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DISSERTATION

MICROBIAL RESPONSES TO BIOSOLIDS TREATMENT OF RANGELANDS

Submitted by

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In partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Summer 2000

COLORADO STATE UNIVERSITY

April 18, 2000

We hereby recommend that the Dissertation prepared under our supervision by Bonnie L. Pierce entitled Microbial Responses to Biosolids Treatment of Rangelands be accepted as fulfilling in part requirements for the degree of Doctor of Philosophy.

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ABSTRACT OF DISSERTATION

MICROBIAL RESPONSES TO BIOSOLIDS TREATMENT OF RANGELANDS

Biosolids application to semiarid rangelands is a change in soil management that may introduce large quantities of organic C and N and varying concentrations of trace elements to soils. Since changes in the physical and chemical characteristics of soil may alter the size and activity of the soil microbial community, this study was initiated to determine how soil microorganisms respond to biosolids treatment. Biosolids were applied once in 1991 at 0 and 40 Mg ha^{-1} to a shrubland soil and at 0 and 30 Mg ha^{-1} to a grassland soil in Colorado. Biosolids application increased the basal respiration rates of shrubland soil by 400% over unamended soil (control soil) 5 yr following treatment, with a return to lower respiration rates, similar to control soil, 6 yr after treatment. Basal respiration rates also increased by 62% 6 yr following biosolids amendment of grassland soils as compared to control soil.

Metabolically active biomass-C ($SIR\text{-}C_{micr}$) showed a 25% increase in biosolids-amended shrubland soil in 1996 and 13 and 12% increases, respectively, in shrubland and grassland biosolids-amended soil relative to control soils in 1997. The specific respiration rate (qCO_2) of biosolids-amended soil increased 300% over control soil at the shrubland site 5 yr after treatment, but returned to a lower value, similar to unamended soil by the sixth year. The qCO_2 value for the grassland biosolids-amended soil also increased by 45% in 1997. The large increase in basal respiration and qCO_2 values for biosolids-amended shrubland soil in 1996 is an indication that microbial efficiency in C cycling was decreased as a result of treatment. The

higher biosolids application rate at the shrubland site in combination with a lower mean annual temperature and shorter mean frost-free period may have increased the adversity index (includes both stress and disturbance) for soil microflora.

The biosolids increased potential C mineralization by 130% over control soil for the shrubland and 71% over control soil for the grassland 6 yr following biosolids addition. An increase of 440% in potential net N mineralization was also found for biosolids-amended shrubland soil in 1997. No significant difference in net N mineralization was shown for biosolids-amended grassland soil in 1997, possibly due to the short duration of the incubation. It appears that biosolids C and N were mineralized to a greater extent during the 6 yr following treatment at the grassland site due to the lower biosolids application rate, coarser soil texture, warmer mean annual temperature, and a longer growing season. These results indicate that when field environmental conditions are optimal for microbial activity, increases in C and N mineralization may still be expected after 6 yr for these biosolids-amended rangeland soils.

In 1997, western wheatgrass (*Pascopyrum smithii* (Rydb.) A. Love) at the shrubland site showed a 33% increase in the percentage of root samples colonized by arbuscular mycorrhizal (AM) fungi as a result of biosolids amendment. A 23% increase was shown in the percentage of blue grama (*Bouteloua gracilis* (H.B.K.) Lag. ex steud) root samples colonized by AM fungi in biosolids-treated soil at the grassland site 6 yr following treatment. The most likely reason for the increase in AM fungal colonization of these economically important forage grasses on biosolids-amended plots is fungal proliferation into soil locations with increased organic C content.

In general, biosolids quality did not impair the size of the active microbial biomass, but biosolids quality and/or quantity lowered microbial efficiency in cycling of C substrates. Biosolids application greatly increased total soil organic C at the shrubland site (130%) and the grassland site (120%) and total soil organic N at the shrubland site (110%) and the grassland site (260%) 6 yr after addition indicating that decomposition of biosolids may not yet be complete. Large additions of biosolids to two semiarid rangelands may alter soil nutrient cycling and C storage longer than previously thought.

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INTRODUCTION

Waste management has become a top environmental concern as the world's human population surpasses six billion. In many regions of the world, land application of municipal biosolids has become an accepted waste disposal practice because other disposal options have caused air and water pollution (USEPA, 1985). Decades of research in the U.S. (USEPA, 1992) and Europe (MAFF, 1993) have generated land application management guidelines and monitoring programs to address concerns over problems of environmental pollution and disturbance. Much of this research has addressed the effects of biosolids on the use of crops or forage species for human or animal consumption and protection of water resources. Because the quality of biosolids can vary greatly in concentrations of nutrients, trace elements, and organic substances, and biosolids may be applied to soils of vastly different ecosystems, environmental concerns still persist.

Government regulations for allowable pollutant loading in soils have been established for land application of biosolids in the U.S. (USEPA, 1992) and Europe (MAFF, 1993) based, in part, on research into phytotoxic and zootoxic effects of constituent trace elements. Although much evidence exists that microorganisms are sensitive to high levels of trace elements *in vitro* (Brynhildsen et. al., 1988; Doelman, 1986; Stotzky and Babich, 1984; Duxbury, 1981), the *in situ* effects of trace elements and other potential organic pollutants on soil microorganisms are less clearly understood. Babich and Stotzky (1985) reviewed the problems associated with research into trace element toxicity on microbe-mediated ecological processes in soils and concluded that *in situ* experiments were complicated by abiotic environmental factors and lack of appropriate quantifiable data at the ecosystem level.

Some researchers have questioned whether regulatory agencies have appropriately addressed the effects of biosolids on the size and physiological functioning of soil microbial populations when setting regulatory limits (Babich and Stotzky, 1985). Concerns include a need for long-term monitoring of the size of the soil microbial biomass following biosolids addition (Brookes and McGrath, 1984), and a better understanding of relative toxicities of trace elements in soils and their effects on the total soil organic matter content (Baath, 1989). Other researchers have suggested the need to use physiological parameters (e.g., the metabolic quotient of CO_2, qCO_2, developed by Anderson and Domsch {1986}) to allow comparisons between different soils, and further investigation of growth characteristics of microbial populations following substrate addition (Dahlin et al., 1997).

The effect of pollution or disturbance on soil microbial activity is related to effects on vegetation and ecosystem productivity because soil microorganisms regulate the availability of most nutrients and soil C storage. In most natural systems, microbial mineralization of soil organic matter provides most nutrients required by plants (Smith and Paul, 1990). Application of biosolids to native rangelands (e.g., soils which have not been in crop production) is a relatively large change in soil management, especially in semiarid regions, because natural C inputs tend to be low (Smith et al., 1994). Manures (McGill et al., 1986) and biosolids (USEPA, 1992) added to arable soils have resulted in temporary, and sometimes large, increases in available C in soils. However, unlike typical manures, biosolids may also contain certain lipophilic organic substances (e.g., fatty acids originating from shampoos, soaps, and the food industry) that form metal complexes with biosolids-bourne trace elements (Carlson-Ekvall and Morrison, 1995). Some lipid-soluble metal complexes (e.g., with Cu) can more rapidly

penetrate biomembranes by passive diffusion versus the slower active uptake of metal ions, thereby potentially increasing bioavailability (Carlson-Ekvall and Morrison, 1995).

Anderson and Domsch (1989) have suggested that the greater the magnitude of change in soil management, the more time is required to reach a state of equilibrium in C cycling. This state of equilibrium is defined as when C input to the soil organic matter pool equals C output from the pool, and there is no further loss or accumulation of organic matter. In order to detect early changes in C turnover in soils under different management, researchers have focused on changes in soil microbial populations and activity. In a survey of 134 agricultural plots in central Europe, Anderson and Domsch (1989) determined that the size of the microbial biomass responds more quickly to changes in soil management (e.g., organic fertilizer amendment) than the total amount of soil organic matter. Van de Werf and Verstraete (1987) concluded, from their study of pesticide effects on soil bio-kinetic parameters, that the size of the active microbial biomass responds even more quickly to soil pollution than the total microbial biomass. The authors estimate that 4 to 49% of the total soil microbial biomass is metabolically active in most soils.

When change in soil management alters the amount of available C, or introduces potential pollutants to soils, an assessment of changes in microbial population size or respiration may offer insight into the effect of management practices on soil C cycling. Any changes in total or active microbial biomass may indicate redirection of C from biosynthesis to cell maintenance, and changes in basal respiration may indicate the rate of C loss via microbial decomposition of soil organic matter or added substrates. Many changes in qCO_2 values (unit of CO_2-C basal respiration per unit of microbial biomass-C), also referred to as the specific

respiration rate of the biomass, are correlated with changes in soil management. For example, increases in qCO_2 values have been reported with herbicide application (Biederbeck et al., 1987), simulated acid rain and osmotic stress (Killham, 1985), substrate addition (Ocio and Brookes, 1990), gradients of increasing soil pollution (Ohtonen, 1994), and heavy metal additions to soils (FlieBbach et al., 1994; Chander and Brookes, 1991; Brookes and McGrath, 1984). Other researchers have documented declines in qCO_2 values during primary succession and following recovery from disturbance (Insam and Haselwandter, 1989; Insam and Domsch, 1988; Anderson and Domsch, 1985). Wardle and Ghani (1995), in a critique of the use of qCO_2, have cautioned that this measure may confound the effects of disturbance (e.g., rapidly changing environmental conditions) with the effects of stress (non-changing, harsh environmental conditions). The authors suggest that qCO_2 values may be most useful as an index of adversity (both stress and disturbance) for the soil microbial population and secondly, as a relative measure of microbial efficiency in metabolizing available C resources.

Any information which aids our understanding of microbial activity, especially at the ecosystem level, is key to determining if soil management practices are affecting nutrient cycling or are causing soils to lose or accumulate organic matter. Researchers have monitored the effects of biosolids on microbial mineralization of N to determine if soil fertility has been adversely impacted by this practice. Cook and Greaves (1987) have suggested that N transformations are among the soil processes most responsive to environmental changes and changes in soil management. In native rangelands, the production of certain perennial grasses is economically important because they are used as forage by domestic animals and wildlife. Many rangeland grasses are known to benefit from mychorrizal colonization of their roots,

particularly in terms of P and water uptake. Investigators can monitor the presence of arbuscular mychorrizal (AM) fungi on the roots of perennial grasses to determine if any constituent, added to soils with biosolids, interferes with this symbiotic relationship.

The objectives of this study were to determine if basal respiration rates, substrate-induced respiration biomass-C (SIR-C_{micr}), and potential C and net N mineralization rates were increased above unamended (control) soils as a result of biosolids treatment of soils. Further objectives were to evaluate if qCO_2 and C_{micr}/C_{org} values were altered as a result of biosolids addition and to determine if there was a change in the extent of root colonization by AM fungi of perennial grasses as a result of biosolids amendment of soils.

CHAPTER 1

INFLUENCE OF BIOSOLIDS ON MICROBIAL SUBSTRATE-INDUCED AND BASAL RESPIRATION

ABSTRACT

Land application of biosolids is practiced in many regions of the world, yet environmental concerns persist because biosolids quality can vary greatly and may be applied to soils of vastly different ecosystems. This study was conducted to determine the effect of surface-applied biosolids, containing moderate amounts of trace elements, on the size and activity of soil microbial populations in a semiarid shrubland and a grassland in Colorado. Biosolids were applied once in 1991 at 0 and 40 Mg ha^{-1} to shrubland soil and at 0 and 30 Mg ha^{-1} to grassland soil. Biosolids application increased the basal respiration rate of shrubland soil over unamended (control) soil by 400% 5 yr following treatment, with a return to lower respiration rates, similar to control soil, 6 yr after treatment. Basal respiration rates also increased 62% over control soil 6 yr following biosolids amendment of grassland soil. Measurements for metabolically active microbial biomass-C ($SIR\text{-}C_{micr}$) show a 25% increase in biosolids-amended shrubland soil in 1996 and 13 and 12% increases, respectively, in shrubland and grassland biosolids-amended soils in 1997. Calculations of the specific respiration of the microbial biomass (qCO_2), indicate a 300% increase over control soil in shrubland soil 5 yr after biosolids treatment, with a return to a lower qCO_2 value, similar to control soil, 6 yr following amendment. A 45% increase was also found in qCO_2 values in biosolids-amended grassland soil

over control soil 6 yr after treatment. SIR-C_{micr} results are consistent with those expected of low-nutrient semiarid ecosystems and indicate no direct adverse impact of biosolids on soil microbial population size in the two semiarid rangelands. The large increase in basal respiration and the qCO_2 value of biosolids-amended shrubland soil in 1996 is an indication that microbial efficiency in C cycling was decreasing (more C was being diverted to cell maintenance than to cell growth) during the decomposition of biosolids. The higher biosolids application rate at the shrubland site, in combination with a lower mean annual temperature and a shorter mean frost-free period than the grassland site, may have increased the adversity index (includes both stress and disturbance) for soil microflora on biosolids-treated soil.

INTRODUCTION

An active soil microflora is essential for efficient nutrient cycling, especially in low-nutrient semiarid ecosystems. The substrate-induced respiration (SIR) method (Anderson and Domsch, 1978) provides a technique for estimating the biomass of the living, non-resting microbial populations. West and Sparling (1986) modified the SIR method for use with dry soil by recommending that glucose be added to soil samples in solution to reduce moisture limitation on respiration. This technique has been successfully used in studies of dry soils by West et al. (1989).

I selected a continuous airflow system (Cheng and Coleman, 1989) to measure basal respiration and SIR to evaluate the long-term impact of biosolids addition to a semiarid shrubland and a grassland in Colorado. The qCO_2 values (Anderson and Domsch, 1985) for both ecosystems were used as an index of adversity of environmental conditions (including both

stress and disturbance) resulting from land application of biosolids. The objectives of this study were to determine if basal respiration and SIR-C_{micr} were increased by biosolids amendment and to determine if qCO_2 and the ratio of microbial biomass C (C_{micr}) to total soil organic C (C_{org}) (C_{micr}/C_{org}) were altered as a result of biosolids treatment of soils.

MATERIALS AND METHODS

Field Sites

This study was conducted at two semiarid rangeland sites that were established in 1991 (Pierce et al., 1998; Harris-Pierce, 1994). The first study site is a shrubland site located in the intermountain region 2 km north of Wolcott, CO, at an elevation of 2225 m and is dominated by mountain big sagebrush (*Artemisia tridentata* Nutt. ssp. *vaseyana* (Rydb) Beetle) and several perennial grasses, including western wheatgrass (*Pascopyrum smithii* (Rydb.) A. Love). The mean annual precipitation is 356 mm, with 40% received as snowfall, the mean annual temperature is 5.6°C, and the mean frost-free period is 85 d (SCS, 1992). The soils are of the Tanna-Pinelli complex consisting of fine, montmorillonitic Aridic Argiborolls and fine, montmorillonitic Borollic Haplargids (SCS, 1992). Slopes range from 12 to 25%, and surface texture is mostly clay loam (SCS, 1992). Selected chemical characteristics of the soil are shown in Table 1.1.

During July 1997, the current study was expanded to include evaluation of soil from a grassland study site located 32 km north of Fort Collins, CO. The site is part of Meadow Springs Ranch, which is owned by the city of Fort Collins and is at an elevation of 1750 m. It is a shortgrass steppe dominated by perennial grasses, including blue grama (*Bouteloua gracilis*

(H.B.K.) Lag. ex steud). The mean annual precipitation is 356 mm, with 25% received as snowfall, the mean annual temperature is 9.4°C, and the mean frost-free period is 125 d. The soil is a deep, well-drained Aridic Argiustoll formed in mixed alluvial deposits underlain by sand and gravel (SCS & FS, 1980). Slopes range from zero to 9%, and the surface soil texture is sandy loam (SCS & FS, 1980). Selected chemical characteristics of the soil are shown in Table 1.2.

