THE IMPACTS OF MUNICIPAL BIOSOLIDS ON SOME INDICATORS OF SOIL HEALTH

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ABSTRACT

The abundance and diversity of soil organisms are considered to be prime indicators of soil health, which has been defined as the *capacity* of soil to sustain productivity. Municipal biosolids are good sources of N, P and micronutrients for short-term crop production. The long-term impacts of biosolids on indicators of soil health are, however, less well understood. As inputs of organic C, biosolids have potential to increase soil biodiversity and improve soil health. However, because they contain greater concentrations of some metals than receiving soil, there is also potential for biosolids to have regressive effects on soil biodiversity and soil health. We have been studying the impacts of biosolids on arbuscular-mycorrhizal fungi (AMF) and diversity of soil microfauna, as well as nutrient availability, at three sites in the southern interior of BC: (1) irrigated alfalfa, (2) crested wheatgrass, and (3) native range vegetation. We have found that high biosolids application rates (e.g. 60 Mg/ha) can cause slight reductions in diversity of microfauna and colonization of alfalfa roots by AMF, relative to untreated soil. The reduced colonization of alfalfa by AMF appears to be the result of enhanced availability of P. Since uptake of P, Cu and Zn is the primary benefit of AMF to crop plants, the implications of reduced AMF colonization (in response to enhanced P availability) are not yet clear. It is also still unclear if the reduced diversity of micro-fauna is the result of nutrient loading or metals. We hypothesize that the use of biosolids in reclamation projects, where the reference point is degraded soil, is more likely to enhance soil health.

INTRODUCTION

The abundance and diversity of soil organisms are considered to be prime indicators of soil health, which has been defined as the *capacity* of soil to sustain productivity (Doran and Safley 1997). Municipal biosolids are good sources of N, P and micronutrients for short-term crop production. The long-term impacts of biosolids on indicators of the health of agricultural soil are less well understood. As inputs of organic C, biosolids have potential to increase soil biodiversity and improve soil health. However, because they contain greater concentrations of some metals than receiving soil, there is also potential for biosolids to have regressive effects on soil biodiversity and soil health. Municipal biosolids are used extensively for mine and landfill reclamation and better knowledge of how biosolids influence the health of agricultural soils should improve understanding of the long-term benefits and constraints of using biosolids in mine and landfill reclamation.

In order to better understand the long-term effects of biosolids on health of agricultural soils, we have been studying the impacts of biosolids on the dynamics of soil organic matter, arbuscular-mycorrhizal

fungi (AMF) and the diversity of soil microfauna, as well as nutrient availability, at three sites in the southern interior of BC: (1) irrigated alfalfa, (2) crested wheatgrass, and (3) native range vegetation.

MATERIALS AND METHODS

Three separate experiments were established at sites of crested wheatgrass (CW), native range vegetation (NR) and irrigated alfalfa (IA) all in the Ashcroft area. At all three sites four replicate strip plots (16 m x 100 m) of six different treatments were arranged in a randomized complete block experimental design. The treatments were biosolids at 0 (control), 20, 40 and 60 Mg/ha, and fertilizer (ammonium phosphate + urea) at 300 kg N/ha + 400 kg P/ha (note: 1 Mg = 1 metric tonne). The fertilizer treatment was calculated to match the inputs of available N and total P in the 20 Mg/ha biosolids treatment. The teatments were surface-applied to CW and NR plots in fall of 2001. Biosolids were applied in spring 2002 to the IA experiment and disked into the soil before replanting alfalfa.

Forage Production:

Forage was harvested from the NR and CW sites in mid-June of each year after application. Six 0.5 m^2 (70.71 cm by 70.71 cm) quadrats of forage were harvested from each treatment plot. The six sub-samples from each plot were combined in a paper bag, dried at 60^{o}C until dry, and weighed to determine dry matter production. Alfalfa was harvested from the IA site twice in each growing season, usually in mid-June and August. Alfalfa yield was determined using the same methods as for NR and CW.

Sampling:

Soil samples were taken from the 0-15 cm depth interval of NR and CW plots twice each year, in April/May and October. Twenty 2.5 cm diameter cores were taken to a depth of 15 cm from each plot. The cores were taken along a 100 m transect running lengthwise down the middle of each plot, i.e. approximately 1 core/5 meters. The twenty cores from each plot were combined to make a single composite sample representing the plot. In spring of each year, additional composite samples were taken from the 15-30 cm depth (NR and CW sites) and 30-45 cm depth (CW only).

