2000 Billings Land Reclamation Symposium

MICROBIAL INOCULATION POTENTIAL OF ORGANIC MATTER AMENDMENTS FOR MINE TAILINGS RECLAMATION

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ABSTRACT

Microorganisms are important for successful reclamation because of their role in nutrient cycling, plant establishment, geochemical transformations, and soil formation. Microbial communities associated with mine disturbance often show decreased density and diversity, relative to undisturbed sites. While the chemical and structural contributions of organic matter amendments to mine tailing reclamation efforts are often considered, little is known about the microbial communities associated with organic amendments. This research examines microbial communities associated with common organic matter amendments, as compared to baseline mine tailings substrates. The microbial communities were characterized with heterotrophic bacteria plate counts, actinomycete plate counts, fungal plate counts, and carbon substrate utilization profiles. Samples included 4 reference mine waste substrates and 4 organic matter types including sawdust, manure, soil, and landfill compost (4 replicates of each). The compost consistently showed the highest plate count numbers and carbon substrate metabolic diversity, followed by the soil sample. The manure had relatively high heterotrophic bacteria counts, but low metabolic diversity. The sawdust had lower plate count numbers than the other organic matter samples, but comparable metabolic diversity. Future research will examine changes in organic matter microbial communities after incubation with mine tailings. Understanding the microbial inoculation potential of organic matter amendments will lead to better reclamation strategies and more complete remediation of mine waste disturbed systems.

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INTRODUCTION

Soil microorganisms are crucial for proper soil functioning and development of plant communities (Bolton et al. 1993). A diverse microbial community can affect plant growth through improved nutrient uptake, transformation of soil properties, nitrogen fixation, pathogen protection, and production of phytohormones (Bolton et al. 1993, Azcon-Aguilar and Barea 1996, Arshad 1993). Plant community composition can be directly affected by the composition of soil microbial communities (van der Heijden et al. 1998, Bever 1988). And soil processes, such as soil aggregate formation, mobilization and immobilization of nutrients, and transformation of soil organic matter are controlled by soil microbes (Bolton et al. 1993, Edgerton et al. 1995, Francis and Dodge 1988, Lovely 1991, Levi-Minzi et al. 1990). For these reasons, plant ecologists and restoration ecologists have paid increasing attention to soil microbial communities.

Successful revegetation of harsh mine waste soils (often dry, acidic, and metal-contaminated) depends on amelioration of the soil environment (Noyd 1995). Common mine waste amelioration strategies include neutralization and the addition of organic matter. Organic matter sources used for reclamation include a wide range of materials such as topsoil, straw, various composts, sawdust, fungal mycelia byproduct, and manure, each of which offers a different suite of benefits to the disturbed soil. For example, Noyd et al. (1995, 1996, 1997) found that composted yard waste added to taconite iron ore tailings increased plant cover, mycorrhizal properties, and accrual of organic matter in soil. Organic additions have also been shown to increase plant cover (DeLuca and Lynch 1997, Meikle et al. 1999, Noyd et al. 1996), reduce soil bulk density (Schoenholtz et al. 1992, Tester 1990), lower penetration resistance (Tester 1990), increase water content (Schoenholtz et al. 1992, Tester 1990), increase total and mineralizable N (Schoenholtz et al. 1992), decrease exchangeable metal levels (DeLuca and Lynch 1997) and alter pH (DeLuca and Lynch 1997, Tester 1990) in a variety of soil types. Such soil alterations can lead to improved conditions for soil biota and plant root growth. Together, these studies show that benefits of organic matter addition depend on the combination of organic matter type and disturbed soil properties (DeLuca and Lynch 1997, Tester 1990).

While previous research has examined the physical and chemical benefits of various organic matter additions, little is known about the relative benefits of these amendments as microbial inoculum sources. Noyd et al. (1995) measured increased microbial biomass and increased numbers of several important soil microbial populations in taconite iron ore tailings in field plots amended with composted yard waste. Other studies have shown that organic matter additions can increase microbial biomass (DeLuca and Lynch 1997, Noyd et al. 1995) and enhance decomposition rates of organic matter (Levi-Minzi et al. 1990).