Experimental Design

Plots were arranged in a randomized complete block design at both study sites with three replications for the Wolcott shrubland site (Pierce et al., 1998) and four replications for the Meadow Springs Ranch grassland site (Harris-Pierce, 1994). Plot sizes at the Wolcott site vary in dimension, but are approximately 31 m by 107 m (3320 m^2). The plots at the Meadow Springs Ranch site are 15 m by 15 m (225 m^2). Plots received 0 and 40 Mg biosolids ha^{-1} at the shrubland site and 0 and 30 Mg biosolids ha^{-1} at the grassland site. Biosolids from nearby municipal sources were surface-applied to both sites in 1991, and the chemical properties of the biosolids are described in Table 1.3.

Sampling Plan

During July 1996, and again in July 1997, I collected ten random soil samples per plot on the 0 and 40 Mg biosolids ha^{-1} plots at Wolcott, in three replicated blocks. A fourth block (replication), was not analyzed due to the extensive presence of immature soil (Entisols) that was not present on the remaining three replicate blocks. I collected five random soil samples per plot on the 0 and 30 Mg biosolids ha^{-1} plots for all four replicates at Meadow Springs Ranch in August 1997.

At both study sites transects were placed from east to west down the center of each plot, and a random number generator was used to locate each successive position along the transects. I used a random number generator to select which direction (south or north of the transect) I would begin taking soil samples. Movement was from east to west, and after the first sample was taken, I alternated south and north directions during sampling. At each randomly located sampling position, I placed a 20 by 25-cm frame on the ground, marked each corner to a 5-cm depth, and then scraped away the top 0.5 cm of soil and litter to avoid soil contamination by remaining biosolids on the soil surface. The soil was collected with a sterilized hand spade (wiped with isopropyl alcohol) to a 5-cm depth, placed in polyethylene bags, and stored at 5°C in the dark until further processing in the lab.

Soil Analyses

I took random subsamples from each field-collected sample and prepared them separately for measurement of pH, electrical conductivity (EC), gravimetric moisture content, soil ammonium bicarbonate-diethylenetriaminepentaacetic acid (AB-DTPA)-extractable metals, total organic C, and total N. The following methods were selected for analyses: pH and EC (Rhoades, 1982), and soil AB-DTPA-extractable metals (Barbarick and Workman, 1987) followed by determination of constituent concentrations on an inductively coupled plasma spectrophotometer (ICP). Soil organic C and total N were measured using a LECO CHN-1000 automated analyzer. I pre-treated the Wolcott soil samples with 10% HCl and oven-dried them at 70° C to remove excess carbonates before measuring total organic C.

Laboratory Incubations

I used the substrate-induced respiration (SIR) method to determine if the size of the living, non-resting microbial population was affected by biosolids amendment at the study sites. The method is based on the concept that when a readily-available substrate is added in a saturating quantity to soil, a steady rate of CO_2 production reflects the energy demand of the metabolically active microbial population. Respiration rates were measured using a soil respiration system with continuous airflow (Cheng and Coleman, 1989) with some modifications. I added a 50-μm filter in the air supply line before the 0.2-μm filter to protect equipment from coarse dust and water. Anhydrone tubes ($MnClO_4$) were added to the air supply line before the incubation bath and on each line leaving the water vapor condenser to extract moisture from the air supply. In addition, a cotton filter was positioned, in line, before the mass flow meter to protect the meter from fine dust. I used a reference gas system to provide air containing 50 ppm CO_2 for calibration of the Lycor infrared gas CO_2 analyzer.

For the SIR technique, glucose is used to induce a maximal respiratory response (MRR) from the soil microbial biomass, which is measured as the maximum amount of evolved CO_2. Anderson and Domsch (1978) determined that following glucose addition to soil, the initial MRR was correlated to the size of the actively metabolizing microbial biomass (SIR-C_{micr}) by the following equation:

$$\text{mg biomass-C } 100\ g^{-1}\ \text{soil} = 40.04 * \text{mL } CO_2\ 100\ g^{-1}\ \text{soil}\ h^{-1} + 0.37 \quad (1)$$

I selected a continuous airflow system (CO_2-free air) to solve the problem of experimental errors due to loss of microbially-respired CO_2 to the liquid phase (HCO_3^-) in calcareous soils of high pH (Martens, 1987).

Sample preparation for all laboratory incubations (i.e., MRR, basal respiration, and SIR) involved using field-moist, coarsely sieved (2 mm) subsamples, after removing recognizable live roots and shoots with tweezers. I placed 25 g fresh, homogenized (hand-mixed) subsamples into 125-mL Erlenmeyer flasks and placed them in a 22^0 C ± 0.5°C water bath for 2 h prior to each incubation.

To determine the C mineralization rate of the shrubland and grassland control soils which best represented the size of the original microbial population, I set up 8-h incubations for three randomly selected soil subsamples from the shrubland site in July 1996 and from both sites in July 1997. Each subsample was amended with a glucose solution (0.01 g glucose mL^{-1} distilled water) and brought up to 35% water-holding capacity (WHC) (i.e., 35% of total by oven-dry weight). In each case, I observed CO_2 evolution curves with initially zero slopes (at least the first 3 h of the incubation) followed by a tendency toward a positive slope. Anderson and Domsch (1978) interpreted a positive slope as indicating biomass synthesis and recommended that measurements be taken before this tendency occurs. Based on these observations, I selected 1-h incubations for determination of MRR.

I amended three soil samples per replication from Wolcott and Meadow Springs Ranch, which had not received biosolids treatment in 1991 (e.g., from the control plots) with a series of glucose concentrations to estimate MRR (Anderson and Domsch, 1978). Average results for 1-h incubations for both soils are shown in Fig. 1.1. In both 1996 and 1997, I determined that a concentration of 0.18 mmole glucose g^{-1} dry soil produced the MRR at 35% WHC in Wolcott soil. My 1997 measurements showed that a concentration of 0.06 mmole glucose g^{-1} dry soil was needed to achieve the MRR at 35% WHC for the Meadow Springs Ranch soil. Although a

higher glucose concentration (0.18 mmole glucose g^{-1} dry soil) was required to stimulate the MRR in the shrubland soil versus the grassland soil, similar levels of respiration (1.2 mL CO_2 h^{-1} 100 g^{-1} dry soil for Wolcott soil and 0.96 mL CO_2 h^{-1} 100 g^{-1} dry soil for Meadow Springs Ranch soil) were obtained following 1-h incubations under identical conditions. The MRR values and the corresponding glucose concentrations fall within the range of values obtained by Anderson and Domsch (1978) for a wide range of soils.

To determine the SIR response for each subsample, I set up new incubations and added the glucose solution, which was selected based on the MRR, by syringe to each flask to bring soil water content up to 35% WHC. The flasks were then connected to the respiration measuring system, and once the CO_2 rate changed less than 1% over a consecutive 3-min period at an air flow rate of 100 mL min^{-1}, I recorded the CO_2 evolution rate as the SIR rate of the sample. Stabilization of the CO_2 evolution rate took 40 to 70 min for all samples. The equation of Anderson and Domsch (1978) (equation 1) was used to convert the SIR rate of each sample to SIR-C_{micr}.

During the analyses of each set of soil samples, an empty flask was connected to the respiration measuring system to determine if CO_2 was leaking into the system and to obtain a baseline CO_2 concentration. All glassware was acid-washed, rinsed three times with distilled water, covered with aluminum foil, and autoclaved at 121^0 C for 20 min followed by a 10-min drying cycle.

I measured basal respiration rates on the same day that I measured each subsample for SIR. The same procedure used to determine SIR was used to determine the basal respiration rate, except that only distilled water was added to bring each sample up to 35% WHC. I ensured

that corresponding samples (SIR and basal) were placed in the same position in the water bath rack (i.e., I used the same air line for corresponding measurements).

Statistical Analyses

Data were analyzed using analysis of variance (ANOVA; SAS, 1994) of SIR-C_{micr} and basal respiration across biosolids rate treatments. When the F-Test from ANOVA was significant for a treatment effect, Fisher's least significant difference (LSD) test was used for mean comparisons ($p \leq 0.05$).

RESULTS AND DISCUSSION

Evaluation of Basal Respiration

Basal soil respiration rates are a measure of the overall activity or the amount of energy spent by the indigenous microbial populations. Results for the shrubland site (Fig. 1.2) show a significant increase in basal respiration rates for soil receiving 40 Mg biosolids ha^{-1} over control soil in 1996, but lower rates, similar to unamended soil, in 1997. The control plots showed no significant differences in respiration rates between the fifth and sixth yr following biosolids treatment. In 1997, respiration rates at the grassland site show a significant increase for soil that received 30 Mg biosolids ha^{-1} over control plots (Fig. 1.3). The basal respiration rates at both the shrubland and grassland sites are relatively low but similar to values reported for young soils (the first 79 yr) on a recessional moraine in Canada investigated along a primary successional gradient of 225 yr (Insam and Haselwandter, 1989).

The shrubland site results indicate a substrate surplus in 1996 which led to increased community respiration. Weather data for this region indicate below average annual and seasonal

precipitation for 1996 and 1997 (USGS, 1999). I, therefore, do not attribute the large release of CO_2-C of microbial origin to an increase in plant production due to higher precipitation. In semiarid shrublands, C inputs are usually low, and turnover of C is slow due to low annual precipitation (Bolton et al., 1990). The colder temperatures at higher elevation, and shorter growing season at the shrubland site also contribute to slower C cycling. It appears that the addition of biosolids to the shrubland site in 1991 provided a labile source of C, which was responsible for the increase in basal respiration seen in 1996, but was not available in summer 1997. An increased supply of labile C was probably also responsible for increased basal respiration rates in biosolids-amended soil at the grassland site in 1997. Carbon inputs from fresh municipal biosolids (USEPA, 1992) differ in chemical composition from plant litter and plant exudates, and following a drastic change in the prime carbon source for the decomposers, a period of adaptation may be required for the indigenous soil microbial populations to achieve high energy use efficiency of the new substrates. Other factors which may affect C availability over time include annual and diurnal periodicity of temperature and the large temporal and spatial variations in precipitation often seen within one growing season during years of average annual precipitation at semiarid locations (Sturges, 1975).

Numerous researchers, while investigating the effects of environmental stresses on microbiota, have postulated that the more efficient the functioning of soil microorganisms (i.e., more C is converted into microbial biomass), the less C is lost via respiration. This concept is an extension of Odum's theory of bioenergetics in ecosystem development (Odum, 1969). Relevant research has included investigations into the effects of substrate quality (Smith, 1993), soil management (Anderson and Domsch, 1990), pH, high trace element concentrations, and

salinity (Killham, 1985) on soil microbial activity. These authors suggest that under conditions of environmental stress, the soil microbial community diverts more energy from growth into maintenance of cell integrity.

The fourfold increase in basal respiration rates for biosolids plots over control plots at the shrubland site 5 yr following application indicates that a change in C cycling occurred. A smaller increase in basal respiration rates for biosolids plots over control plots (62%) occurred at the grassland site 6 yr following biosolids treatment. Basal respiration appears to be a sensitive measure of changes in microbial community activity as much as 6 yr following biosolids addition to these semiarid soils.

Biosolids Effect on Substrate-Induced Respiration

In both 1996 and 1997, soil which received 40 Mg biosolids ha^{-1} at the shrubland site showed significant increases in metabolically active microbial biomass (SIR-C_{micr})(Fig. 1.4). The results for the grassland site also indicate a significant increase in SIR-C_{micr} over control soil in 1997 for soil which received 30 Mg biosolids ha^{-1} (Fig. 1.5). The increases in the size of the metabolically active microbial biomass 5 and 6 yr following biosolids application are positively correlated with the increases in soil organic C content (Tables 1.1 and 1.2) at both locations ($r = 0.87$ in 1996 and $r = 0.76$ in 1997 for the shrubland site and $r = 0.71$ in 1997 for the grassland site). Correlation coefficients were significant at $p \leq 0.05$ and were calculated using $n = 60$ for the shrubland site and $n = 40$ for the grassland site.

The mean SIR-C_{micr} values (mg biomass-C kg^{-1} dry soil) for all replications on unamended plots for the shrubland soil (397 in 1996 and 434 in 1997) and the grassland soil (399 in 1997) are lower than those reported by Smith and Paul (1990) for typical arable soils

(700) and forest soils (850). However, Santruckova and Straskraba (1991) reported the following SIR-C_{micr} values (mg biomass-C kg^{-1} dry soil): fallow plots (340), cultivated fields (370), meadow soils (550), and forest soils (460) in southern Bohemia. Smith et al. (1994) reported a mean SIR-C_{micr} value (mg biomass-C kg^{-1} dry soil) of 748 for an arid shrubland soil in Washington state. These authors found this value to be unusually high for an arid soil but reported sufficient soluble soil C to support the microbial population. In comparison, mean SIR-C_{micr} values for the biosolids-amended soils show a 25% increase over control plots for the shrubland soil in 1996 and 13 and 12% increases, respectively, for biosolids-amended shrubland and grassland soils over control soils in 1997. As expected, soil organic C content was increased by biosolids treatment. I did not expect that it would take 6 yr for microbial mineralization of the organic constituents, added with the biosolids, at the shrubland site because of results reported by Fresquez et al. (1988) for biosolids amendment of a similar semiarid rangeland. The authors reported a higher rate of decomposition of biosolids over a four month growing season in a New Mexico soil at a lower application rate (22.4 Mg amendment ha^{-1}).

Dahlin et al. (1997) suggested that the effects of trace elements contained in biosolids on microbial biomass may be masked by the increase in soil organic C which would increase available substrate C. A comparison of total soil trace element concentrations (4 M HNO_3 extractions of trace elements) following biosolids application between the soil at the Brunnby site in Sweden (Dahlin et al., 1997) and the shrubland soil (Table 1.1) indicates that the Brunnby soil contains 290% more Cr, 180% more Cu, 19% more Ni, and 240% more Pb than the Wolcott shrubland soil. However, the shrubland soil has 270% more Cd and 41% more Zn than the Brunnby soil. The grassland soil (Table 1.2) contains far lower concentrations of total soil Cd,

Cr, Cu, Ni, Pb, and Zn than the Brunnby soil. Trace element concentrations are up to five times higher in the Brunnby soil than the grassland soil. Dahlin et al. (1997) found a negative correlation between soil Cu concentrations and SIR-C_{micr}, but no statistically significant effect on microbial basal respiration. I report no decreases in SIR-C_{micr} or basal respiration at either study site during the present investigation.

In addition to higher trace element concentrations in Brunnby soil, soil pH is lower at Brunnby (5.8) than the shrubland (7.1) on biosolids-treated plots, which also influences bioavailability of these constituents. The pH values of soil at the grassland site and the Brunnby site are similar, indicating conditions for similar availability of trace elements, except that the grassland soil contains far lower concentrations of the same trace elements. Because total concentrations of trace elements (4 M HNO_3 extractions of trace elements) do not account for metal speciation in the presence of organic substances, either added with biosolids or already present in the soil, and therefore do not reflect the bioavailability of trace elements, I suggest that ethylenediaminetetraacetic acid (EDTA) or AB-DTPA extractions of trace elements are more suitable methods for evaluating metal availability in biosolids-amended soil.

Brookes and McGrath (1984) reported decreases in soil SIR-C_{micr} with biosolids treatment on soils in Britain, which they attributed to elevated Cu and Ni soil concentrations. The soil concentrations the authors report for Cu and Ni (EDTA extractions of trace elements) 20 yr following biosolids amendment are approximately 500 and 530% higher, respectively, than the shrubland soil Cu and Ni concentrations (AB-DTPA extractions of trace elements)(Table 1.1) measured 6 yr following biosolids application.

A comparison of the total soil trace element concentrations of the shrubland soil (Table 1.1) to the arable soils in Germany investigated by Flieβbach et al. (1994), show similar concentrations (4 M HNO_3 extractions of trace elements) of Cu, Zn, and Ni in the shrubland 40 Mg biosolids ha^{-1} plots and the German low-metal biosolids plots. However, the concentration of Cd in the shrubland soil receiving 40 Mg biosolids ha^{-1} is 9.8% higher than the German soil treated with high-metal biosolids. The soil pH values (6.0 to 6.6) reported by FlieBbach et al. (1994) are also lower than that of the shrubland soil pH (7.1). All soil trace element concentrations at the grassland site (Table 1.2) are much lower for the 30 Mg biosolids ha^{-1} plots than the low-metal biosolids plots in Germany. Flieβbach et al. (1994) concluded that the low-metal biosolids had beneficial effects on SIR-C_{micr} and on the soil microbial activity, compared to the large decrease in SIR-C_{micr} they found in soil receiving high-metal biosolids.