IA plots were sampled to a depth of 0-30 cm in April/May, mid-June (after first harvest), and in October of each year. The sampling procedure was similar to that described for NR and CW plots. In spring of each year, additional composite samples were taken from the 30-60 cm depth.

Each sample was passed through a coarse sieve (5 mm) to remove stones and large root fragments and the resin-P and biological analyses were initiated immediately thereafter.

Chemical Analyses of Soil:

Phosphorus adsorbed onto anion exchange resin strips (resin-P) was determined on fresh soil samples using an adaptation of the procedure of Schoenau and Wang (1991). Field moist soil was also extracted with 0.01 M $CaCl_2$ (1:10 soil:solution). The $CaCl_2$ extracts were sent to a commercial analytical

laboratory (Norwest Labs) for analyses of "$CaCl_2$-extractable" trace elements via ICP spectophotometry. The remaining soil was dried at 60°C. Percent organic C, total N and total S were determined on the dried soil samples collected each fall using a LECO combustion analyzer. Dried subsamples collected in fall of 2004 were sent to a commercial analytical laboratory (Norwest Labs) for determination of "total" trace elements via nitric acid extraction and ICP spectrophotometry.

Nematode Analyses:

Soil nematodes were extracted from 50 g fresh (field-moist) aliquots of each sample using a Baermann pan method. After extraction the nematodes were preserved in 4% formalin. For routine analyses, each sample was reduced to 4 ml, placed in a counting chamber on an inverted microscope, and the total number of nematodes in one-tenth of the sample was determined at 40X. Transects were then made through each sample at 200X and 400X and the first 100 nematodes observed in each sample were classified to the genus level of resolution as described in the 2002 report. Nematode counts for each taxon were adjusted to the number of nematodes per 100 g dry soil.

The nematode taxa were assigned 'c-p' values from 1 to 5, corresponding to their positions along the colonizer-persister continuum of nematode life-history strategies (Bongers 1999; 1990), and then assigned functional group weightings as described by Ferris et al. (2001) for calculation of the Enrichment Index (EI) and Structure Index (SI), in addition to other community indices such as Simpson's index of diversity/evenness and the Shannon-Weiner index of diversity.

Mycorrhizal Analyses:

Roots of alfalfa were dug from the IA plots in April/May and June of 2003 and 2004. Roots of crested wheatgrass were dug in April/May of each year. Viable fine roots were trimmed from each of the six to ten root systems from each plot, combined into one composite sample and washed vigorously over a 2 mm sieve. The pieces of root were chopped into approximately 2 cm sections and cleared and stained. Ten root fragments from each sample were randomly picked and mounted on microscope slides. Six fields of view (0.4 mm) at 100X were observed at random locations along each of the ten root fragments (total of 60 fields-of-view), and the number of fungal structures (primarily vesicles) counted. The data were expressed as the number of fungal structures per mm root and the proportion of (0.4 mm) fields-of-view that were positive for colonization.

RESULTS

Crop Response:

CW yield was nearly doubled by fertilizer and biosolids at 20 Mg/ha in 2003; these two treatments were projected to have the same inputs of mineral N and total P, and did not differ significantly from each other (Fig. 1). In the 60 Mg/ha treatment, CW yield was nearly 3X the control in 2003 (Fig. 1). In the IA experiment, cumulative yield was increased by fertilizer and all biosolids treatments except the 10 Mg/ha treatment (data not shown). Yield in the 60 Mg/ha treatment was nearly 1.5X the control. The yield of

forage from the native rangeland site is overall low, and ranged from 300 kg/ha in the control to 533 kg/ha in the high biosolids treatment (data not shown). Vegetation in the NR experiment is very patchy, which has translated to extremely variable yield data, and no statistically significant effect of biosolids on yield of native range grasses was observed for 2003 or 2004 (data not shown).

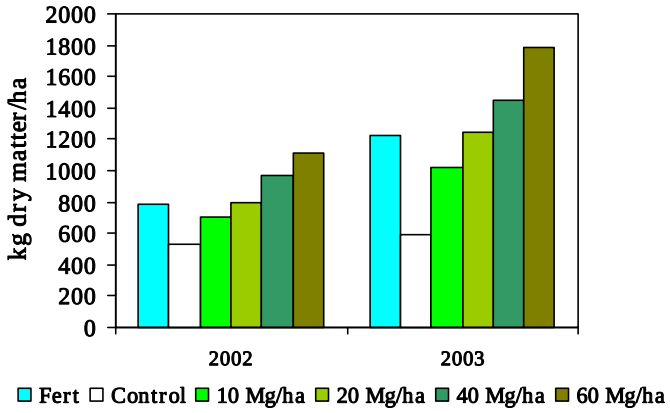

Figure 1. Yield of crested wheatgrass in response to fertilizer and increasing amounts of biosolids.