The objective of this research was to directly compare microbial communities associated with four common organic matter amendments and with several different mine tailings substrates. A diversity of microorganisms is required for soil functioning and improved plant growth. For example, actinomycetes are common soil bacteria that are important for decomposition of complex carbonaceous substrates such as chitin, celluloses and hemicelluloses (Killham 1994). A variety of heterotrophic bacteria are responsible for many other soil functions, such as non-symbiotic nitrogen fixation, plant mutualisms, and decomposition. Soil fungi have a number of important roles in the soil (such as plant symbionts and pathogens), the most important of which is the decomposition of organic matter. They metabolize both simple and complex carbohydrates, and fungal biomass in the soil is important for soil structure and stability (Killham 1994). A primary function of many soil microorganisms during soil restoration is to promote organic matter turnover and nutrient cycling through diverse metabolic functioning. Carbon-utilization profiles, generated with Biolog microtiter plates, can be used to estimate potential metabolic diversity on a wide array of carbon sources (Garland and Mills 1991, Lehman et al. 1995, Zak et al. 1994). These microplates consist of 96 wells, 95 that contain unique sole carbon sources and one control well with no carbon. Minimal salts for growth and tetrazolium redox indicator dye also are included in each well. The carbon-utilization patterns that develop provide a metabolic profile for the sample community, and serve as an indicator of the carbon-based metabolic diversity of the microbial community. Data from these different microbial assessments can be used to compare the microbial communities associated with organic matter sources and tailings types.

METHODS

Sample Collection

The experiment compared five organic matter treatments and five mine tailings substrates. The organic matter treatments were 1) fresh sawdust from a lumber mill; 2) cattle manure; 3) soil from an undisturbed range site near Missoula, MT; and 4) landfill compost. The substrates included 1) silica sand (control); 2) acidic mine tailings; 3) new tailings, freshly processed and de-watered at an active mine near Butte, MT; 4) "clean" tailings, in which pyrite was removed through an extra separation step; and 5) an old tailings substrate, taken from a 10 year old marginally vegetated test plot at the same active mine. Plant material was detected in the old tailings, but none of the other substrates. Substrates and organic matter additions were collected, sieved (2mm mesh), and homogenized for sampling. Four subsamples were taken from each organic matter and substrate type and placed in sterile sample bags for immediate processing. Sample soil not used for immediate processing was dried and stored for future analyses.

Soil Parameters

Soil moisture content was determined gravimetrically in both organic amendments and soil substrates. Twenty-five grams of each sample was weighed, dried at 60° C for three days, and re-weighed. Measurement of soil pH was performed by addition of 20 ml deionized water to 10 grams dry soil followed by a 5 minute equilibriation period prior to measurement with a pH probe.

Culturable Bacteria and Fungi

Soil samples were homogenized, diluted 1:10 in 0.1% sodium pyrophosphate (Sigma Chemicals Co.), sonicated for ten minutes (Bransonic Ultrasonic Cleaner 3210, Danbury, CT), and used for the various microbiological assays. Heterotrophic bacteria and actinomycetes (as detected by colony and microscopic morphology) were determined on R2A agar (Difco Laboratories). Total fungal counts were determined on Rose Bengal agar (Difco Laboratories) supplemented with 0.1g/L chloramphenicol (Sigma Chemicals Company, St. Louis, MO).

Carbon-utilization Pattern Analysis

Heterotrophic plate count data were used to adjust the samples to a standard cell density for Biolog plate inoculation. A second soil slurry was prepared as described above. This slurry was further diluted in phosphate-buffered saline to a standard density of $1x10^4$ CFU/ml. 150µl of each sample was inoculated into Biolog GN microplates and incubated at 23°C for 300 hours. The optical density of the wells was measured approximately every 24 hours with an ELISA microplate reader at 570nm (Molecular Devices, Menlo Park, CA).

Biolog data was analyzed by calculating average well color development (AWCD) across time, averaging the absorbance readings for each well in a single plate after subtracting the time zero reading (Garland and Mills 1991). The number of active wells on a given plate was determined by quantification of the number of positive wells (>0.100 absorbance units above the time zero reading) in each sample. Plates were analyzed at 144 h because this was the time at which patterns were established and stabilized, but visible fungal biomass had not appeared any of in the plates (Zak et al. 1994).