It is difficult to make direct comparisons of effects of trace element constituents added to soils with biosolids on microbial populations and activity in separate studies for many reasons. The most complicating factors may be the chemical form of the added trace elements (e.g., organically-complexed versus metal-salts) and the method of trace element extraction (e.g., HNO_3, EDTA, or AB-DTPA extractions). Other important factors are frequency of biosolids application, soil pH, and length of monitoring of microbial responses. All of these factors can potentially affect metal availability in soils, and consequently, may affect microbial responses. It is also possible that low concentrations of trace elements contained in biosolids may alleviate trace element deficiencies in soil microorganisms or plant species instead of creating toxicities. It is difficult to isolate these effects because of the varying composition of biosolids from different sources and the complex interactions of trace elements in soil systems.

Evaluation of Physiological Parameters

In an effort to study the relationship between microbial metabolic activity and microbial biomass in soils, Anderson and Domsch (1985) used measurements of specific respiration rate (qCO_2). Insam and Domsch (1988) found that qCO_2 values were unrelated to soil organic C content and soil moisture but may reflect the response of the soil microbial biomass to disturbance or stress. An increase in qCO_2 values in a soil indicates a decreased efficiency of conversion of C into new biomass C (Brookes, 1995).

My results indicate a large increase (300%) relative to control soil in the qCO_2 values at the shrubland site in 1996 for soil which was amended 5 yr earlier with 40 Mg biosolids ha^{-1} (Table 1.4). By 1997 the qCO_2 values of biosolids-amended soil at the shrubland site were reduced to a lower rate identical to unamended soil. Results for the grassland soil in 1997 indicate a higher qCO_2 value (45%) for soil which received 30 Mg biosolids ha^{-1} 6 yr earlier as compared to control soil (Table 1.4). The mean qCO_2 values for the shrubland soil which received no biosolids compare favorably with values reported by Smith et al. (1994) for a shrub steppe in Washington State (1.2×10^{-3}). Smith (1993) also reported a mean qCO_2 value of 0.5×10^{-3} for an annual grassland, which is similar to the 1997 value I report for the grassland soil which received no biosolids (Table 1.4). Anderson and Domsch (1990), in a survey of 134 agricultural plots in central Europe, reported mean qCO_2 values of 1.097×10^{-3} for plots under long-term monoculture and 0.0645×10^{-3} for plots under long-term continuous rotation. It appears that the large increase in qCO_2 values for biosolids-amended shrubland soil in 1996 is an indication that microbial efficiency in C-cycling was decreasing during the decomposition of biosolids. This may be an indication that environmental conditions for soil microflora have

become more adverse as a result of a one-time, large addition of biosolids, containing moderate amounts of trace elements, possibly due to low substrate quality (e.g., heavy metal stress or an imbalance of C and N) or due to the high biosolids application rates.

I also calculated the ratio of microbial biomass-C to total soil organic C (C_{micr}/C_{org}) to evaluate the effect of biosolids treatment on soil C storage in both ecosystems. My 1997 results (Table 1.4) indicate a 46% decrease in this ratio for the shrubland soil and a 67% decrease for the grassland soil relative to control soils. The overall C_{micr}/C_{org} value for the grassland soil appears to be unusually low compared to values for similar semiarid areas (Insam, 1990).

The C_{micr}/C_{org} ratio has been used extensively for soil systems under agricultural management (Mann, 1986) to assess whether organic matter is declining or accumulating over time. Anderson and Domsch (1989), in evaluating soils from long-term agricultural experiments, found no universal equilibrium constants for C_{micr}/C_{org} for various soil management practices, but reported that manuring practices did have a direct influence on the ratio, causing a temporary (8-month) increase. Most reported C_{micr}/C_{org} values are for agroecosystems or temperate soils and range from 0.7 to 4.0 (Brookes, 1995; Anderson and Domsch, 1989). It is difficult to compare results for semiarid rangelands to these. Insam and Domsch (1988), in a study on chronosequences of reclamation sites, found that C_{micr}/C_{org} decreased with time. They suggested that soils with a C_{micr}/C_{org} above an empirical average for the same type of soil management, are considered "developing" soils with a net accumulation of organic C, and those with lower values are losing organic C from a pool of relatively stable humic materials.

Brookes (1995) reported that there is accumulating evidence that biosolids containing trace elements near the mandatory European Union (EU) limits cause decreases in C_{micr}/C_{org} in

soils. Chander and Brookes (1991) reported C_{micr}/C_{org} ratios of 1.5 to 2.0 in non-biosolids treated soil and 0.7 to 1.0 in soil treated with biosolids contaminated with Cu or Zn. In the present study, the relative decreases in C_{micr}/C_{org} indicate that high biosolids application rates reduced microbial efficiency in C-cycling, resulting in a higher proportion of C lost via microbial respiration than C added to the microbial biomass-C pools in both rangeland systems.

CONCLUSIONS

Biosolids application rates of 40 Mg ha^{-1} at a shrubland site and 30 Mg ha^{-1} at a grassland site significantly increased active microbial biomass-C over control plots 5 and 6 yr following treatment at the shrubland site and 6 yr following treatment at the grassland site. Basal respiration rates were increased over control plots for biosolids-amended soils 5 yr after treatment at the shrubland site and 6 yr after amendment at the grassland site. Land application of biosolids increased the specific respiration value (qCO_2) of shrubland soil after 5 yr, but values were similar to unamended soil by the sixth yr. The biosolids-amended grassland soil also showed an increase in qCO_2 after 6 yr. These results indicate that biosolids treatment did not adversely affect the size of soil microbial populations but decreased their efficiency in cycling of C substrates. The decrease in soil C_{micr}/C_{org} values at both sites 6 yr following biosolids amendments also indicates that microbial efficiency in C cycling decreased, resulting in a smaller proportion of C being added to the soil microbial biomass-C pools than found in control plots. In view of these changes in C cycling following biosolids treatment, further research is required at these sites beyond 6 yr to determine if irreversible changes in the quantity and quality of soil organic matter may occur at high biosolids treatment rates.

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Table 1.1. Selected properties of soils at Wolcott, CO. Soil data were collected in July 1993 (Pierce et al., 1998) and in July 1997.

		1997		1993	
Constituent	**Units**	**0 Mg Biosolids ha^{-1}**	**40 Mg Biosolids ha^{-1}**	**0 Mg Biosolids ha^{-1}**	**40 Mg Biosolids ha^{-1}**
pH		7.3	7.1	6.9	7.1
EC	dS m^{-1}	0.71	0.73	0.50	0.70
Organic C	%	0.03	0.07	na	na
Total N	%	0.16	0.34	na	na
		AB-DTPA Extraction[+]		**4 M HNO_3 Extraction**[∓]	
P	g kg^{-1}	0.01	0.06	0.32	0.64
K	g kg^{-1}	0.25	0.48	1.33	1.81
Al	g kg^{-1}	0.01	0.01	4.57	6.27
Cd	mg kg^{-1}	0.47	1.48	2.35	2.90
Cr	mg kg^{-1}	0.08	0.09	6.20	8.00
Cu	mg kg^{-1}	4.62	15.1	13.0	21.3
Fe	g kg^{-1}	0.01	0.02	6.62	8.08
Mo	mg kg^{-1}	0.01	0.04	5.05	4.60
Ni	mg kg^{-1}	1.94	1.43	16.6	19.7
Pb	mg kg^{-1}	0.88	1.88	5.55	7.26
Zn	mg kg^{-1}	5.21	33.4	88.3	165

na = parameter not measured

[+] Barbarick and Workman, 1987

[∓] Bradford et al., 1975

Table 1.2. Selected properties of soils at Meadow Springs Ranch, CO. Soil data were collected in August 1997. Data collected for total metal analyses in July 1993 (Harris-Pierce, 1994).

Constituent	Units	0 Mg Biosolids ha^{-1}	30 Mg Biosolids ha^{-1}
pH		5.9	5.8
EC	dS m^{-1}	0.32	0.60
Organic C	%	1.54	3.46
Total N	%	0.09	0.32
		1993 4 M HNO_3 Extraction[Ŧ]	
P	g kg^{-1}	0.23	1.16
K	g kg^{-1}	1.49	1.47
Al	g kg^{-1}	na	na
Cd	mg kg^{-1}	0.5	0.5
Cr	mg kg^{-1}	4.9	6.2
Cu	mg kg^{-1}	6.6	48.2
Fe	g kg^{-1}	4.45	4.55
Mo	mg kg^{-1}	0.01	1.00
Ni	mg kg^{-1}	4.7	5.6
Pb	mg kg^{-1}	6.1	10.3
Zn	mg kg^{-1}	19.5	64.4

na = parameter not measured

Ŧ Bradford et al., 1975

Table 1.3. Selected properties of biosolids used for surface treatment in 1991 at Wolcott (shrubland) and Meadow Springs Ranch (grassland). Trace element extraction by H_2SO_4-$HClO_4$ digest.

Constituent	Units	Shrubland	Grassland
pH		7.7	7.3
EC	dS m^{-1}	13.0	5.00
Organic N	g kg^{-1}	0.12	0.46
NO_3-N	mg kg^{-1}	0.16	3963
NH_4-N	mg kg^{-1}	168	102
P	g kg^{-1}	32.0	16.0
K	g kg^{-1}	3.6	1.95
Al	g kg^{-1}	0.16	8.7
Cd	mg kg^{-1}	4.8	5.06
Cr	mg kg^{-1}	22.1	39.7
Cu	mg kg^{-1}	567	553
Fe	g kg^{-1}	0.12	4.81
Mo	mg kg^{-1}	2.9	16.1
Ni	mg kg^{-1}	14.7	19.2
Pb	mg kg^{-1}	47.1	117
Zn	mg kg^{-1}	1210	776

Table 1.4. Selected parameters for the soil systems at Wolcott (shrubland) and Meadow Springs Ranch (grassland). Soil data were collected in August 1996 and 1997.

			Shrubland		Grassland	
Year	**Parameter**	**Units**	**0 Mg Biosolids ha^{-1}**	**40 Mg Biosolids ha^{-1}**	**0 Mg Biosolids ha^{-1}**	**30 Mg Biosolids ha^{-1}**
1996	qCO_2	mg CO_2-C kg^{-1} C_{micr}	$1.09x10^{-3}$	$4.37x10^{-3}$	na	na
1997	qCO_2	mg CO_2-C kg^{-1} C_{micr}	$0.73x10^{-3}$	$0.73x10^{-3}$	$0.60x10^{-3}$	$0.87x10^{-3}$
1997	C_{micr}/C_{org}	x 100	1.3	0.71	0.03	0.01

na = parameter not measured

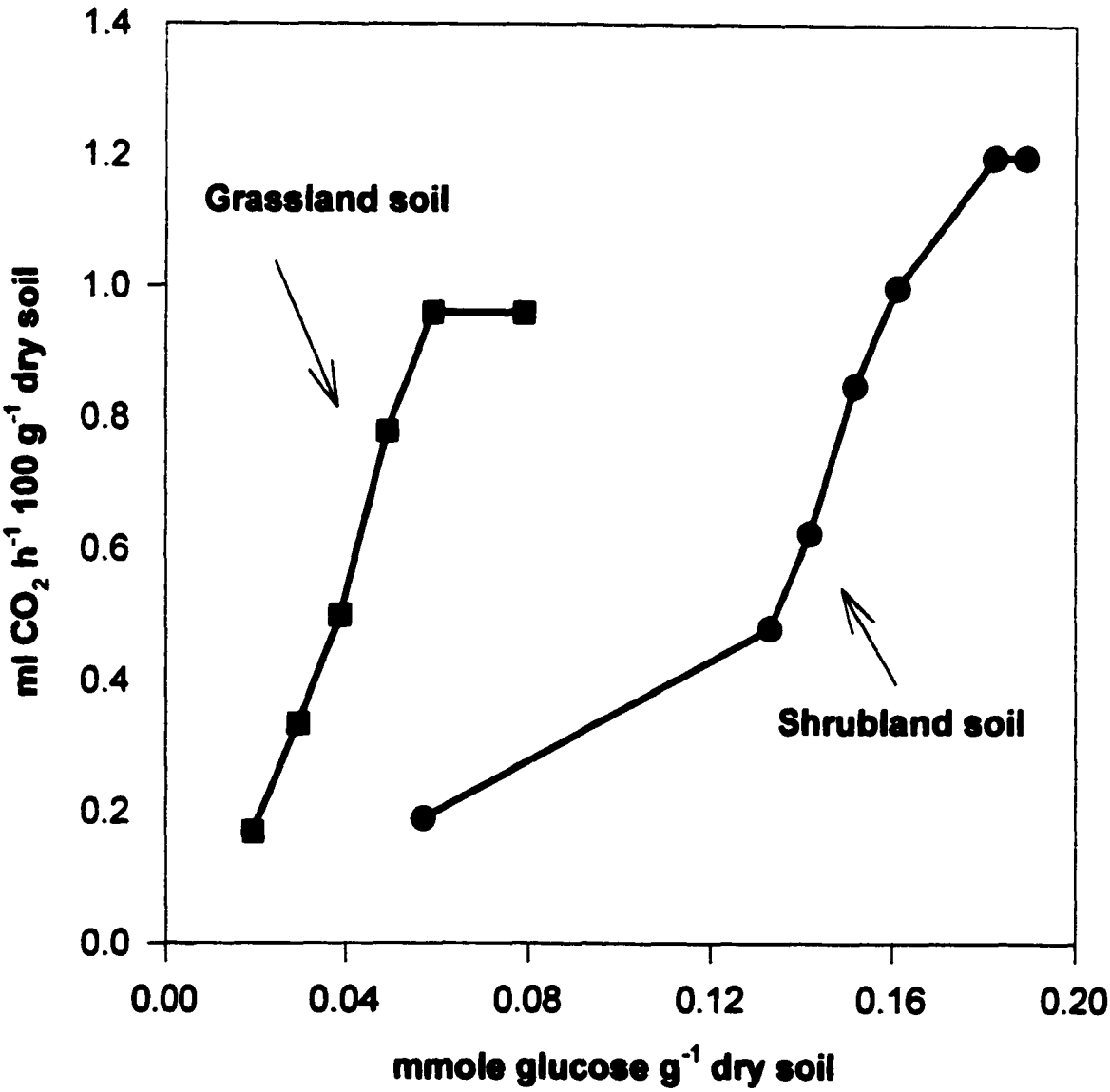

Figure 1.1. Microbial respiratory responses in relation to glucose concentrations. Respiration values were collected for 1-h incubations in July 1997.