Soil Carbon and Nitrogen:

Soil organic carbon (C) and total nitrogen (N) in surface soil increased with biosolids application rates (Fig. 3). The effects of biosolids treatments on soil C and N were statistically significant in the CW experiment but not in the IA experiment (Fig. 3). The combination of higher baseline (control) soil organic C concentrations at the IA site, and incorporation of biosolids into a 30 cm profile, probably diluted the added C and N more than at the CW site where the biosolids were surface-applied and samples were only taken to 15 cm.

Concentrations of C and N in biosolids-amended soil have not decreased with time since application, indicating that the organic matter in biosolids is either relatively resistant to decomposition, or enhanced plant productivity and root/rhizosphere inputs of C and N have offset the biosolids-C and N lost through decomposition. Previous studies indicate that about 30% of biosolids C and N are usually lost in the first year of decomposition (Gilmour et al. 2003), indicating that enhanced plant productivity probably contributed to the apparent stability of soil organic matter levels in biosolids-amended soil.

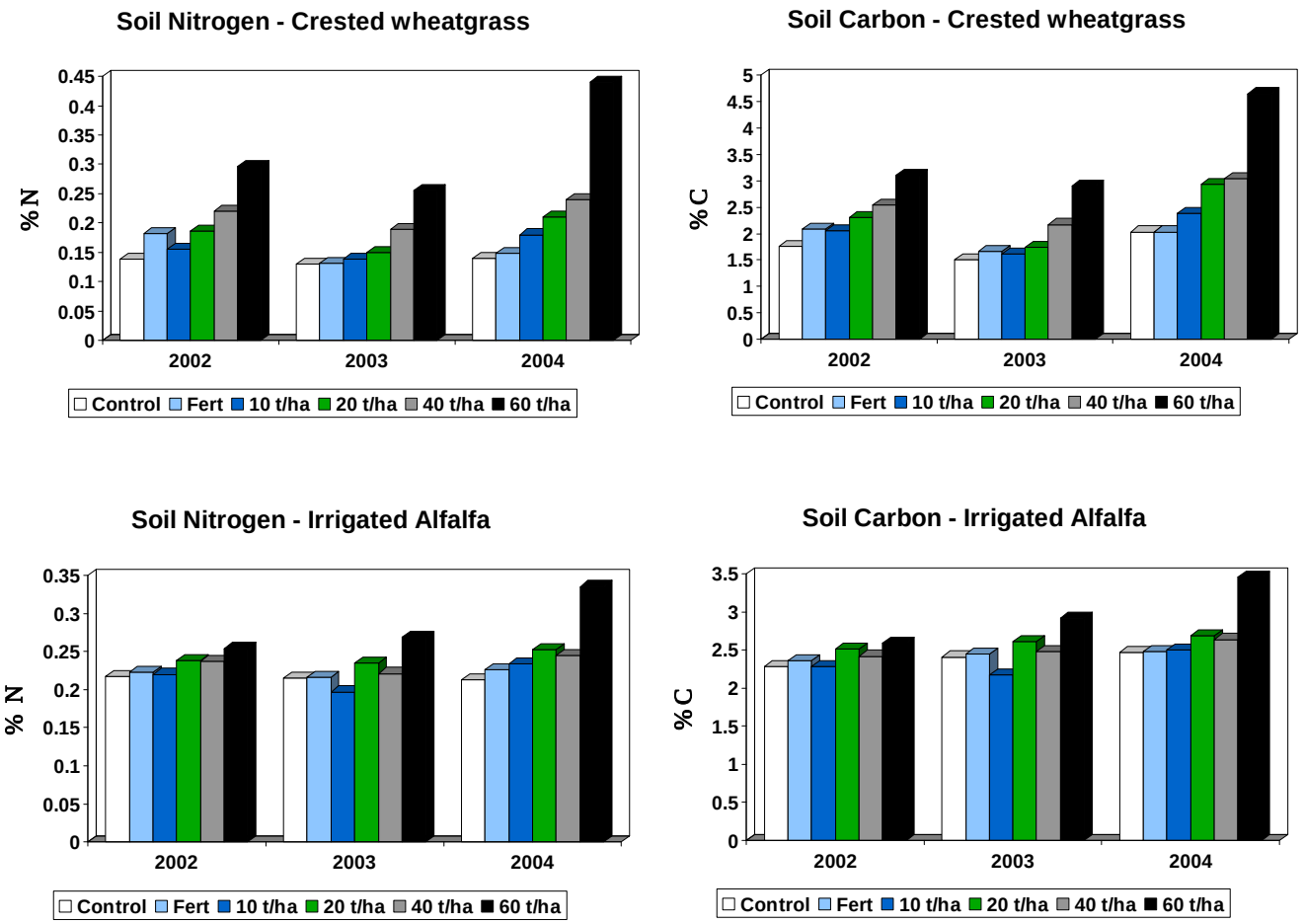

Figure 3. Effects of fertilizer and biosolids application rates on total soil N and soil organic C in 2002, 2003 and 2004, in the crested wheatgrass (top) and irrigated alfalfa (bottom) experiments.

Soil Phosphorus:

Most P added to soil is fixed via processes of adsorption onto amorphous Fe and Al and precipitation with Ca, and generally only a small portion of added P remains readily available for crop uptake. Adsorption of P onto anion exchange resins (resin-P) is a common method of measuring the pool of readily available P, and resin-P is correlated with plant uptake of P (Schoenau and Wang 1991).

