	100 (8.9)	107 (18.8)	108 (9.6)	33 (4.9)	53 (5.8)	45 (5.5)	29 (1.2)
Ammonium oxidizers (10^2 g^{-1})	14 (1.3)	16 (8.3)	142 (26.3)	129 (37.3)	1 (0.6)	60 (14.1)	212 (74.3)	893 (353.3)

Table 4. Distribution and relative density of fungal groups in an untreated soil and sewage-sludge-amended soils (Mg ha^{-1}) measured in August 1985 and 1986

Fungal groups	August 1985				August 1986			
	0	22.5	45	90	0	22.5	45	90
<i>Absidia</i> spp.	—	—	—	—	—	—	1	2
<i>Acremonium</i> spp.	4	—	—	—	4	—	—	—
<i>Alternaria</i> spp.	3	—	—	—	—	2	2	—
<i>Ascomycete</i> spp.	4	—	—	—	3	5	4	7
<i>Aspergillus</i>								
<i>alliaceous</i>	6	—	—	—	—	—	—	—
<i>candidus</i>	(24)	—	—	—	—	2	2	2
<i>flavus</i>	—	—	1	—	—	—	—	—
<i>fumigatus</i>	(9)	12	3	—	8	30	11	20
<i>ochraceous</i>	—	—	—	—	(38)	(43)	15	(25)
<i>Cephalophora</i> spp.	—	—	—	—	—	—	9	3
<i>Chaetomium</i> spp.	4	1	—	—	4	5	1	1
<i>Chrysosporium</i>								
<i>irregularis</i>	2	—	—	—	1	—	—	—
<i>Curvularia</i> spp.	—	—	—	—	1	—	—	—
<i>Fusarium</i> spp.	7	15	16	3	22	(45)	(41)	5
<i>Gliocladium</i> spp.	—	—	—	—	—	—	1	—
<i>Humicola</i> spp.	1	—	—	—	—	—	—	—
<i>Mucor</i> spp.	—	(44)	(121)	(224)	5	6	8	9
<i>Mycelia sterilia</i>	5	—	—	—	3	2	—	—
<i>Pencillium</i>								
<i>chrysogenum</i>	—	(26)	(65)	(32)	—	—	15	6
<i>cyclopium</i>	(32)	(26)	18	8	(39)	(50)	25	(26)
<i>frequentans</i>	—	—	—	—	—	1	2	1
<i>funiculosum</i>	2	—	—	—	1	27	8	11
<i>janthinellum</i>	—	—	—	—	—	—	(104)	(223)
<i>lilacinum</i>	—	—	—	—	1	—	—	—
<i>monoverte</i>	—	—	—	—	8	2	21	4
<i>Scopulariopsis</i> spp.	—	1	—	—	—	—	22	4
<i>Sepdonium</i> spp.	—	—	—	—	2	—	1	—
Unidentified isolate	4	—	—	—	—	—	—	—
No. of isolates	107	125	224	267	140	220	293	349
No. of groups	14	7	6	4	16	13	19	16
Diversity	0.943	0.685	0.506	0.242	0.876	0.871	0.967	0.658
Evenness	0.823	0.811	0.650	0.402	0.727	0.782	0.756	0.547

of the sludge itself by the soil microbial community also contributed to the overall amounts of nutrients available for plant growth. One net effect was the significant increases in plant-available N and reductions in the C:N ratio in the treated soils. While pretreatment C:N ratios in this semi-arid grassland soil were within the limits for effective decomposition of organic substrates (Pugh 1974), the low C and N levels probably limited the amounts of microorganisms that could be supported (Taylor 1979; Fresquez et al. 1988a). Increased microbial populations and a decrease in the diversity of fungal groups following the sewage sludge applications indicated that the C and N limitation was at least reduced, if not eliminated.

Conclusions

Significant increases in microbial populations and in many macro- and micro-nutrients with the application of sewage sludge suggest that the surface applications of dried sewage sludge may have improved the fertility of this degraded semi-arid grassland soil. The improvement in soil fertility was reflected in the populations, diversity, and composition of the soil microbial community, and, later, in the quantity and quality of above-ground plant biomass produced on these same sewage-sludge-amended plots (Fresquez, Francis and Dennis 1989, unpublished data). Moreover, the continued increases in N and P during the 2nd year after the sludge applications combined with the maintenance of high fungal populations, despite recovering but low fungal diversities, suggest that a single sludge application has the potential to serve as a low-grade, slow-release fertilizer for this soil for several years.