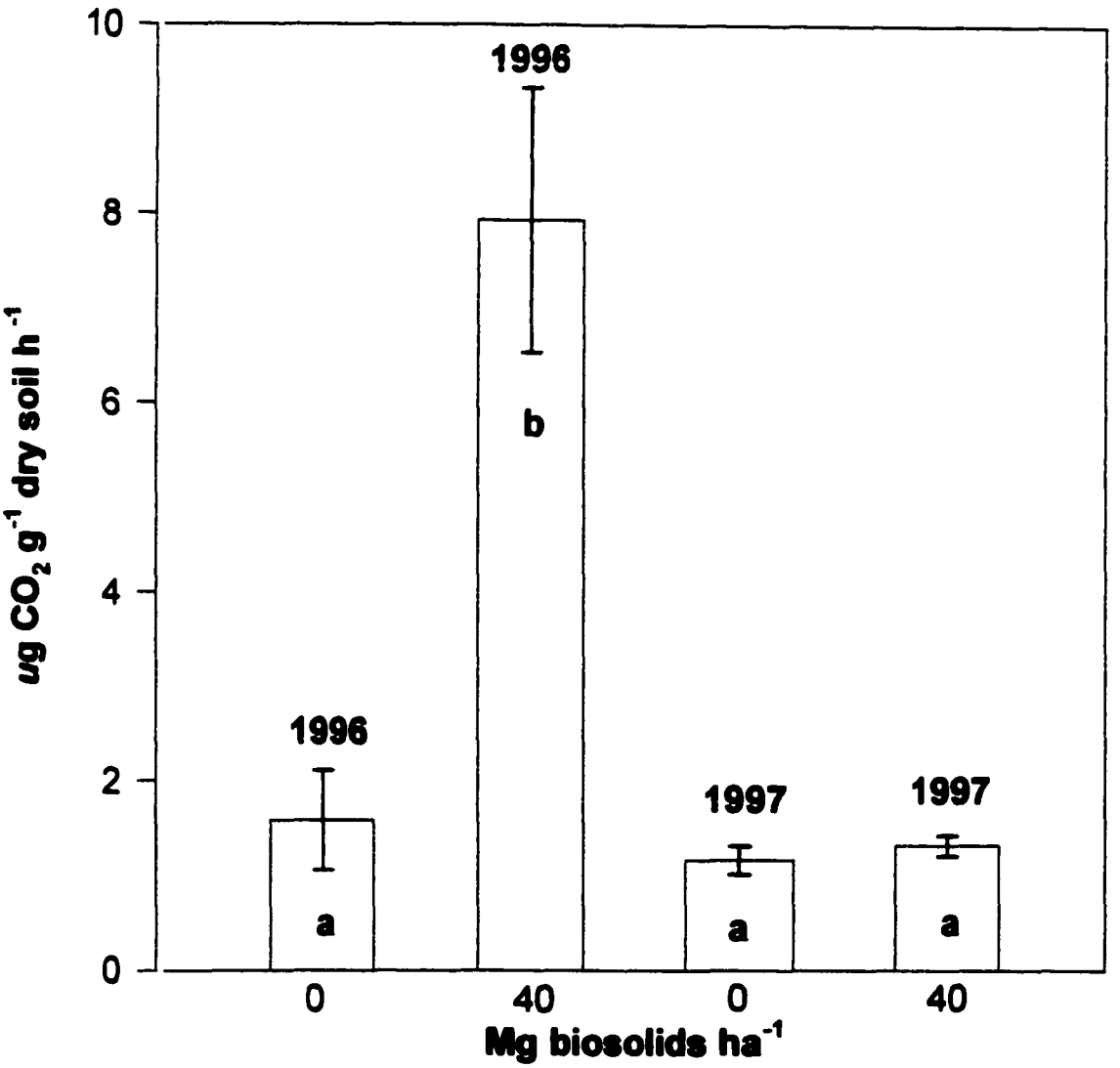

Figure 1.2. Microbial basal respiration responses to biosolids at the shrubland site in July 1996 and 1997. Histogram bars represent the mean of n=30. Different letters indicate significant differences at $p<0.05$ (Fisher's LSD). Error bars show 95% CI.

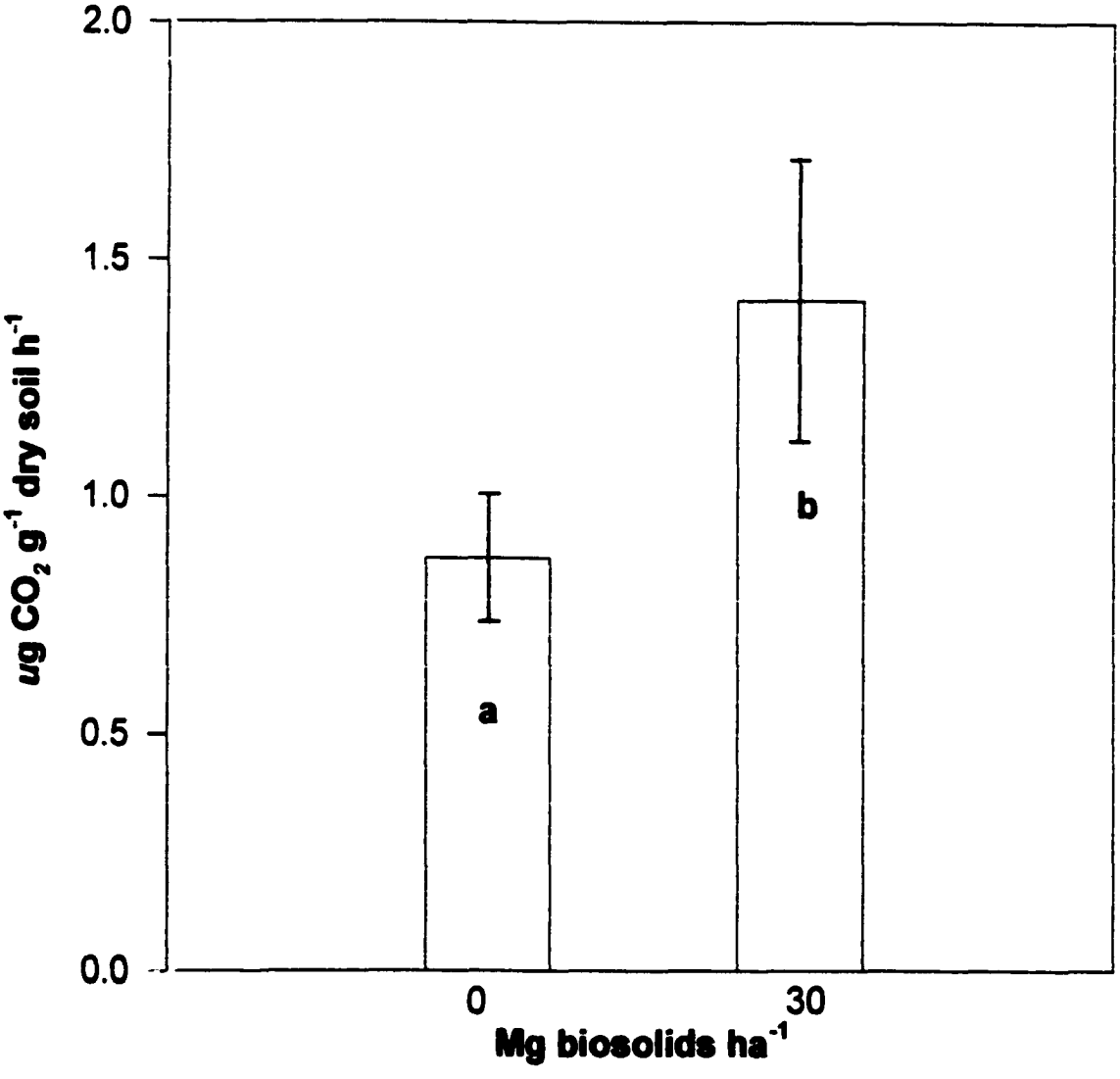

Figure 1.3. Microbial basal respiration responses to biosolids at the grassland site in August 1997. Histogram bars represent the mean of n=20. Different letters indicate significant differences at $p<0.05$ (Fisher's LSD). Error bars show 95% CI.

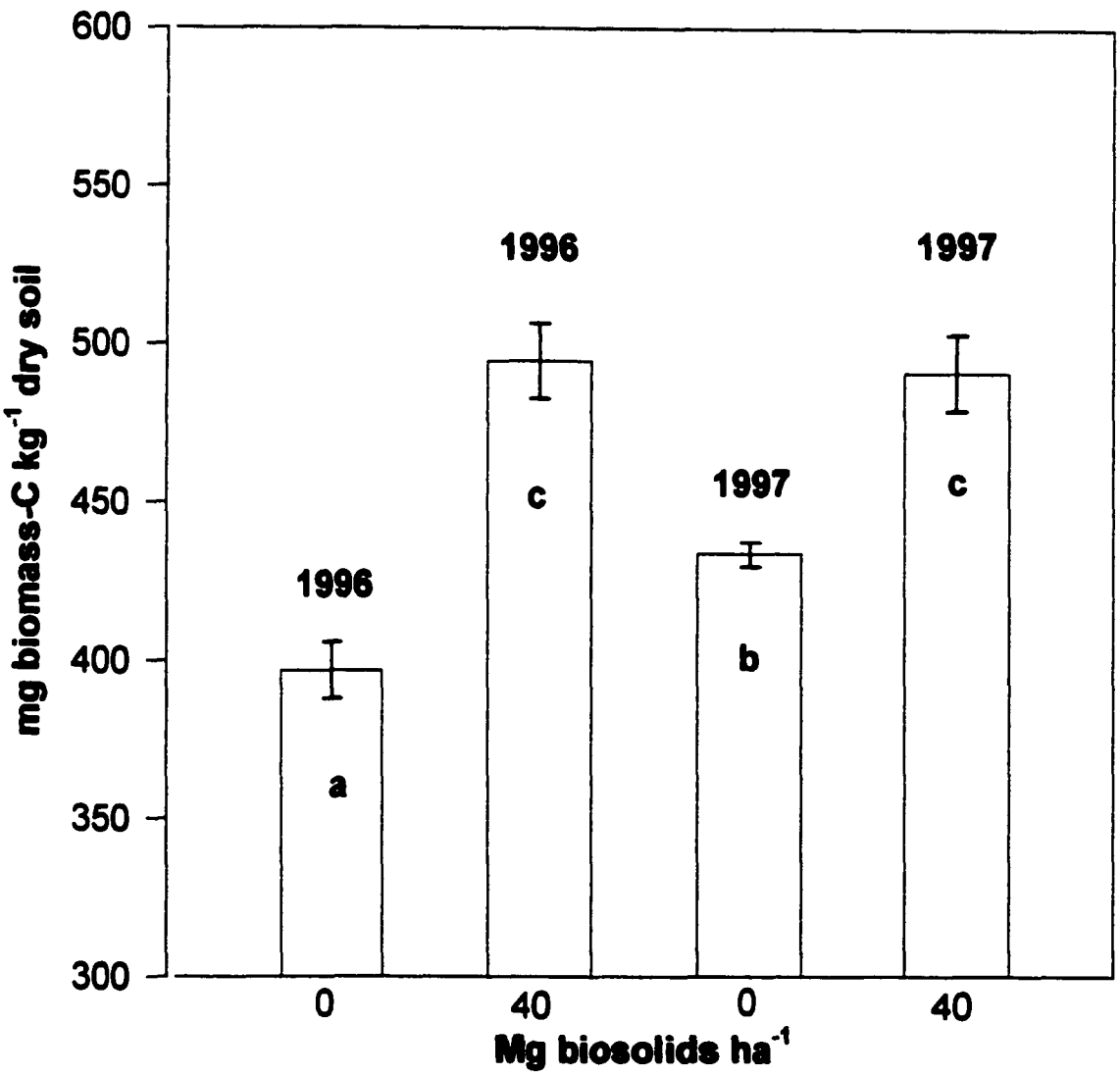

Figure 1.4. Active microbial biomass-C responses to biosolids at the shrubland site in July 1996 and 1997. Histogram bars represent the mean of n=30. Different letters indicate significant differences at $p<0.05$ (Fisher's LSD). Error bars show 95% CI.

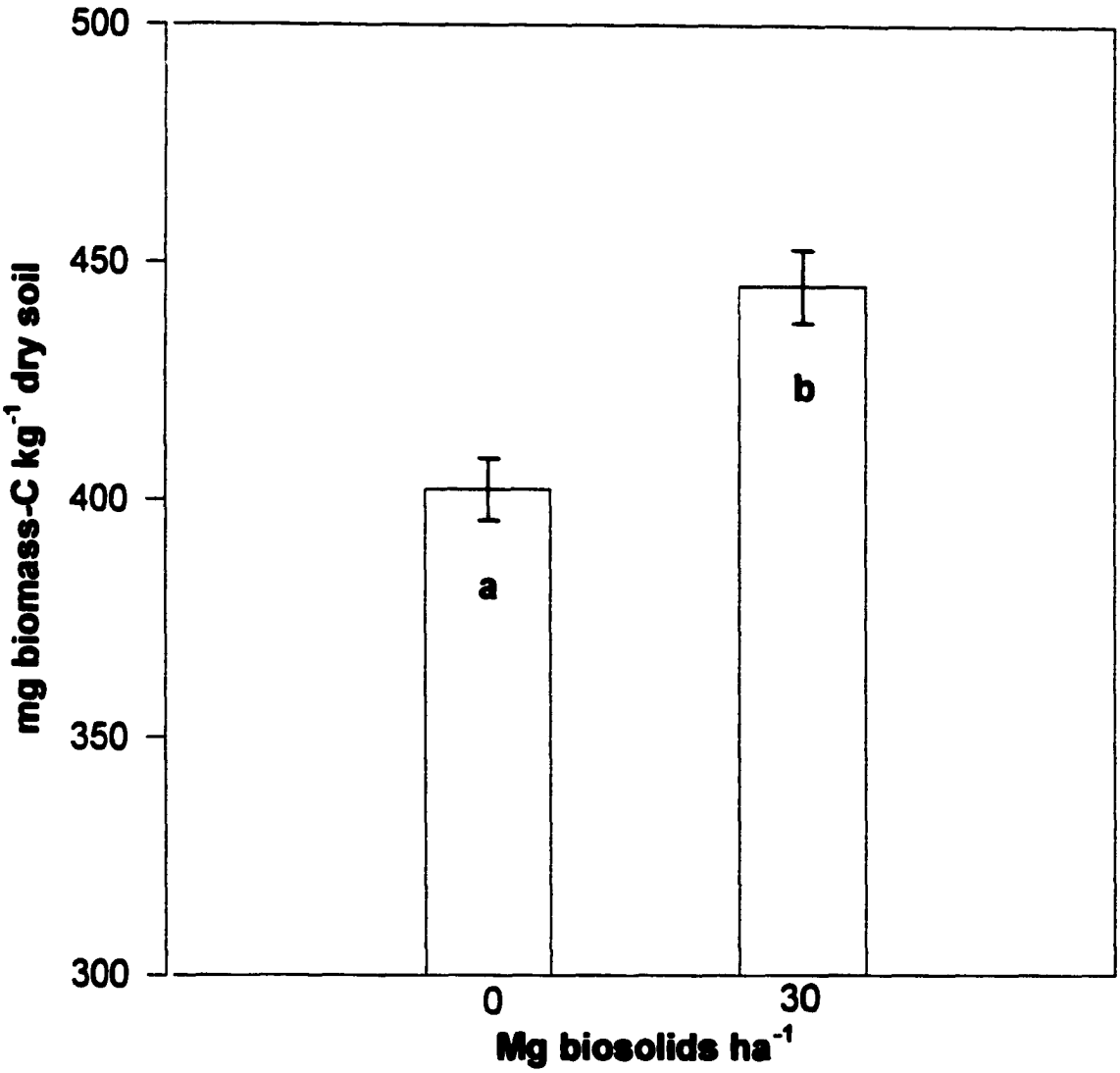

Figure 1.5. Active microbial biomass-C responses to biosolids at the grassland site in August 1997. Histogram bars represent the mean of n=20. Different letters indicate significant differences at $p<0.05$ (Fisher's LSD). Error bars show 95% CI.

CHAPTER 2

EFFECT OF BIOSOLIDS ON POTENTIAL CARBON AND NET NITROGEN MINERALIZATION

ABSTRACT

Carbon and N mineralization of soil organic materials are important ecosystem-level processes which may be impacted by biosolids amendment due to the addition of organic matter, nutrients, and trace elements. This study was conducted to determine the effect of surface-applied biosolids on potential C and net N mineralization in soils of semiarid rangelands in Colorado. Biosolids were applied once in 1991 at 0 and 40 Mg ha^{-1} to a shrubland soil and at 0 and 30 Mg ha^{-1} to a grassland soil and analyzed 6 yr later. During a 28-d incubation, biosolids-amended shrubland and grassland soils showed 130% and 71% increases, respectively, in potential C mineralization over unamended soil (control soil) 6 yr following treatment. These increases are positively correlated ($r = 0.70$ for the shrubland and $r = 0.46$ for the grassland) at $p \leq 0.05$ with increases in total soil organic C. Biosolids amendment of the shrubland resulted in a 440% increase in potential net N mineralization over control soil in 1997, and although a similar trend was seen in the grassland soil, the results were not statistically significant. The incubation period was not sufficiently long to allow stabilization of the biosolids-amended soil systems which would have provided more information on mineralization of organic matter during the later stages of decomposition. The results may also mean that the biosolids C and N were mineralized to a greater extent during the 6 yr following treatment at the grassland site due

to the lower biosolids application rate, coarser soil texture, warmer mean annual temperature, and a longer growing season. The net N mineralized and the total soil N are positively correlated with $r = 0.67$ for the shrubland and $r = 0.47$ for the grassland at $p \leq 0.05$. These results indicate that when field environmental conditions are optimal for microbial activity, increases in C and N mineralization may still be expected after 6 yr for these biosolids-amended rangeland soils.