Total soil P (HNO_3-extractable) in the surface soil was doubled by the 60 Mg/ha biosolids application in the IA and CW experiments (data not shown). Concentrations of total P in lower soil layers were not affected by biosolids treatments, indicating that little if any P had moved down the soil profile in high biosolids treatments. The fertilizer treatment and the 20 Mg/ha biosolids treatment were intended to result in the same addition of total P (400 kg/ha), and effects of these treatments on total soil P were indeed similar.

For the IA and CW experiments, the fertilizer treatment resulted in the most resin-P (Fig. 4). The main-factor effect of treatment was significant for both experiments. Relative to the controls (baseline), available P in the fertilizer treatment tended to decline through time, while available P in biosolids treatments tended to increase through time (Fig. 4). However, even at the end of 2004, resin-P in the fertilizer treatment was significantly greater than in the 20 Mg/ha biosolids treatment, which had originally received the same total amount of P (Fig. 4). Data from the NR experiment have been extraordinarily variable, and no statistically significant differences were obtained in 2003 and 2004 (data not shown).

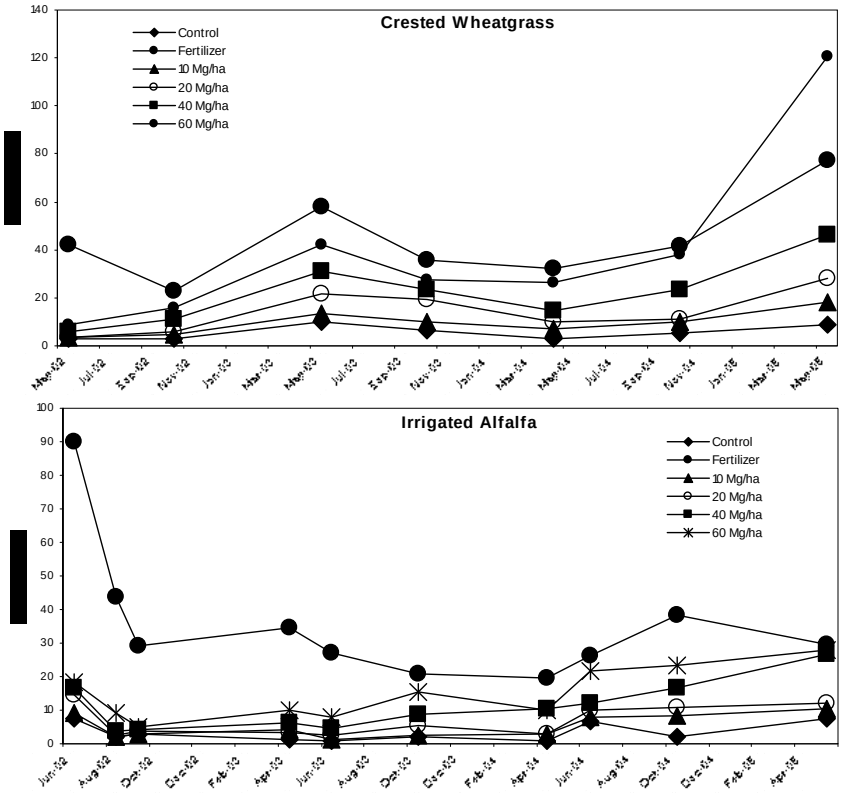

Figure 4. Effects of fertilizer and biosolids application rates on resin-extractable phosphorus in soil from plots of crested wheatgrass (top) and irrigated alfalfa (bottom), from spring 2002 through spring 2005.