References

- Aldon EF (1966) Deferred grazing and soil ripping improves forage on New Mexico's Rio Puerco drainage. *NM Stockman* 31:44–46
- Aldon EF, Garcia G (1972) Vegetation changes as a result of soil ripping in the Rio Puerco in New Mexico. *J Range Manage* 25:381–383
- Alexander M, Clark FE (1965) Nitrifying bacteria. In: Black CA et al. (eds) *Methods of soil analysis*. Am Soc Agron, Madison, Wisc, pp 1477–1483
- Allison LE (1965) Organic carbon. In: Black CA et al. (eds) *Methods of soil analysis*. Am Soc Agron, Madison, Wisc, pp 1367–1378
- Association of Official Analytical Chemists (1980) *Official methods of analysis*, 13th edn. AOAC, Washington, DC
- Atalay A, Blanchar RW (1984) Evaluation of methane generator sludge as a soil amendment. *J Environ Qual* 13:341–344
- Atlas RM, Bartha R (1981) *Microbial ecology: fundamentals and applications*. Addison-Wesley, Menlo Park
- Barnett HL, Hunter BB (1972) *Illustrated genera of imperfect fungi*, 3rd edn. Burgess Publishing, Minneapolis
- Barron GL (1968) The genera of *Hyphomycetes* from soil. Williams & Wilkins, Baltimore MD
- Biondini ME, Redente EF (1986) Interactive effect of stimulus and stress on plant community diversity in reclaimed lands. *Reclam Reveg Res* 4:211–222
- Black AL, Wight JR (1979) Range fertilization: Nitrogen and phosphorus uptake and recovery over time. *J Range Manage* 32:349–353
- Bohn H, McNeal B, O'Conner G (1979) *Soil chemistry*. Wiley, New York
- Branson FA (1985) Vegetation changes on western rangelands. Soc Range Manage, Denver, Colo (Range monogr. No. 2)
- Chaney RL (1983) Potential effects of waste constituents on the food chain. In: Parr JF et al. (eds) *Land treatment of hazardous wastes*. Noyes Data Corporation, Park Ridge, NJ, pp 13–22
- Clark FE (1977) Internal cycling of ¹⁵nitrogen in a shortgrass prairie. *Ecology* 58:1322–1333
- Coleman DC, Reid CPP, Cole CV (1983) Biological strategies of nutrient cycling in soil systems. *Adv Ecol Res* 13:1–55
- Cooke WG, Pipes WO (1970) The occurrence of fungi in activated sludge. *Mycopathol Mycol Appl* 40:429–470
- Detling JK (1979) Processes controlling blue grama production on the shortgrass prairie. In: French N (ed) *Perspectives in grassland ecology*. Springer, New York Heidelberg Berlin, pp 25–40
- Dodd JL, Lauenroth WK (1979) Analysis of the response of a grassland ecosystem to stress. In: French N (ed) *Perspectives in grassland ecology*. Springer, New York Heidelberg Berlin, pp 43–58
- Domsch KH, Gams W, Anderson T (1980) *Compendium of soil fungi*. Academic Press, London
- Elkins NZ, Parker LW, Aldon EF, Whitford WG (1984) Responses of soil biota to organic amendments in stripmine spoils in northwestern New Mexico. *J Environ Qual* 13:215–219
- Elliott HA (1986) Land application of municipal sewage sludge. *J Soil Water Conserv* 1:5–10
- EPA (1974) *Methods for chemical analysis of water and wastes*. National Environmental Research Center, Cincinnati, Ohio (No. 6004-79-020)
- Francis RE (1986) Phyto-edaphic communities of the Upper Rio Puerco Watershed, New Mexico. USDA Forest Service Research Paper RM-272, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colo
- Fresquez PR, Aldon EF (1984) Distribution of fungal genera in stockpiled topsoil and coal mine spoil overburden. U.S. Department of Agriculture Forest Service Research Note RM-447, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colo
- Fresquez PR, Lindemann WC (1983) Greenhouse and laboratory evaluations of amended coal-mine spoils. *Reclam Reveg Res* 2:205–215
- Fresquez PR, Aldon EF, Lindemann WC (1986) Microbial reestablishment and the diversity of fungal genera in reclaimed coal mine spoils and soils. *Reclam Reveg Res* 4:245–258
- Fresquez PR, Aldon EF, Dennis GL (1988a) Carbon dioxide evolution from an organically amended Rio Puerco soil. U.S. Department of Agriculture Forest Service Research Note RM-447, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colo
- Fresquez PR, Francis RE, Dennis GL (1988b) Fungal communities associated with phytoedaphic communities in the semiarid Southwest. *Arid Soil Res Rehab* 2:187–202
- Gilman JC (1968) *A manual of soil fungi*. Iowa State University Press, Ames
- Guillemat J, Montegut J (1960) The effect of mineral fertilizers on some soil fungi. In: Parkinson D, Waid JS (eds) *The ecology of soil fungi*. Liverpool University Press, Liverpool, pp 98–111