INTRODUCTION

Land application of biosolids is often reported to have a beneficial impact on soil fertility in arable land (Dar and Mishra, 1994) because the mineralization of organic materials in the biosolids gradually releases essential plant nutrients and produces stable soil organic matter. Primary productivity in semiarid rangelands is limited by N as well as soil moisture (Bolton et al., 1990), so efficient retention and cycling of nutrients is necessary to maintain ecosystem productivity. Biosolids amendment of soil can increase soil organic matter content (Logan et al., 1997), N supply (Wen et al., 1995), and soil water-holding capacity (Catroux et al., 1981). Biosolids may also provide trace element concentrations sufficient to overcome trace element deficiencies in plants or soil microorganisms. However, the quality of the biosolids can vary greatly due to varying concentrations of trace elements (USEPA, 1985), relative proportions of inorganic and organic N (Jarvis et al., 1996), and the recalcitrance of the organic fraction resulting from the biosolids production process (Smith et al., 1992). Because biosolids addition to soil may affect the functioning of soil microorganisms, I investigated its effect on microbially-mediated mineralization processes. The objectives of this study were to determine if large

additions of biosolids containing moderate levels of trace elements increased potential C and net N mineralization of shrubland and grassland soils over control soils.

MATERIALS AND METHODS

Field Sites

This study was conducted at two semiarid rangeland sites that were established in 1991 (Pierce et al.; 1998; Harris-Pierce, 1994). The shrubland study site is a sagebrush steppe located in the intermountain region 2 km north of Wolcott, CO at an elevation of 2225 m. The grassland study site is part of Meadow Springs Ranch and is a shortgrass steppe located 32 km north of Fort Collins, CO at an elevation of 1750 m. Soils are of the Tanna-Pinelli complex consisting of Aridic Argiborolls and Borollic Haplargids (SCS, 1992) at Wolcott, and the soil is an Aridic Argiustoll at Meadow Springs Ranch (SCS & FS, 1980). The mean annual precipitation at Wolcott is 356 mm, with 40% received as snowfall, the mean annual temperature is 5.6 °C, and the mean frost-free period is 85 d (SCS, 1992). The mean annual precipitation at Meadow Springs Ranch is 356 mm, with 25% received as snowfall, the mean annual temperature is 9.4 °C, and the mean frost-free period is 125 d (SCS & FS, 1980). I collected soil for laboratory incubations from Wolcott and Meadow Springs Ranch in May 1997. Selected chemical characteristics of the soil are shown in Tables 2.1 and 2.2.

Experimental Design

Plots were arranged in a randomized complete block design at both study sites with three replications for the shrubland site (Pierce et al., 1998) and four replications for the grassland site (Harris-Pierce, 1994). Plot sizes at the Wolcott site vary in dimension, but are approximately

31 m by 107 m (3320 m^2). The plots at the Meadow Springs Ranch site are 15 m by 15 m (225 m^2). Plots received 0 and 40 Mg biosolids ha^{-1} at the shrubland site and 0 and 30 Mg biosolids ha^{-1} at the grassland site. Both study sites received surface-applied biosolids from nearby municipal sources in 1991, and the chemical properties of the biosolids are described in Table 2.3.

Sampling Plan

During May 1997, I collected composite soil samples (ten samples per plot, 0-5 cm depth) on the three replications of the 0 and 40 Mg biosolids ha^{-1} treatments at Wolcott. In May 1997, I also collected composite soil samples (five samples per plot, 0-5 cm depth) on the four replications of the 0 and 30 Mg biosolids ha^{-1} treatments at Meadow Springs Ranch. The composite soil samples were used for the laboratory incubations to determine potential C and net N mineralization. Additional random soil samples were collected in July 1997 at the Wolcott site (ten samples per plot, 0-5 cm depth, three replications) and in August 1997 at the Meadow Springs Ranch site (five samples per plot, 0-5 cm depth, four replications) for determination of selected chemical characteristics (Tables 2.1 and 2.2).

At both study sites transects were placed from east to west down the center of each plot, and a random number generator was used to locate each successive position along the transects. I used a random number generator to select which direction (south or north of the transect) I would begin taking soil samples. Movement was from east to west, and after the first sample was taken, I alternated south and north directions during sampling. At each randomly located sampling position, I placed a 20 by 25-cm frame on the ground, marked each corner to a 5-cm depth, and then scraped away the top 0.5 cm of soil and litter to avoid soil contamination by

remaining biosolids on the soil surface. The soil was collected with a sterilized hand spade (wiped with isopropyl alcohol) to a 5-cm depth, placed in polyethylene bags, and stored at 5°C in the dark until further processing in the lab.

Soil Analyses

I took random subsamples from each field-collected sample taken in July and August 1997 and prepared them separately for measurement of pH, electrical conductivity (EC), gravimetric soil moisture content, soil ammonium bicarbonate-diethylenetriaminepentaacetic acid (AB-DTPA)-extractable metals, total organic C, and total N. The following methods were selected for analyses: pH and EC (Rhoades, 1982), and soil AB-DTPA-extractable metals (Barbarick and Workman, 1987) followed by determination of constituent concentrations on an inductively coupled plasma spectrophotometer (ICP). Soil organic C and total N were measured using a LECO CHN-1000 automated analyzer. I pre-treated the Wolcott soil samples with 10% HCl and oven-dried them at 70° C to remove excess carbonates before measuring total organic C.

Laboratory Incubations

I prepared 21 field-moist subsamples from each composite soil sample taken in May 1997 for laboratory incubations to determine potential C and net N mineralization using the method of Stanford and Smith (1972) with some modifications. Triplicate incubations were prepared to determine potential net N mineralization on day 0, 2, 4, 6, 10, 14, and 28 of the incubation study. During each incubation period three subsamples per treatment were randomly selected for destructive sampling of inorganic N. A total of 126 incubations were used for the Wolcott soil and 168 for the Meadow Springs Ranch soil. The subsamples were coarsely sieved (< 8 mm), hand mixed, and roots and stubble were manually removed.

I first determined the gravimetric moisture content (the percentage of the total by oven-dry weight) of the field-moist soil samples. These values ranged from 7.2% to 21%. I then determined the gravimetric moisture content for each treatment at field capacity by placing separate subsamples in graduated cylinders with holes in the bottom to allow leaching of distilled water which was added to bring soil moisture content up to saturation. The top of each cylinder was sealed with polyethylene permeable to air but impermeable to water, and the cylinders were allowed to drain for 24 h. I measured gravimetric moisture content at field capacity for each treatment and found values ranging from 31% to 55%.

The incubations were set up in 0.95 L airtight glass Mason jars and incubated at 25° C in the dark. I used 25-g field-moist samples adjusted to gravimetric moisture content at field capacity. The incubations were adjusted for moisture content at 7, 14, and 21 d, as needed. Potential net N mineralization during each incubation period was calculated as an average of the triplicate subsamples per treatment using the combined amounts of NO_3-N and NH_4-N in the soil. Inorganic N present in the soil at the beginning of the incubation period was subtracted from the sum of inorganic N present in the soil sampled at the end of the incubation period (equation 2).

$$\textit{Net N mineralized} = \textit{Final soil inorganic N} - \textit{Initial soil inorganic N} \quad (2)$$

The soil was extracted with 50 mL of 2 M KCl and shaken for 1 h. The soil extracts were filtered through Whatman #5 filter paper that was rinsed with 20-25 mL of 2 M KCl. The filtrates were analyzed using "The FLOW Solution™ " segmented flow analysis system (Perstorp Analytical Methodology, 1994) for NO_3-N and NH_4-N . Nitrate-N was determined using the Cd column reduction method outlined in Perstorp Analytical method no. P/N 001094. Ammonium-

N was determined using the salicylate-nitroprusside-cyanurate method outlined in Perstorp Analytical method no. P/N 001146.

Concurrent measurement of CO_2 production was accomplished during the N mineralization incubation experiment using an alkali trap method. I placed vials containing 5 mL of 1 M NaOH in each glass incubation jar at the beginning of the 28-d incubation period. Additional empty jars (in triplicate) were set up containing vials with 5 mL of 1 M NaOH to determine background levels of CO_2 for each incubation period. Trapped CO_2 was precipitated as carbonate with excess $BaCl_2$. The CO_2 evolved during incubation was measured by titration against a standard acid solution (0.2 M HCl) with three drops of phenolphthalein as an indicator. The amount of CO_2 evolved was calculated using the relationship that 1 mL of 1 M NaOH = 22 mg CO_2. All values for CO_2 produced were corrected for background CO_2 levels.

Statistical Analysis

Data were analyzed using the GLM procedure (SAS, 1994) to evaluate biosolids treatment effect. When the F-Test was significant for a treatment effect, Fisher's least significant difference (LSD) test was used for mean comparisons ($p \leq 0.05$).

RESULTS AND DISCUSSION

Impact of Biosolids on Potential Carbon Mineralization

Laboratory incubations to determine potential C mineralization provide a method to measure the ability of the soil microbial populations to decompose existing soil organic materials under optimal conditions. The addition of 40 Mg biosolids ha^{-1} to shrubland soil in 1991 significantly increased potential C mineralization over control plots from day 4 through

day 28 of the laboratory incubation (Fig. 2.1). Fig. 2.2 indicates a significantly higher rate of CO_2-C evolution for grassland biosolids-amended soil over control soil only on day 28 of the incubation. The 28-d incubation period was not sufficiently long to allow stabilization of soil systems which had received biosolids. Fig. 2.1 and 2.2 show that CO_2 evolution for biosolids-amended soil is still exhibiting a positive slope by the end of the incubation, and it is not known when the values would reach a plateau. After 28 d, biosolids-amended shrubland and grassland soils showed 130% and 71% increases, respectively, in potential C mineralization over unamended soils.

Other researchers have reported smaller increases in CO_2-C evolution by 30 days during laboratory incubations of soil receiving non-metal-contaminated biosolids of 15-39% (Dar and Mishra, 1994), 30% (Oba and Nguyen, 1981), and 50% (Hattori and Mukai, 1986). Carbon mineralization is a sensitive indicator for metal pollution, even at low trace element concentrations. Inhibitory effects have been reported for soil additions of 10 mg Cd kg^{-1} soil (Necker and Kunze, 1986), 10-50 mg Cd kg^{-1} (Doelman and Haanstra, 1984), 25 and 50 mg Cd kg^{-1} soil (Dar and Mishra, 1994), 375 mg Pb kg^{-1} soil (Doelman and Haanstra, 1979), and 100 mg Cu kg^{-1} soil (Bhuiya and Cornfield, 1972).

When both elevated trace element concentrations and biosolids are present in soil, results have been less clear. Dar and Mishra (1994) observed no significant changes in C mineralization for biosolids-amended soil containing 10 mg Cd kg^{-1} soil during a 60-d incubation. When available Cd concentrations (DTPA extraction) were increased in biosolids-treated soil to 25 and 50 mg Cd kg^{-1} soil, they reported significant decreases in CO_2-C evolution. When two biosolids of differing Cd concentrations (2 mg Cd kg^{-1} soil and 815 mg Cd kg^{-1} soil)

were added at 20 Mg biosolids ha^{-1} to an arid soil, Moreno et al. (1999) reported no significant differences in CO_2-C evolution. The authors did report a sizeable increase in C mineralization when the Cd-contaminated biosolids (815 mg Cd kg^{-1} soil) were added at 80 Mg biosolids ha^{-1}. Total and available Cd concentrations in the shrubland and grassland soils (Tables 2.1 and 2.2) are far lower than the values reported by Dar and Mishra (1994) and Moreno et al. (1999). In a separate study, Dar (1997) reported that the addition of 100 mg Pb kg^{-1} soil had no significant influence on C mineralization in a biosolids-amended soil, whereas the addition of 250 mg Pb kg^{-1} soil caused a significant decrease in C mineralization in a sandy loam soil amended with biosolids. Again, the shrubland and grassland soil which received biosolids treatment had far lower concentrations of Pb (Tables 2.1 and 2.2) than the soil investigated by Dar (1997).

The large increases in potential C mineralization for biosolids-amended shrubland and grassland soils indicate that the treated soils have larger pools of available C substrates than unamended soil. These results are positively correlated with increases in total soil organic C ($r = 0.70$ for the shrubland and $r = 0.46$ for the grassland). Correlation coefficients are significant at $p \leq 0.05$ and were calculated using $n = 18$ at the shrubland site and $n = 24$ at the grassland site. Total soil organic C is also positively correlated with increases in the size of the metabolically active microbial biomass ($SIR\text{-}C_{micr}$) on biosolids-amended soil in 1996 ($r = 0.87$ for the shrubland) and 1997 ($r = 0.76$ for the shrubland and $r = 0.71$ for the grassland) indicating microbial assimilation of biosolids C. These correlation coefficients are significant at $p \leq 0.05$ and were calculated using $n = 60$ for the shrubland site and $n = 40$ for the grassland site. In addition, the increase in total soil organic C on biosolids treated plots may be due to increases in

organic C inputs as a result of earlier reported increases in aboveground perennial grass biomass (summer 1993) at the shrubland (Pierce et al., 1998) and grassland sites (Harris-Pierce, 1994).

It appears that decomposition of organic materials added with the biosolids is not yet complete. Decomposition of organic materials in semiarid rangelands is typically slow due to moisture limitations (Sturges, 1975). The increases in CO_2-C evolution of biosolids-amended shrubland and grassland soils represent potential changes which could occur in C cycling under field conditions when moisture is not limiting soil organic matter decomposition or plant production. The large variability in results (95% CI) for CO_2-C evolution on biosolids-treated plots (Fig. 2.1 and 2.2) may also indicate greater spatial heterogeneity resulting from uneven placement or subsequent movement of organic materials contained in the biosolids across the landscape.

Biosolids Effect on Potential Net Nitrogen Mineralization

Nitrogen mineralization of soil organic materials is an important activity of soil microorganisms relevant at the ecosystem level as a key process controlling primary production. Addition of 40 Mg biosolids ha^{-1} at the shrubland site caused an increase in potential net N mineralization evident by day 28 of the laboratory incubation (Fig. 2.3). The cumulative N mineralized for biosolids-amended soil was 440% higher than control plots 6 yr following addition of the biosolids. Results for the grassland soil indicate no significant difference in the amount of N mineralized between the 30 Mg biosolids ha^{-1} soil and the soil from control plots 6 yr following treatment (Fig. 2.4). A trend towards higher net N mineralization for the biosolids-amended grassland soil is clearly evident in Fig. 2.4 and is similar to the results reported for the shrubland soil; however, the incubation period was not sufficiently long to allow stabilization of

the soil system. Both Fig. 2.3 and 2.4 show that the cumulative amount of mineralized N has not reached its maximum (the slope is positive) for biosolids treated soils by day 28 of the incubation. The net N mineralized and the total soil N (Tables 2.1 and 2.2) are positively correlated with $r = 0.67$ for the shrubland and $r = 0.47$ for the grassland. The correlation coefficients are significant at $p \leq 0.05$ and were calculated using $n = 18$ at the shrubland site and $n = 24$ at the grassland site. When field environmental conditions are optimal for microbial activity at the shrubland site, increased N mineralization may occur on biosolids-amended soil.

Microbial transformations of organic N have been reported to be closely related to those of organic C (Paul and Juma, 1981). Comparison of the C mineralization curve (Fig. 2.1) to the N mineralization curve (Fig. 2.3) for the biosolids-amended soil at the shrubland site indicates that an increase in net N mineralization was not evident until after day 14, although an increase in C mineralization began to occur by day 4 of the incubation period. A similar trend is seen in grassland biosolids-amended soil (Fig. 2.2 and 2.4), although the results are not statistically significant. This delay in release of inorganic N may be due to microbial immobilization during the early stages of decomposition.