Trace Elements:

Trace element concentrations in soil were below OMRR approval limits for soil in all cases except for Cu at the 60 Mg/ha application to IA (Fig. 5). The Cu concentration in that treatment, 103 mg/kg soil, was just over the OMRR limit of 100 mg/kg soil. It appears that the potential for Cu loading in the field used for the IA experiment could limit biosolids inputs to only two to three applications at the standard "agronomic rate" of 20 Mg/ha.

Concentrations of Cu extracted with 0.01M $CaCl_2$ increased with biosolids application rate at both sites (Fig. 5). In contrast, concentrations of $CaCl_2$-extractable Zn did not increase with biosolids application rate in the IA experiment.

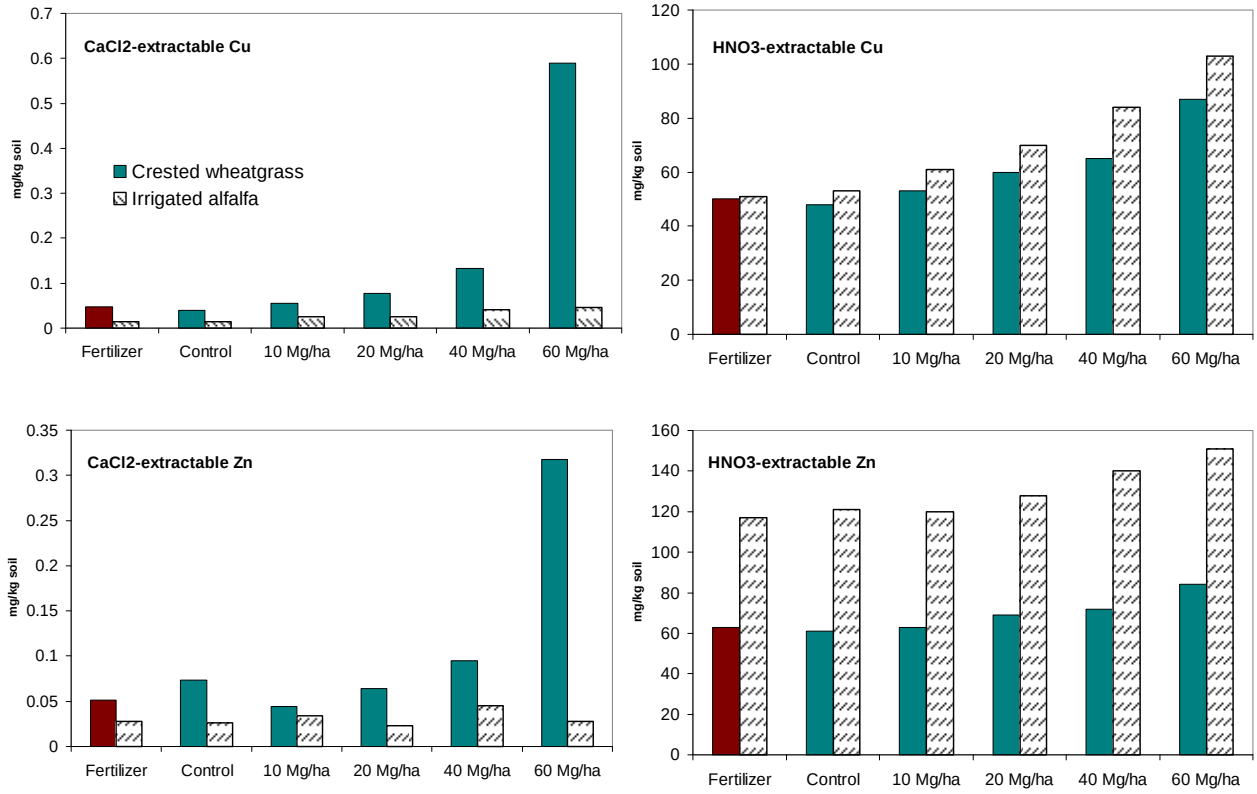

Figure 5. Effects of fertilizer and biosolids application rates on $CaCl_2$-extractable and nitric acid-extractable Cu and Zn from the crested wheatgrass experiment (solid bars) and irrigated alfalfa experiment (hatched bars).

Mycorrhizal Colonization

Mycorrhizal fungi are important symbionts of most crop plants, improving uptake of water, P and micronutrients (e.g. Cu and Zn) when they are scarce. The hyphae of mycorrhizal fungi also appear to make important contributions to soil aggregation and aggregate stability via production of the glycoprotein, glomalin.