- Huston J (1979) A general hypothesis of species diversity. *Am Nat* 113:81–101
- Joffe AZ (1967) The mycoflora of a light soil in a citrus fertilizer trial in Israel. *Mycologia* 52:535–544
- Khaleel R, Reddy KR, Overcash MR (1981) Changes in soil physical properties due to organic waste applications: a review. *J Environ Qual* 10:133–141
- Kiekens L (1983) Behavior of heavy metals in soil. In: Berglund S et al. (eds) *Utilization of sewage sludge on land: Rates of application and long-term effects on metals*, Reidel, Boston, Mass, pp 126–134
- Knudtsen K, O'Connor GA (1987) Characterization of iron and zinc in Albuquerque sewage sludge. *J Environ Qual* 16:85–90
- Küster E, Williams ST (1964) *Soil organic matter – its nature its role in soil formation and fertility*. Pergamon Press, New York
- Lauenroth WK, Sims PL (1976) Evapotranspiration from a shortgrass prairie subjected to water and nitrogen treatments. *Water Resources Res* 12:437–442
- Martin JP (1950) Use of acid, rosebengal, and streptomycin on the plate count method for estimating soil fungi. *Soil Sci* 69:215–233
- McCaslin BD, O'Connor GA (1982) Potential fertilizer value of gamma-irradiated sewage sludge on calcareous soil. *NM Agric Exp St Bull* 692
- Miller RH (1973) Soil microbiological aspects of recycling sewage sludge and waste effluents on land. In: *Proceedings of the Joint Conference on Recycling Municipal Sludge and Effluents on Land*. Nat Tech Infor Ser US PB-227106, US Dept of Commerce, Champaign, Ill, pp 79–88
- Mueller-Dombois D, Ellenberg H (1974) *Aims and methods of vegetation ecology*. Wiley, New York
- Parker LW, Elkins NZ, Aldon EF, Whitford WG (1987) Development of soil biota and nutrient cycles on reclaimed coal mine spoils in the arid southwest. *Biol Fertil Soils* 4:129–135
- Power JF, Alessi J (1971) Nitrogen fertilization of semiarid grasslands: Plant growth and soil mineral N levels. *Agron J* 63:277–280
- Pugh BJF (1974) Terrestrial fungi. In: Dickinson CH, Pugh BJF (eds) *Biology of plant litter decomposition*. Academic Press, New York
- Sommers LW (1977) Chemical composition of sewage sludges and analysis of their potential use as fertilizers. *J Environ Qual* 6:225–232
- Stark JM, Redente EF (1985) Soil-plant diversity relationships on a disturbed site in northwestern Colorado. *Soil Sci Soc Am J* 49:1028–1034
- Taylor EC (1979) Seasonal distribution and abundance of fungi in two desert grassland communities. *J Arid Environ* 2:295–312
- USDA (1954) *Methods of soil characterization*. In: Richards LA (ed) *Diagnosis and improvement of saline and alkali soils*. Agricultural Handbook 60, Washington, DC
- USDA (1972) *Soil survey laboratory methods and procedures for collection of soil samples*. Soil Conserv Am, Soil Surv Invest Rep No 1, Washington, DC
- Wight JR, Black AL (1979) Range fertilization: Plant response and water use. *J Range Manage* 32:345–348
- Wollum AG (1982) Cultural methods for soil microorganisms. In: Page AL et al. (eds) *Methods of soil analyses*. Am Soc Agron, Madison, Wisc, pp 781–801
- Zar JH (1974) *Biostatistical analysis*. Prentice-Hall, Englewood Cliffs, NJ

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