The variables affecting trace element bioavailability in soils are numerous and complex. Soil variables such as texture (Hattori, 1989), organic matter content, pH, cation exchange capacity (CEC), and $CaCO_3$ content (Dar, 1995; Baath, 1989) influence the toxic effects of trace elements on soil microorganisms and their activities. Therefore, it is not surprising that trace element additions to soils have been reported to have stimulatory (Dar, 1997; Premi and Cornfield, 1969), inhibitory (Dar and Mishra, 1994; Chang and Broadbent, 1982; Liang and

Tabatabai, 1978), and no effects (Dar, 1997; Minnich and McBride, 1986; Mather and Preston, 1981) on net N mineralization in soils.

Minnich and McBride (1986), in a study of soils which had received Cu-enriched biosolids both 3 and 26 yr prior to their study, found no significant effect of between 6 and 40 total mg Cu kg^{-1} soil on net N mineralization rates, when compared to control soils. In my study, in 1993 the shrubland biosolids-amended soil contained a total concentration of 21.3 mg Cu kg^{-1} soil, and the grassland biosolids-amended soil contained 48.2 mg Cu kg^{-1} soil (Tables 2.1 and 2.2). In 1994, Dar and Mishra investigated the effects of Cd on N mineralization in biosolids-amended and unamended soils. The authors report that 10 mg Cd kg^{-1} soil (DTPA extraction) had no significant effect on N mineralization, but reported that 25 and 50 mg Cd kg^{-1} soil (DTPA extraction) did cause significant decreases in N mineralization over control soil, both with and without the addition of biosolids. They investigated a range of soil textures and concluded that clay content was a more important factor than organic matter content in detoxification of Cd added to soils. The shrubland soil has a higher clay content than the grassland soil which may provide some level of protection from the toxic effects of Cd, but the available concentration of 1.48 mg Cd kg^{-1} soil (AB-DTPA extraction) for the shrubland soil in 1997 was much lower than the levels evaluated by Dar and Mishra (1994).

In a separate study, Dar (1997) reported a decline in N mineralization at 500 mg Pb kg^{-1} soil (DTPA extraction) during a 60-d incubation of 48, 35, and 26% in sandy loam, loam, and clay loam soils, respectively. When the respective soils were treated with biosolids and 500 mg Pb kg^{-1} soil, they showed corresponding reductions of 38, 28, and 24% in N mineralization. Dar (1997) suggested that Pb was not significantly immobilized by biosolids but was by soil

phosphate. In comparison, both the shrubland and grassland biosolids-amended soils contain far lower concentrations of Pb (Tables 2.1 and 2.2), and the shrubland soil has a higher phosphate concentration (AB-DTPA) than those examined by Dar (1997).

CONCLUSIONS

Biosolids application rates of 40 Mg ha^{-1} at a shrubland site and 30 Mg ha^{-1} at a grassland site significantly increased potential C mineralization over unamended (control) soils 6 yr following treatment. Land application of biosolids increased potential net N mineralization of shrubland soil, but not grassland soil, over control soil 6 yr following biosolids addition. It appears that C and N mineralization of biosolids is more extensive in the grassland soil possibly due to a lower biosolids application rate, coarser soil texture, warmer mean annual temperature, and shorter growing season. Increases in total soil organic C ($r = 0.70$ for the shrubland and $r = 0.46$ for the grassland) and total soil organic N ($r = 0.67$ for the shrubland and $r = 0.47$ for the grassland) were positively correlated at a significance level of $p \leq 0.05$ with potential C mineralization and net N mineralization, respectively. These results indicate that when environmental conditions are optimal for microbial activity in these semiarid rangelands, increased microbial mineralization of organic C and N may be expected on biosolids-treated soils. Because the 28-d incubation period was too short to allow stabilization of biosolids-amended soil systems, I was not able to determine how decomposition of more recalcitrant materials was affected in later stages of decomposition.

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Table 2.1. Selected properties of soils at Wolcott, CO. Soil data were collected in July 1993 (Pierce et al., 1998) and in July 1997.

		1997		1993	
Constituent	**Units**	**0 Mg Biosolids ha^{-1}**	**40 Mg Biosolids ha^{-1}**	**0 Mg Biosolids ha^{-1}**	**40 Mg Biosolids ha^{-1}**
pH		7.3	7.1	6.9	7.1
EC	dS m^{-1}	0.71	0.73	0.50	0.70
Organic C	%	0.03	0.07	na	na
Total N	%	0.16	0.34	na	na
		AB-DTPA Extraction[+]		**4 M HNO_3 Extraction**[∓]	
P	g kg^{-1}	0.01	0.06	0.32	0.64
K	g kg^{-1}	0.25	0.48	1.33	1.81
Al	g kg^{-1}	0.01	0.01	4.57	6.27
Cd	mg kg^{-1}	0.47	1.48	2.35	2.90
Cr	mg kg^{-1}	0.08	0.09	6.20	8.00
Cu	mg kg^{-1}	4.62	15.1	13.0	21.3
Fe	g kg^{-1}	0.01	0.02	6.62	8.08
Mo	mg kg^{-1}	0.01	0.04	5.05	4.60
Ni	mg kg^{-1}	1.94	1.43	16.6	19.7
Pb	mg kg^{-1}	0.88	1.88	5.55	7.26
Zn	mg kg^{-1}	5.21	33.4	88.3	165

na = parameter not measured

[+] Barbarick and Workman, 1987

[∓] Bradford et al., 1975

Table 2.2. Selected properties of soils at Meadow Springs Ranch, CO. Soil data were collected in August 1997. Data collected for total metal analyses in July 1993 (Harris-Pierce, 1994).

Constituent	Units	0 Mg Biosolids ha^{-1}	30 Mg Biosolids ha^{-1}
pH		5.9	5.8
EC	dS m^{-1}	0.32	0.60
Organic C	%	1.54	3.46
Total N	%	0.09	0.32
		1993 4 M HNO_3 Extraction[∓]	
P	g kg^{-1}	0.23	1.16
K	g kg^{-1}	1.49	1.47
Al	g kg^{-1}	na	na
Cd	mg kg^{-1}	0.5	0.5
Cr	mg kg^{-1}	4.9	6.2
Cu	mg kg^{-1}	6.6	48.2
Fe	g kg^{-1}	4.45	4.55
Mo	mg kg^{-1}	0.01	1.00
Ni	mg kg^{-1}	4.7	5.6
Pb	mg kg^{-1}	6.1	10.3
Zn	mg kg^{-1}	19.5	64.4

na = parameter not measured

∓ Bradford et al., 1975

Table 2.3. Selected properties of biosolids used for surface treatment in 1991 at Wolcott (shrubland) and Meadow Springs Ranch (grassland). Trace element extraction by H_2SO_4-$HClO_4$ digest.

Constituent	Units	Shrubland	Grassland
pH		7.7	7.3
EC	dS m^{-1}	13.0	5.00
Organic N	g kg^{-1}	0.12	0.46
NO_3-N	mg kg^{-1}	0.16	3963
NH_4-N	mg kg^{-1}	168	102
P	g kg^{-1}	32.0	16.0
K	g kg^{-1}	3.6	1.95
Al	g kg^{-1}	0.16	8.7
Cd	mg kg^{-1}	4.8	5.06
Cr	mg kg^{-1}	22.1	39.7
Cu	mg kg^{-1}	567	553
Fe	g kg^{-1}	0.12	4.81
Mo	mg kg^{-1}	2.9	16.1
Ni	mg kg^{-1}	14.7	19.2
Pb	mg kg^{-1}	47.1	117
Zn	mg kg^{-1}	1210	776

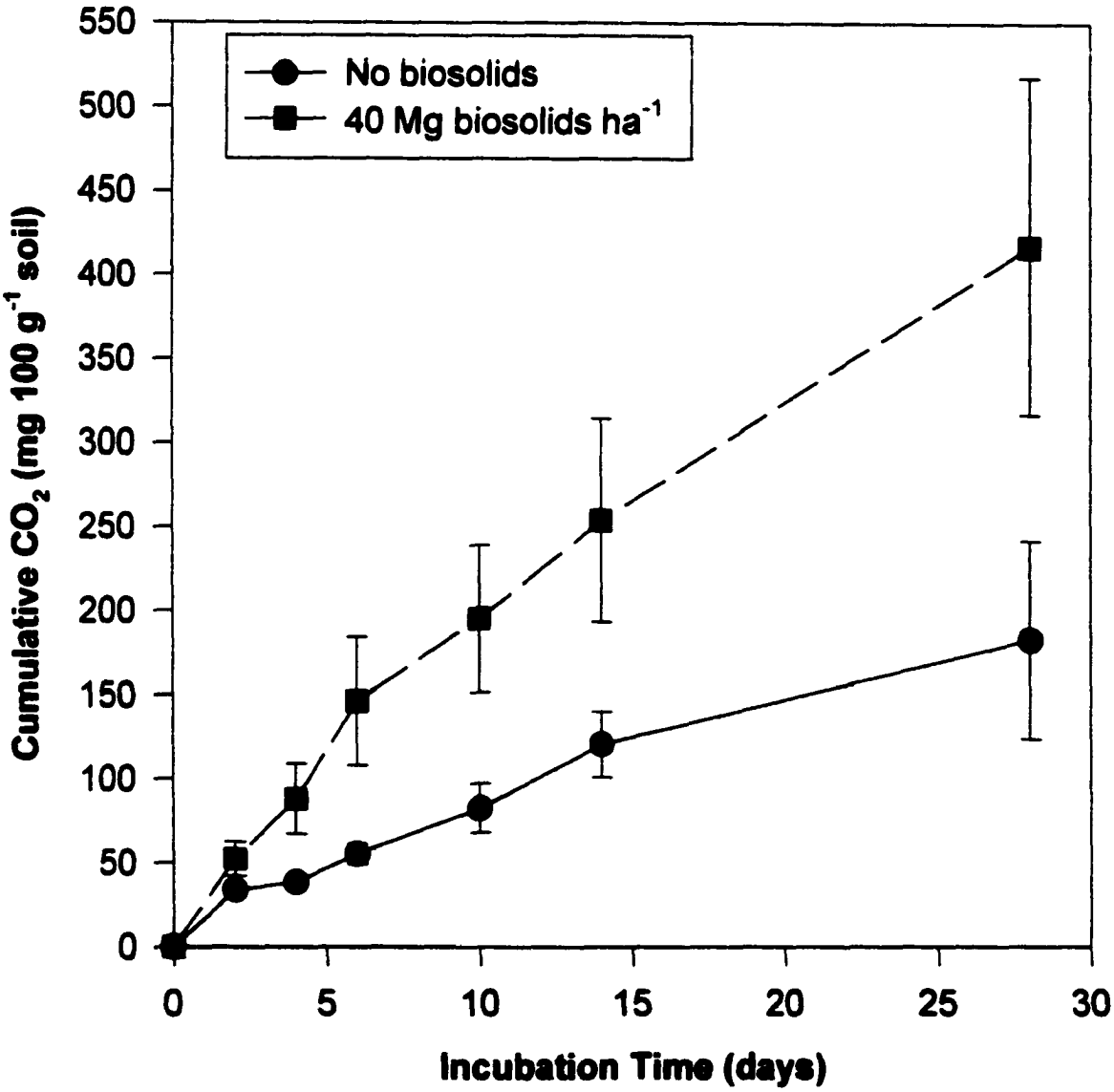

Figure 2.1. Biosolids effect on potential CO_2 evolution at the shrubland site in 1997. Points represent the mean of n=9. Error bars represent 95% CI.

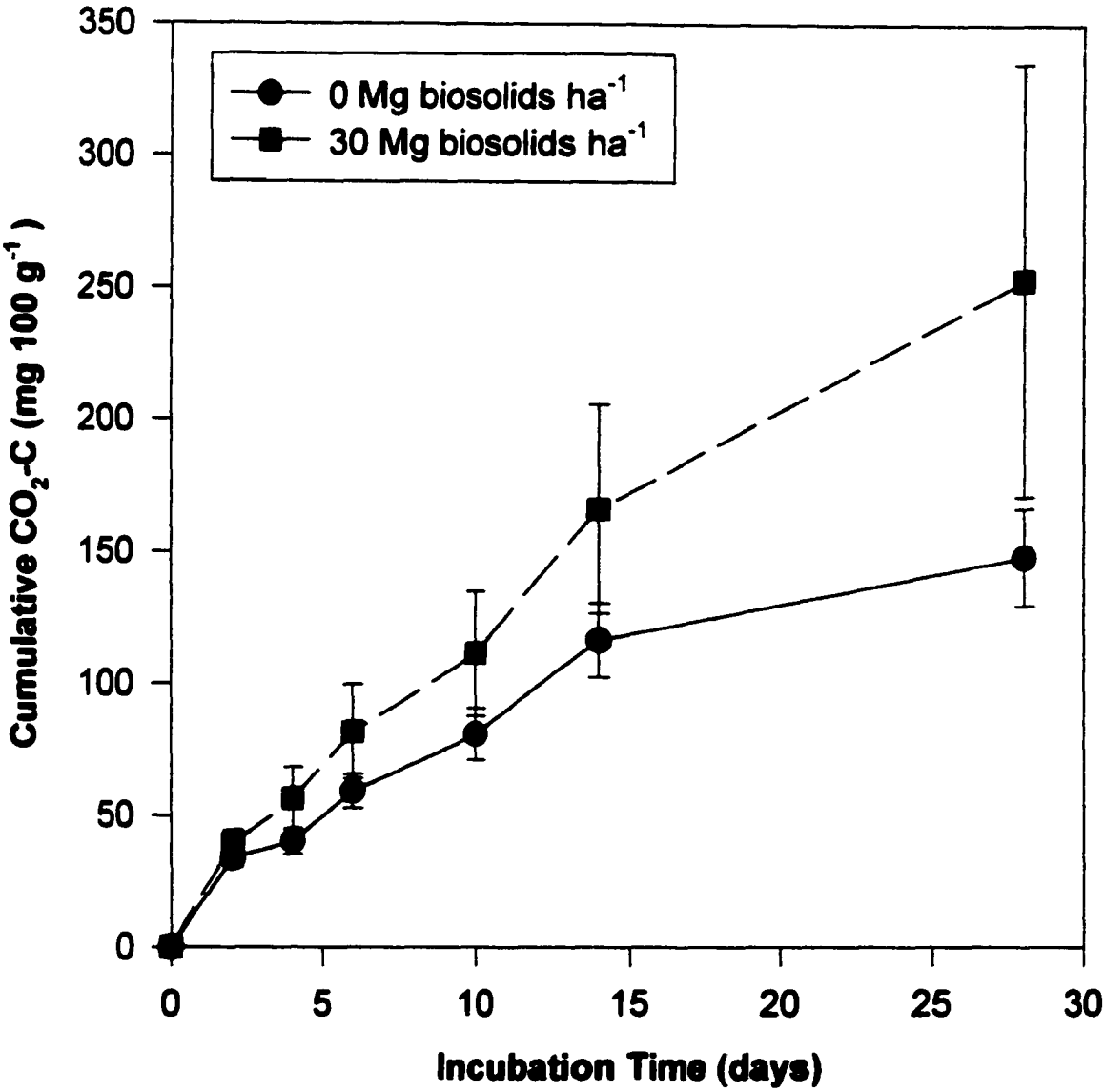

Figure 2.2. Biosolids effect on potential CO_2 evolution at the grassland site in 1997. Points represent the mean of n=12. Error bars represent 95% CI.