Mycorrhizal colonization of the roots of alfalfa (IA experiment) was negatively affected by the fertilizer treatment as well as the 40 and 60 Mg/ha biosolids treatments (Fig. 6). Mycorrhizal colonization is known to be suppressed when there are high concentrations of available P as well as trace elements such as Cu and Zn (e.g. Del Val et al. 1999). Suppression of mycorrhizae by excess nutrients obviously will not have an immediate impact on ability of the crop to take up nutrients that are in excess. However, it could reduce plant drought tolerance. Also, long-term suppression of colonization will probably impact on the diversity and abundance of the inoculum reservoir in soil, reducing capacity of the soil to provide

adequate mycorrhizal symbionts after the elevated nutrient availability has been drawn down. The negative effect of fertilizer on mycorrhizal colonization was most likely due to high P availability.

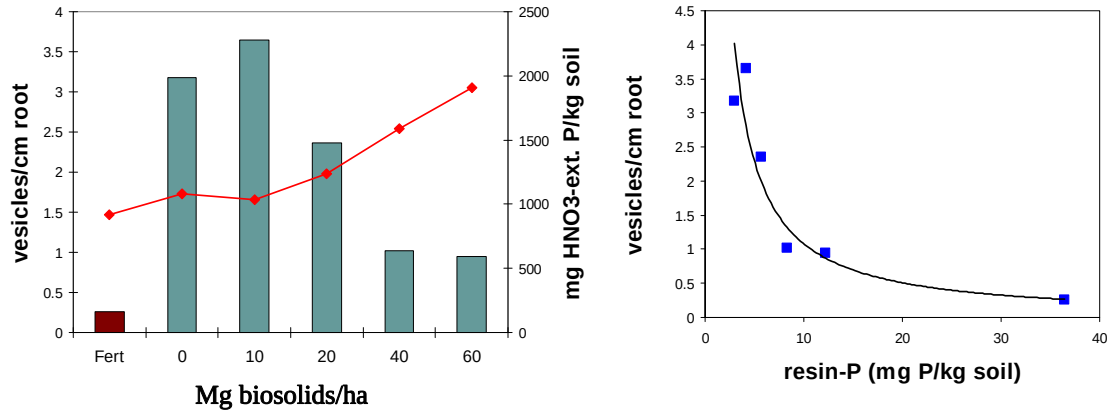

Figure 6. Effects of fertilizer and increasing biosolids application rates on arbuscular-mycorrhizal colonization of alfalfa roots (bars) and total P (left graph), and the relationship between resin-P and AMF colonization (right graph). Data shown are averages over the 2003 and 2004 samples.

Soil Nematodes:

Soil biodiversity is critical for soil ecosystem functioning (Nannipieri et al. 2003), and some measurements of soil biodiversity as well as certain soil processes have been negatively impacted by excess trace element loading from biosolids (Georgieva et al. 2002; Giller et al. 1998). However, most of the aforementioned studies utilized sites with acidic soils or that had received particularly high metal loadings. The objective of our research is to use changes in nematode community structure as an indicator of changes in overall soil biodiversity, structure of the decomposition soil food web and potential changes in soil functioning. The benefits of using nematode communities as indicators has been reviewed extensively (e.g. Bongers and Ferris 1999; Ferris et al. 2001).

A particular group of free-living nematodes, the enrichment opportunists, are known to multiply rapidly whenever soil is enriched with labile organic substrates supporting high rates of microbial growth (Bongers and Ferris 1999; Ferris et al. 2001). Thus, the enrichment opportunists are indicative of enhanced turnover of the microbial biomass and nutrients, and the abundance of this group of nematodes has been correlated with N mineralization in previous studies (Ferris and Matute 2003; Forge and Simard 2001; Hassink et al. 1993). This group of nematodes also appears to be less sensitive to heavy metal toxicity than other groups of nematodes (e.g. Georgieva et al. 2002; Korthals et al. 2000), so they would be expected to increase with nutrient enrichment even in the presence of metal concentrations that may affect other groups of soil organisms.

The nematode Enrichment Index (EI) is a dimensionless measure of the extent to which the nematode community is represented by enrichment opportunists. The EI was increased by biosolids, relative to the control and fertilizer treatments, particularly in the 40 and 60 Mg/ha treatments (Fig. 7). The main-factor effect of treatment was significant but the treatment x sample date term was not significant in the overall

ANOVA, and data presented in Fig. 7 are main-factor means (averaged over all sample dates from 2002 through 2004).