Statistical Analyses

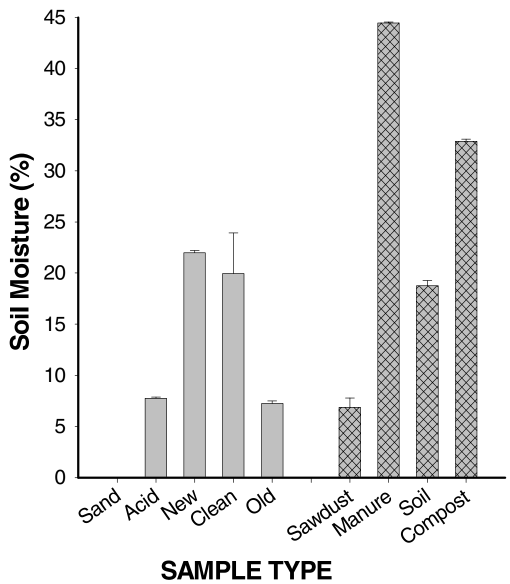
Treatment differences were detected with Kruskal-Wallis analysis of variance (anova) on ranks, followed by a Student-Newman-Keuls post-hoc test for multiple comparisons. Analysis of variance was used to measure differences between substrates separately from differences between organic matter sources. An adjusted alpha level of 0.025 was used to judge significance of the multiple tests.

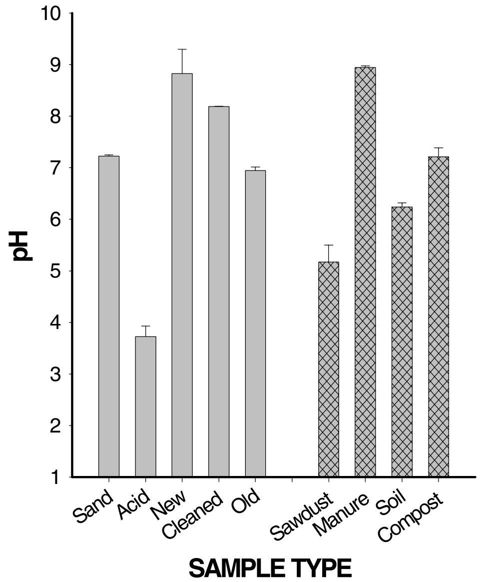
RESULTS

Soil measurements

Soil moisture contents were significantly different between substrates and organic matter types (Figure 1). Sand moisture was undetectable, acid and old tailings had low moisture (approx. 5%), and the new and clean tailings had the highest moisture content (approx. 20%). Moisture was significantly lowest in the sawdust (approx. 4%), higher in soil, higher still in compost, and highest in manure samples.

The pH of the acid tailings was significantly lower than all of the other substrates, while the old tailings was more acidic than the sand, clean tailings and new tailings (Figure 2) The pH was significantly higher in the manure than the sawdust, but the soil and compost were not different from any of the other treatments.





Heterotrophic Plate Counts

Heterotrophic plate counts are a measure of culturable bacteria, or the total number of colony forming units that are able to grow on agar medium in the laboratory. Since the growing requirements of many bacteria may include conditions unique to a soil environment that are not well simulated within the lab, heterotrophic plate counts are thought to represent only a small portion (less than ten percent) of the total bacteria in the sample. Nevertheless, plate counts provide a relative measure of culturable bacterial numbers between samples.

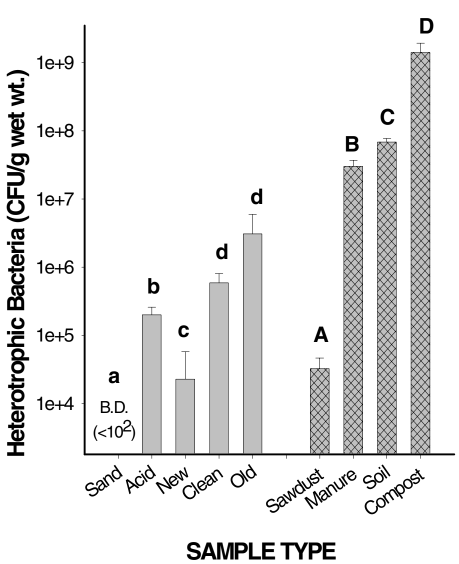


Figure 3. Heterotrophic bacteria plate counts for all samples. Lowercase letters designate statistically significant substrate treatment differences ($p<0.001$), and uppercase letters designate organic matter treatment differences ($p=0.003$). n=4. Error bars represent ± SE.