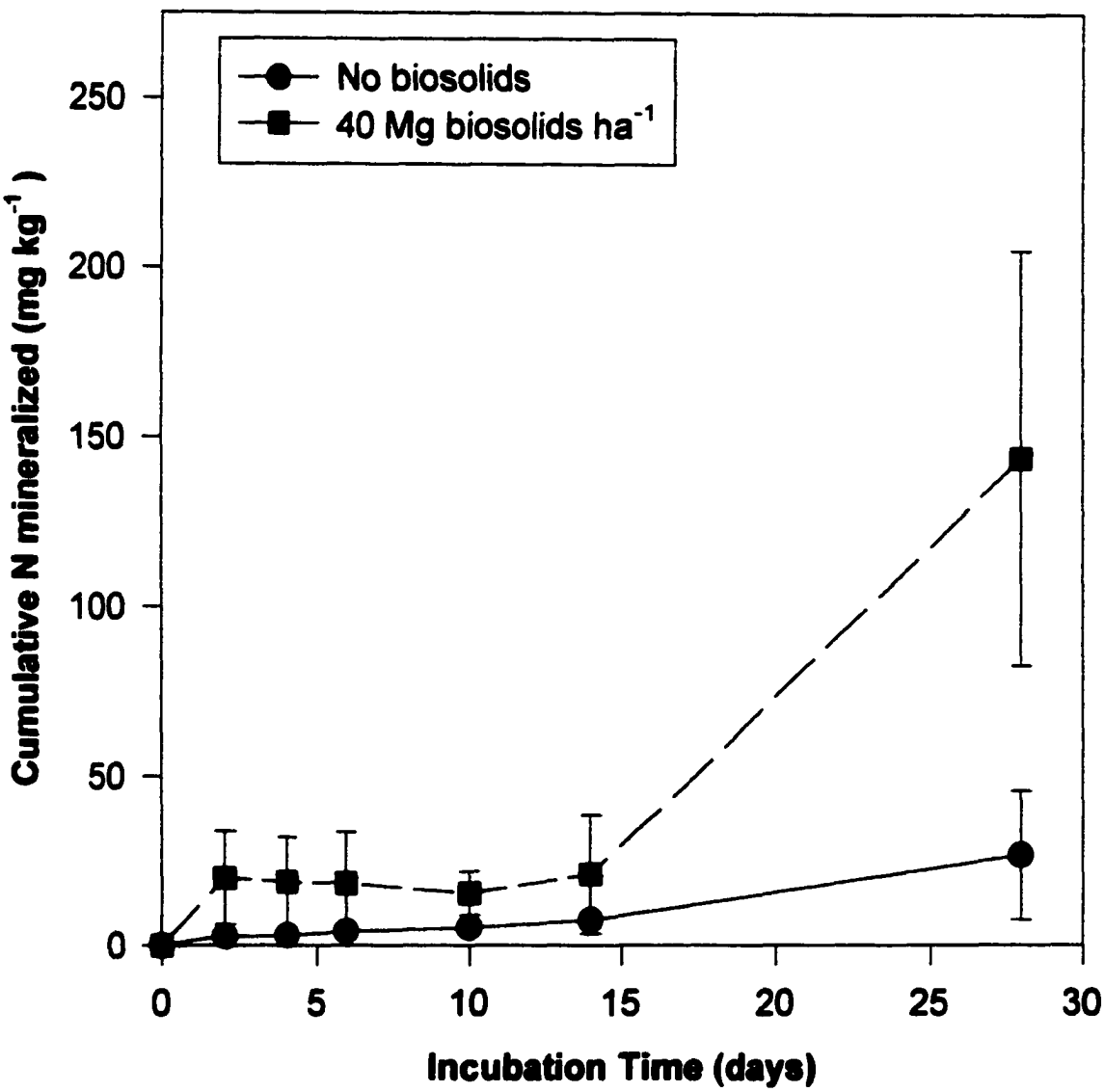

Figure 2.3. Effect of biosolids on potential nitrogen mineralization in shrubland soils in 1997. Points represent the mean of n=9. Error bars represent 95% CI.

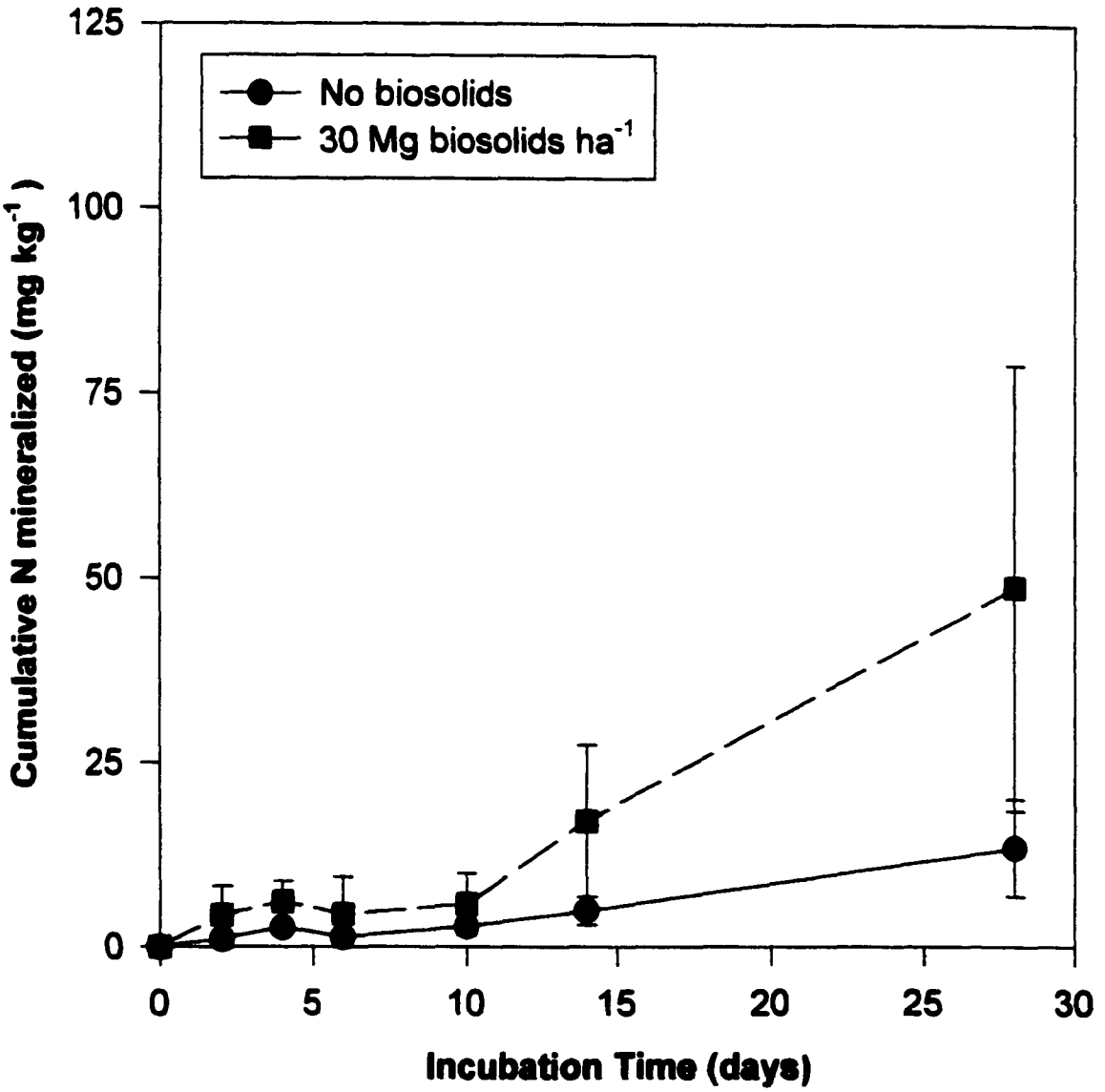

Figure 2.4. Effect of biosolids on potential nitrogen mineralization in grassland soils in 1997. Points represent the mean of n=12. Error bars represent 95% CI.

CHAPTER 3

BIOSOLIDS EFFECT ON MYCORRHIZAL COLONIZATION OF PERENNIAL GRASSES

ABSTRACT

Biosolids treatment of rangelands may alter the nutrient and trace element concentrations of rhizosphere soils thereby affecting arbuscular mycorrhizal (AM) development. Since most grasses in these ecosystems are mycotrophic, this study was initiated to determine if surface-applied biosolids, containing moderate amounts of trace elements, affected the percentage of root samples of western wheatgrass (*Pascopyrum smithii* (Rydb.) A. Love) and blue grama (*Bouteloua gracilis* (H.B.K.) Lag. ex steud) colonized by AM fungi. Biosolids were applied once in 1991 at 0 and 40 Mg ha^{-1} to shrubland soil and at 0 and 30 Mg ha^{-1} to grassland soil and analyzed 6 yr later. In 1997, western wheatgrass at the shrubland site showed a significant increase of 33% in the percentage of root samples colonized by AM fungi as a result of biosolids amendment. A significant increase of 23% was also found in the percentage of blue grama root samples of biosolids-treated soil colonized by AM fungi 6 yr following biosolids application. Biosolids treatment of both rangeland soils did not adversely affect the extent of root colonization by AM fungi for these economically important forage grasses.

INTRODUCTION

The most common type of mycorrhizal colonization of plant roots is by arbuscular mycorrhizal (AM) fungi (Gerdemann and Nicolson, 1963). Stahl and Christensen (1991) have estimated that 85 to 95% of grasses in natural grasslands are mycotrophic. Host plant and fungal interdependence can range from facultatively to obligately mycotrophic (Janos, 1980). The physical and chemical characteristics of rhizosphere soils are profoundly influenced by microbial activity, where AM fungi are often the predominant organisms. The most commonly reported benefits of AM colonization for host plants are gains in phosphate, N, and water uptake (Tate, 1995), increased uptake of poorly mobile ions (Sanders and Fitter, 1992), increases in aboveground biomass (Hoflich et al., 1994), improved drought resistance (Nelsen and Safir, 1982), and protection from plant pathogens (Newsham et al., 1995). Stahl and Christensen (1991) have suggested that AM fungi may have the greatest impact in soils with environmentally-stressed conditions which limit plant growth. Because biosolids addition to soil may alter nutrient and trace element concentrations and organic matter content in the rhizosphere, I investigated its effects on mycorrhizal colonization of perennial grasses in two semiarid rangelands. The objective of this study was to determine if additions of biosolids containing moderate levels of trace elements altered the percentage of western wheatgrass and blue grama root samples colonized by AM fungi in a semiarid shrubland and a grassland, respectively.

MATERIALS AND METHODS

Field Sites

This study was conducted at two semiarid rangeland sites that were established in 1991 (Pierce et al., 1998; Harris-Pierce, 1994). The shrubland study site is a sagebrush steppe located in the intermountain region 2 km north of Wolcott, CO at an elevation of 2225 m. The grassland study site is part of Meadow Springs Ranch and is a shortgrass steppe located 32 km north of Fort Collins, CO at an elevation of 1750 m. Soils are of the Tanna-Pinelli complex consisting of Aridic Argiborolls and Borollic Haplargids (SCS, 1992) at the Wolcott site, and the soil is an Aridic Argiustoll at the Meadow Springs Ranch site (SCS & FS, 1980). Western wheatgrass (*Pascopyrum smithii* (Rydb.) A. Love) (SCS, 1992) is a dominant grass at Wolcott, and blue grama (*Bouteloua gracilis* (H.B.K.) Lag. ex steud) is a dominant grass at Meadow Springs Ranch (SCS & FS, 1980). Both perennial grasses are late successional mycotrophic species (Allen and Allen, 1984). Selected chemical characteristics of the soils are shown in Tables 3.1 and 3.2.

Experimental Design

Plots were arranged in a randomized complete block design at both study sites with three replications for the shrubland site (Pierce et al., 1998) and four replications for the grassland site (Harris-Pierce, 1994). Plot sizes at the Wolcott site vary in dimension, but are approximately 31 m by 107 m (3320 m^2). The plots at the Meadow Springs Ranch site are 15 m by 15 m (225 m^2). Plots received 0 and 40 Mg biosolids ha^{-1} at the shrubland site and 0 and 30 Mg biosolids ha^{-1} at the grassland site. Both study sites received surface-applied biosolids from nearby municipal sources in 1991, and the chemical characteristics of the biosolids are described in Table 3.3.

Sampling Plan

During June 1997, I collected thirty random root samples per plot from western wheatgrass on 0 and 40 Mg biosolids ha^{-1} treatment plots at Wolcott, which were replicated three times. I also sampled thirty random root samples per plot from blue grama on 0 and 30 Mg biosolids ha^{-1} treatment plots at Meadow Springs Ranch on all four replicates during June 1997. At both study sites, transects were placed from east to west down the center of each plot, and a random number generator was used to locate each successive position along the transects. I used a random number generator to select which direction (south or north of the transect) I would begin taking soil samples. Movement was from east to west, and after the first sample was taken, I alternated south and north directions during sampling. At each randomly located sampling position, I found the nearest patch of the targeted grass species and used several tools (hand spade and screwdriver) to manually remove the smallest diameter root samples possible. I removed excess soil debris and placed the root samples in polyethylene bags, stored in an electric cooler at 5°C until further processing in the lab.

During July 1997, I collected ten random soil samples per plot on the 0 and 40 Mg biosolids ha^{-1} treatment plots at Wolcott, which were replicated three times. I collected five random soil samples per plot on the 0 and 30 Mg biosolids ha^{-1} treatment plots for all four replicates at Meadow Springs Ranch in August 1997. Transects were placed across each treatment plot in the same manner I used to collect root samples. At each randomly located sampling position, I placed a 20 by 25-cm frame on the ground, marked each corner to a 5-cm depth, and then scraped away the top 0.5 cm of soil and litter to avoid soil contamination by remaining biosolids on the soil surface. The soil was collected with a sterilized hand spade

(wiped with isopropyl alcohol) to a 5-cm depth, placed in polyethylene bags, and stored at 5 °C in the dark until further processing in the lab.

Soil Analyses

I took random subsamples from each field-collected sample taken in July 1997 and prepared them separately for measurement of pH, electrical conductivity (EC), gravimetric soil moisture content, soil ammonium bicarbonate-diethylenetriaminepentaacetic acid (AB-DTPA)-extractable metals, total organic C, and total N. The following methods were selected for analyses: pH and EC (Rhoades, 1982), and soil AB-DTPA-extractable metals (Barbarick and Workman, 1987) followed by determination of constituent concentrations on an inductively coupled plasma spectrophotometer (ICP). Soil organic C and total N were measured using a LECO CHN-1000 automated analyzer. I pre-treated the Wolcott soil samples with 10% HCl and oven-dried them at 70° C to remove excess carbonates before measuring total organic C.

Plant Root Analyses

The method of Trappe et al. (1973), with some modifications, was used for examination of AM fungi in root samples. I rinsed the root samples with distilled water to remove soil particles and organic matter and removed a 2-cm length section containing the smallest diameter roots. I placed the root segments into separate glass vials and added enough chlorox solution (seven drops of 2 M HCl in 100 mL of 3% chlorox solution) to cover the sample. After 5 min the root samples were rinsed twice with distilled water and then returned to the vials. I then added lactophenol-trypan blue solution to the vials and placed them in a water bath at 90° C for 5 min. A clear lactophenol solution was used to de-stain most root samples in a 90° C water bath for up to 10 min. Each sample was then mounted in lactophenol on a microscope slide. A total

of 420 microscope slides were examined under 100X and 1000X magnification between August 1997 and September 1998. I rated root samples for presence or absence of AM fungi based on arbuscules or vesicles plus interradical hyphae. If these conditions did not exist, the root sample was determined to have no AM fungal colonization. I chose arbuscules, vesicles, and interradical hyphae as the most important diagnostic structures for AM fungi because they are directly involved in nutrient exchange, storage, and translocation within the root cortical cells. Results are expressed as the percentage of total root samples per treatment colonized by AM fungi ($n = 120$ for each shrubland treatment and $n = 90$ for each grassland treatment).

Statistical Analyses

Data were analyzed using Student's t-test (SAS, 1994) to determine significance for a biosolids treatment effect. Significant differences were identified by Fisher's least significance difference (LSD) at $p \leq 0.05$.