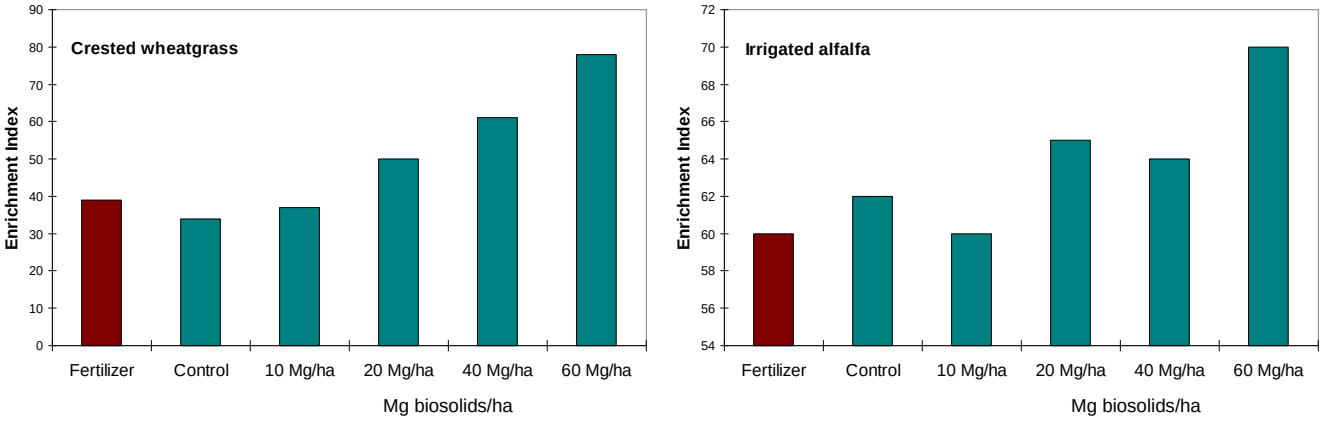

Figure 7. Effects of fertilizer and biosolids application rates on the nematode community Enrichment Index (EI) in the crested wheatgrass (left) and irrigated alfalfa (right) experiments.

The nematode Structure Index (SI) is a measure of functional diversity of the nematode community and structure of the soil food web it represents. Taxa assigned high weightings in computation of the SI are also known to be sensitive to chemical stressors, particularly heavy metals (Korthals et al. 2000). The main-factor effect of treatment was significant but the treatment x sample date interaction was not significant in the CW experiment (Fig. 8). The effect of treatment was not significant in the IA experiment (Fig. 8). In the CW experiment, the SI decreased with increasing biosolids application rate (Figure 8). Similar results were obtained with the Shannon-Weiner and Simpson's indices of diversity (genus level) (data not shown).

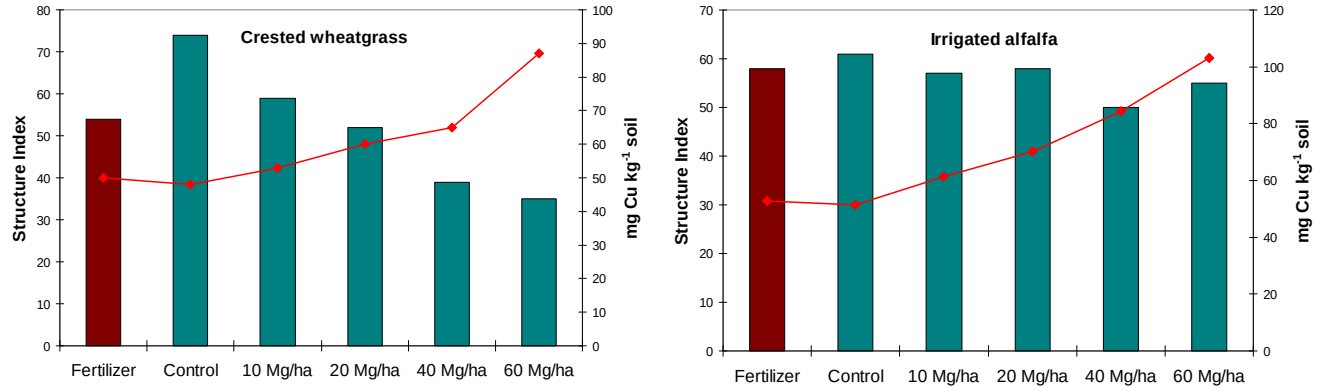

Figure 8. Effects of fertilizer and biosolids application rates on the nematode community Structure Index (SI) in the crested wheatgrass (left) and irrigated alfalfa (right) experiments.