RESULTS AND DISCUSSION

Evaluation of mycorrhizal colonization of perennial grasses

Giovannetti and Mosse (1980), in a comparison of techniques for measuring AM colonization in roots, determined that it was generally preferable to examine the largest possible number of replicates (≥ 30) of root samples ≥ 1 cm in length to reduce the standard error for any chosen method. My results (Fig. 3.1) support their recommendation for root sample length and sample number with standard errors ranging from $\pm$ 2% to $\pm$ 4%. I report significant increases in the percentage of root samples colonized by AM fungi for both the shrubland and grassland perennial grasses examined 6 yr following biosolids treatment (Fig. 3.1). In 1997, western

wheatgrass at the shrubland site showed a 33% increase in the percentage of root samples colonized by AM fungi as a result of biosolids amendment. I also found a 23% increase in the percentage of blue grama root samples of biosolids-treated soil colonized by AM fungi 6 yr following biosolids application. These increases are positively correlated with increases in total soil organic C content (Tables 3.1 and 3.2) at both sites ($r = 0.96$ for the shrubland and $r = 0.84$ for the grassland). The correlation coefficients are significant at $p \leq 0.05$ and were calculated using $n = 180$ at the shrubland site and $n = 240$ at the grassland site. Biosolids addition to both rangeland soils has positively affected the quantity of AM fungi able to colonize western wheatgrass and blue grama.

Field studies of the effects of organic matter additions to soil on AM root colonization have yielded conflicting results. Jensen and Jakobsen (1980) reported decreased colonization of barley and wheat resulting from additions of farm-yard manure. Harinikumar and Bagyaraj (1989), however, reported increases in densities of AM fungi in various crops resulting from additions of farm-yard manure. In studies using biosolids as an organic amendment, results have been equally conflicting. Arnold and Kapustka (1987) reported no effect of metal-containing biosolids on AM development in abandoned agricultural field plots 5 yr after biosolids application, whereas Koomen et al. (1990) reported suppression of AM development in clover in soil with long-term biosolids treatment in a greenhouse experiment.

In a recent study, Weissenhorn et al. (1995) investigated the effect of Zn-Mn and Cd-Ni contaminated biosolids on AM colonization of maize. Their results suggest greater tolerance of the indigenous population of AM fungi to elevated metal concentrations than to high phosphate concentrations. Because chemical extractions of trace elements only show relative differences

between treatments (Brummer et al., 1986), it is difficult to determine actual metal exposure of plants and soil microorganisms in the field. Bulk soil samples do not accurately reflect the heterogeneity of physical and chemical characteristics at microsites where soil microorganisms exist. For example, differences in physical protection of soil organic matter and adsorbed inorganic constituents from decomposition have been associated with entrapment in small pores in aggregates inaccessible to soil microorganisms (Elliott and Coleman, 1988) and encapsulation between clay particles (Tisdall and Oades, 1982).

The most likely reason for the increases in AM colonization of the grasses on biosolids-amended plots at both sites is fungal proliferation into soil locations with increased organic C content. The 130% increase in total soil organic C at the shrubland site and the 120% increase in total soil organic C at the grassland site 6 yr following biosolids addition has provided surplus substrate C in the top 5-cm of these rangeland soils. St. John et al. (1983) suggested that growth and placement of extraradical AM hyphae is affected by the amount and quality of soil organic matter in the rhizosphere.

CONCLUSIONS

Biosolids application rates of 40 Mg ha^{-1} at a shrubland site and 30 Mg ha^{-1} at a grassland site significantly increased the percentage of western wheatgrass and blue grama roots, respectively, that were colonized by AM fungi 6 yr following biosolids treatment. These increases are positively correlated at $p \leq 0.05$ with increases in total soil organic C content at both sites ($r = 0.96$ for the shrubland and $r = 0.84$ for the grassland). It is likely that fungal

proliferation into soil locations with increased organic C content is responsible for the quantitative increase in AM development at both rangeland sites. The increased AM development in these perennial grasses may benefit grass quality and production in these semiarid rangelands by improving drought resistance, aboveground biomass production, and plant tissue quality (i.e., higher protein content) for use as forage. It is also possible that the potential increase in uptake of poorly mobile ions may alleviate plant nutrient deficiencies or create toxicities from the trace elements added to soils by biosolids amendment.

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Table 3.1. Selected properties of soils at Wolcott, CO. Soil data were collected in July 1993 (Pierce et al., 1998) and in July 1997.

		1997		1993	
Constituent	**Units**	**0 Mg Biosolids ha^{-1}**	**40 Mg Biosolids ha^{-1}**	**0 Mg Biosolids ha^{-1}**	**40 Mg Biosolids ha^{-1}**
pH		7.3	7.1	6.9	7.1
EC	dS m^{-1}	0.71	0.73	0.50	0.70
Organic C	%	0.03	0.07	na	na
Total N	%	0.16	0.34	na	na
		AB-DTPA Extraction[+]		**4 M HNO_3 Extraction**[∓]	
P	g kg^{-1}	0.01	0.06	0.32	0.64
K	g kg^{-1}	0.25	0.48	1.33	1.81
Al	g kg^{-1}	0.01	0.01	4.57	6.27
Cd	mg kg^{-1}	0.47	1.48	2.35	2.90
Cr	mg kg^{-1}	0.08	0.09	6.20	8.00
Cu	mg kg^{-1}	4.62	15.1	13.0	21.3
Fe	g kg^{-1}	0.01	0.02	6.62	8.08
Mo	mg kg^{-1}	0.01	0.04	5.05	4.60
Ni	mg kg^{-1}	1.94	1.43	16.6	19.7
Pb	mg kg^{-1}	0.88	1.88	5.55	7.26
Zn	mg kg^{-1}	5.21	33.4	88.3	165

na = parameter not measured

[+] Barbarick and Workman, 1987

[∓] Bradford et al., 1975

Table 3.2. Selected properties of soils at Meadow Springs Ranch, CO. Soil data were collected in August 1997. Data collected for total metal analyses in July 1993 (Harris-Pierce, 1994).

Constituent	Units	0 Mg Biosolids ha^{-1}	30 Mg Biosolids ha^{-1}
pH		5.9	5.8
EC	dS m^{-1}	0.32	0.60
Organic C	%	1.54	3.46
Total N	%	0.09	0.32
		1993	
		4 M HNO_3 Extraction[∓]	
P	g kg^{-1}	0.23	1.16
K	g kg^{-1}	1.49	1.47
Al	g kg^{-1}	na	na
Cd	mg kg^{-1}	0.5	0.5
Cr	mg kg^{-1}	4.9	6.2
Cu	mg kg^{-1}	6.6	48.2
Fe	g kg^{-1}	4.45	4.55
Mo	mg kg^{-1}	0.01	1.00
Ni	mg kg^{-1}	4.7	5.6
Pb	mg kg^{-1}	6.1	10.3
Zn	mg kg^{-1}	19.5	64.4

na = parameter not measured

∓ Bradford et al., 1975

Table 3.3. Selected properties of biosolids used for surface treatment in 1991 at Wolcott (shrubland) and Meadow Springs Ranch (grassland). Trace element extraction by H_2SO_4-$HClO_4$ digest.

Constituent	Units	Shrubland	Grassland
pH		7.7	7.3
EC	dS m^{-1}	13.0	5.00
Organic N	g kg^{-1}	0.12	0.46
NO_3-N	mg kg^{-1}	0.16	3963
NH_4-N	mg kg^{-1}	168	102
P	g kg^{-1}	32.0	16.0
K	g kg^{-1}	3.6	1.95
Al	g kg^{-1}	0.16	8.7
Cd	mg kg^{-1}	4.8	5.06
Cr	mg kg^{-1}	22.1	39.7
Cu	mg kg^{-1}	567	553
Fe	g kg^{-1}	0.12	4.81
Mo	mg kg^{-1}	2.9	16.1
Ni	mg kg^{-1}	14.7	19.2
Pb	mg kg^{-1}	47.1	117
Zn	mg kg^{-1}	1210	776

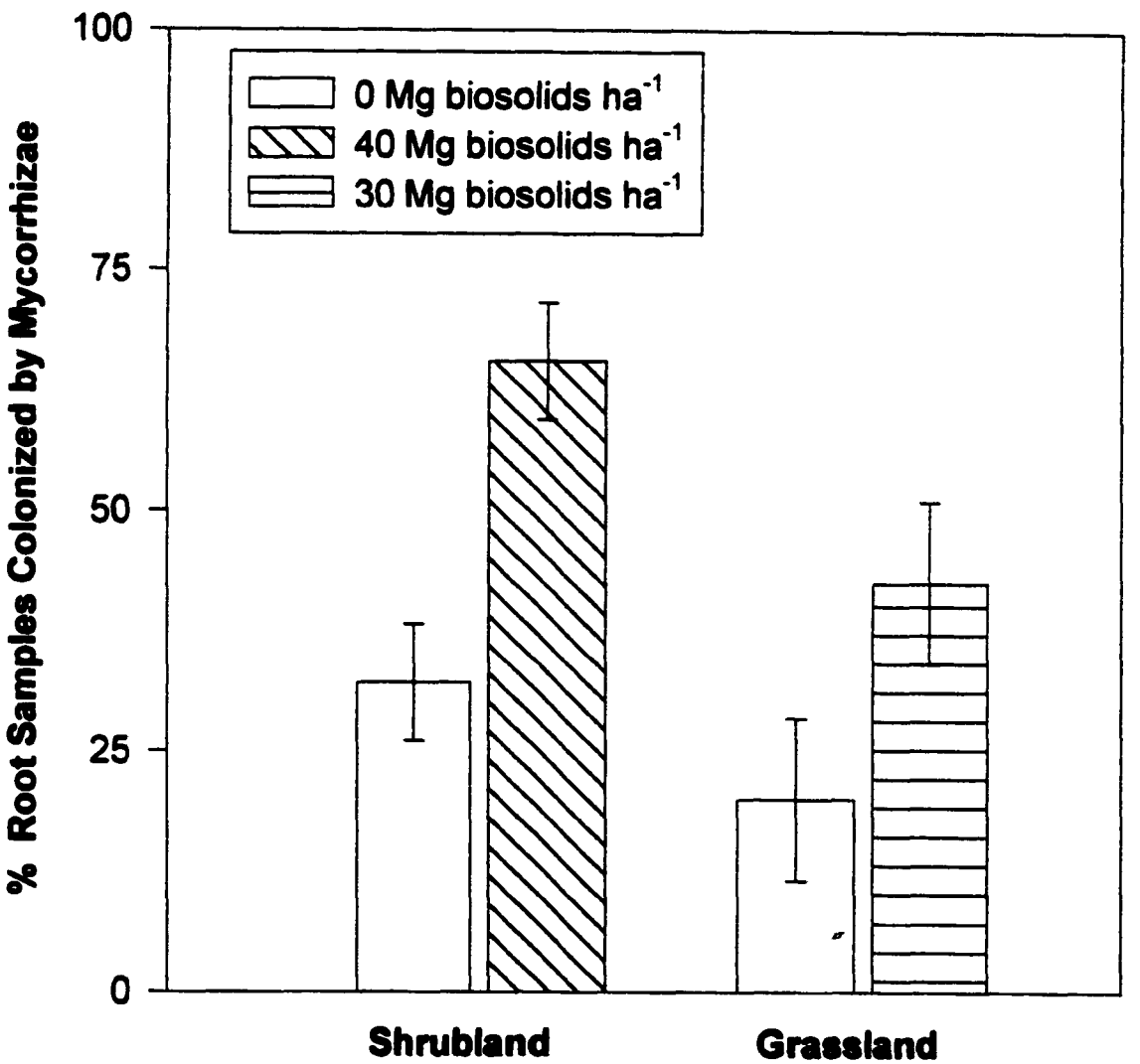

Figure 3.1. Effect of biosolids on percentage of plant root samples colonized by mycorrhizae in western wheatgrass (shrubland site) and blue grama (grassland site) in July 1997. Error bars represent 95% CI.

APPENDIX A

Table A.1. Mean microbial basal respiration rates for Wolcott, CO for July 1996 and 1997.

		0 Mg biosolids ha^{-1}	40 Mg biosolids ha^{-1}
Year	**Block**	**$\mu g\ CO_2\ g^{-1}$ dry soil h^{-1}**	**$\mu g\ CO_2\ g^{-1}$ dry soil h^{-1}**
1996	2	2.1	8.9
1996	3	0.57	9.1
1996	4	2.1	8.5
1997	2	1.2	1.5
1997	3	0.8	1.1
1997	4	1.6	1.3

Table A.2. Mean microbial basal respiration rates for Meadow Springs Ranch, CO for August 1997.

	0 Mg biosolids ha^{-1}	30 Mg biosolids ha^{-1}
Block	**$\mu g\ CO_2\ g^{-1}$ dry soil h^{-1}**	**$\mu g\ CO_2\ g^{-1}$ dry soil h^{-1}**
1	1.1	2.3
2	0.9	1.4
3	0.8	1.3
4	0.6	0.7

Table A.3. Mean active microbial biomass-C responses to biosolids at Wolcott, CO in July 1996 and 1997.

		0 Mg biosolids ha^{-1}	40 Mg biosolids ha^{-1}
Year	**Block**	**mg biomass-C kg^{-1} dry soil**	**mg biomass-C kg^{-1} dry soil**
1996	2	404.5	483.6
1996	3	378.8	495.4
1996	4	407.2	505.1
1997	2	433.1	470.0
1997	3	426.8	516.0
1997	4	441.4	488.0

Table A.4. Mean active microbial biomass-C responses to biosolids at Meadow Springs Ranch, CO in August 1997.

	0 Mg biosolids ha^{-1}	30 Mg biosolids ha^{-1}
Block	**mg biomass-C kg^{-1} dry soil**	**mg biomass-C kg^{-1} dry soil**
1	421.7	476.2
2	398.3	444.4
3	389.3	438.0
4	399.3	421.6

Table A.5. Mean potential CO_2 evolution of soil at Wolcott, CO during a 28-d incubation of soil collected in May 1997.

	0 Mg biosolids ha^{-1}	40 Mg biosolids ha^{-1}
Day	**mg CO_2-C 100 g^{-1} soil**	**mg CO_2-C 100 g^{-1} soil**
2	33.7	52.4
4	38.5	88.1
6	55.2	146.2
10	82.4	195.5
14	120.4	253.7
28	182.1	416.9

Table A.6. Mean potential CO_2 evolution of soil at Meadow Springs Ranch, CO during a 28-d incubation of soil collected in May 1997.

	0 Mg biosolids ha^{-1}	30 Mg biosolids ha^{-1}
Day	**mg CO_2-C 100 g^{-1} soil**	**mg CO_2-C 100 g^{-1} soil**
2	33.5	39.4
4	40.1	56.4
6	59.1	81.7
10	80.8	111.3
14	116.1	165.9
28	147.6	253.0

Table A.7. Mean potential net nitrogen mineralization of soil at Wolcott, CO during a 28-d incubation of soil collected in May 1997.

	0 Mg biosolids ha^{-1}	40 Mg biosolids ha^{-1}
Day	**mg inorganic N kg^{-1} soil**	**mg inorganic N kg^{-1} soil**
2	2.67	20.0
4	2.92	18.7
6	4.17	18.3
10	5.25	15.4
14	7.45	21.0
28	26.7	143.8

Table A.8. Mean potential net nitrogen mineralization of soil at Meadow Springs Ranch, CO during a 28-d incubation of soil collected in May 1997.

	0 Mg biosolids ha^{-1}	30 Mg biosolids ha^{-1}
Day	**mg inorganic N kg^{-1} soil**	**mg inorganic N kg^{-1} soil**
2	1.00	4.31
4	2.61	6.08
6	1.31	4.42
10	2.73	5.77
14	4.88	17.03
28	13.35	48.56

Table A.9. Mean percentage of plant root samples colonized by arbuscular mycorrhizae (AM) fungi in shrubland forage (western wheatgrass) and grassland forage (blue grama) in July 1997.

	Shrubland		Grassland	
Block	**% Root Samples with AM fungi**		**% Root Samples with AM fungi**	
	0 Mg biosolids ha^{-1}	**40 Mg biosolids ha^{-1}**	**0 Mg biosolids ha^{-1}**	**30 Mg biosolids ha^{-1}**
1	na	na	23.0	50.0
2	36.7	73.3	26.7	40.0
3	25.7	60.0	10.0	30.0
4	33.0	63.3	20.0	50.0