The pattern of nematode community responses to biosolids that we have observed is identical to responses to elevated soil Zn and/or Cu concentrations observed in previous studies with experimentally metal-contaminated soil (e.g. Bakonyi et al. 2003; Korthals et al. 1998; Smit et al. 2002) or soil treated

with biosolids spiked with variable levels of metals (Georgieva et al. 2002; Weiss and Larink, 1991). However, it is unclear if the reduced diversity of micro-fauna is the result of nutrient loading or metals. Recent studies have shown that members of the Dorylaimida are also more sensitive than other nematode taxa to nitrate, nitrite and ammonium in solution (Tenuta and Ferris 2004), raising the possibility that nutrient inputs alone could generate similar responses in the nematode community. Finally, it is unclear whether the statistically significant reductions in diversity and food web structure that we have observed reflect significant reductions in function of the soil food web (e.g. decomposition, mineralization), or whether the effects are reversible. Ongoing studies in British Columbia and Washington are attempting to determine the reversibility of changes in nematode diversity after the cessation of biosolids applications. We hypothesize that the use of biosolids in reclamation projects, where the reference point is degraded soil, is more likely to enhance indicators of soil health.

LITERATURE CITED

Bakonyi, G., Nagy, P. and Kadar, I. 2003. Long-term effects of heavy metals and microelements on nematode assemblage. Toxicology Letters 140-140: 390-401.

Bongers, T., and Ferris, H. 1999. Nematode community structure as a bioindicator in environmental monitoring. Trends in Ecology and Evolution 14: 224-228

Del Val, C., Barea, J.M., and Azcon-Aguilar, C. 1999. Diversity of arbuscular mycorrhizal fungus populations in heavy metal contaminated soil. Applied and Environmental Microbiology 65: 718-723.

Ferris, H., Bongers, T., and de Goede, R.G.M. 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. Applied Soil Ecology 18: 13-29.

Ferris, H., and Matute, M.M. 2003. Structural and functional succession in the nematode fauna of a soil food web. Applied Soil Ecology 23: 93-110.

Forge, T.A., and Simard, S.W. 2001. Structure of nematode communities in forest soils of southern British Columbia: relationships to nitrogen mineralization and effects of clearcut harvesting and fertilization. Biology and Fertility of Soils 34: 170-178.

Forge, T.A., Bittman, S., and Kowalenko, C.G. 2005. Responses of grassland soil nematodes and protozoa to multi-year and single-year applications of dairy manure slurry and fertilizer. Soil Biology and Biochemistry 37: 1751-1762.

Georgieva, S.S., McGrath, S.P., Hooper, D.J., Chambers, B.S. 2002. Nematode communities under stress: the long-term effects of heavy metals in soil treated with sewage sludge. Applied Soil Ecology 20: 27-42.

Giller, K.E., Witter, E., and McGrath, S.P. 1998. Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. Soil Biology and Biochemistry 30: 1389-1414.

Gilmour, J.T., Cogger, C.G., Jacobs, L.W., Evanylo, G.K. and Sullivan, D.M. 2003. Decomposition and plant available N in biosolids: Laboratory studies, field studies, and computer simulation. Journal of Environmental Quality 32: 1498-1507.

Hassink, J., Bouwman, L.A., Zwart, K.B., and Brussaard, L. 1993. Relationships between habitable pore space, soil biota and mineralization rates in grassland soils. Soil Biology and Biochemistry 25: 47-55.

Korthals, G.W., Bongers, M., Fokkema, A., Dueck, T.A., and Lexmond, T.M. 2000. Joint toxicity of copper and zinc to a terrestrial nematode community in an acid sandy soil. Ecotoxicology 9: 219-228.

Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., and Renella, G. 2003. Microbial diversity and soil functions. European Journal of Soil Science 54: 655-670.

Schoenau, J.J., and Huang, W.Z. 1991. Anion-exchange membrane, water, and sodium bicarbonate extractkons as soil tests for phosphorus. Commun. In Soil Sci. Plant Anal. 22: 465-492.

Smit, C.E., Schouten, A.J., Van den Brink, P.J., van Esbroek, M.L.P. and Posthuma, L. 2002. Effects of zinc contamination on a natural nematode community in outdoor soil mesocosms. Arch. Environ. Contam. Toxicol. 42: 205-216.

Tenuta, M. and Ferris, H. 2004. Sensitivity of nematode life-history groups to ions and osmotic tensions of nitrogenous solutions. Journal of Nematology 36: 85-94.

Weiss, B. and Larink, O. 1991. Influence of sewage sludge and heavy metals on nematodes in an arable soil. Biology and Fertility of Soils 12: 5-9.