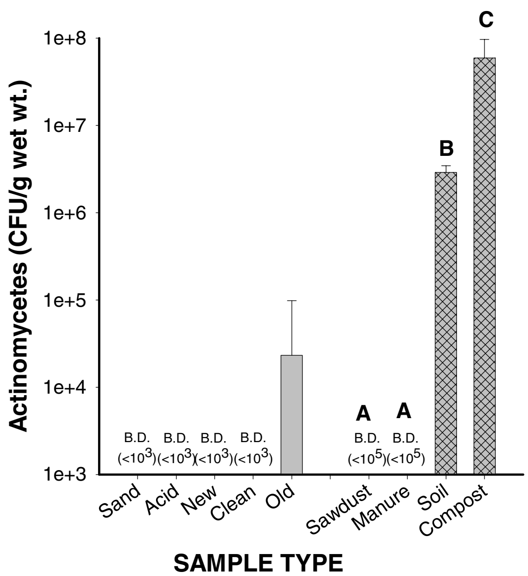
Culturable heterotrophic bacterial counts differed across substrates with the lowest numbers (below detection level of 10^2 CFU/g wet weight of substrate) in samples of sand (Figure 3). Both clean tailings and old tailings showed the highest number of CFU's, and were not significantly different from one another. New tailings and acid tailings were intermediate, with higher levels in the acid tailings than the new tailings. Plate counts of organic amendments were lowest in the sawdust (7.75×10^4 CFU/g substrate), followed by manure, soil, and compost, which were between 10^4 and 10^5 times higher than sawdust (Figure 3).

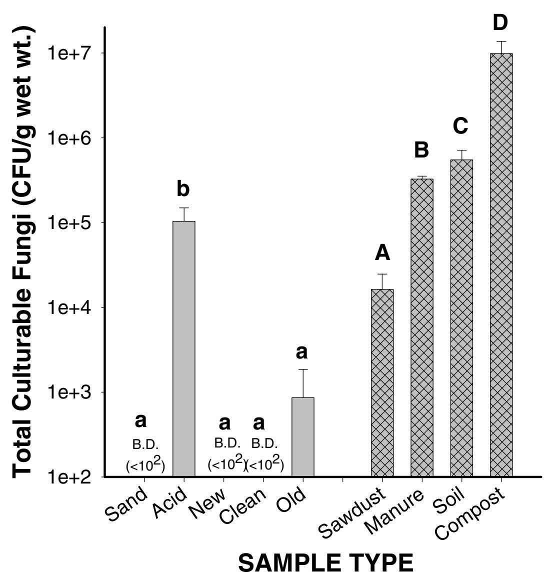
Actinomycete Plate Counts

Actinomycetes are a category of important soil bacteria that are thought to be especially important for the decomposition of complex substrates. Only one of the substrates, old tailings, showed any detectable (>10^3 CFU/g) actinomycete activity (Figure 4). The 'old tailing' and 'soil' samples supported plant growth at the time of sample collection unlike any of the other treatments. Because actinomycete numbers in the organic matter were expected to be higher than in substrates, the level of detection for the organic amendments was set at 10^5 CFU/g. Only the soil and compost had actinomycete activity, which was significantly higher in the compost than in the soil (Figure 4). It should be noted that the 'soil' treatment is not strictly an organic amendment since it is dominantly inorganic material, yet was included among the set of organic amendments since it contained abundant organic matter.

Culturable Fungi Plate Counts

Soil fungi are especially important for the breakdown of complex organic matter and maintaining soil structure. Culturable fungi were significantly higher in acid tailings than any of the other substrates (Figure 5). The mean number of culturable fungi was 9.7 x 10^4 CFU/g in the acid tailings and either below detection (<10^2 CFU/g) or 1.5 x 10^3 CFU/g in the old tailings. All of the organic amendments contained culturable fungi, with the lowest level in the sawdust, followed by manure, and soil, and the highest level in the compost (Figure 5).





Number of Carbons Used

The number of carbons used by each sample indicates how many unique carbon sources were metabolized, giving an absorbance reading greater than 0.100. This reflects diversity of carbon-based metabolism of the microbial community in each sample. It is important to note that this is a functional measure, not a measure of genetic or taxonomic diversity.

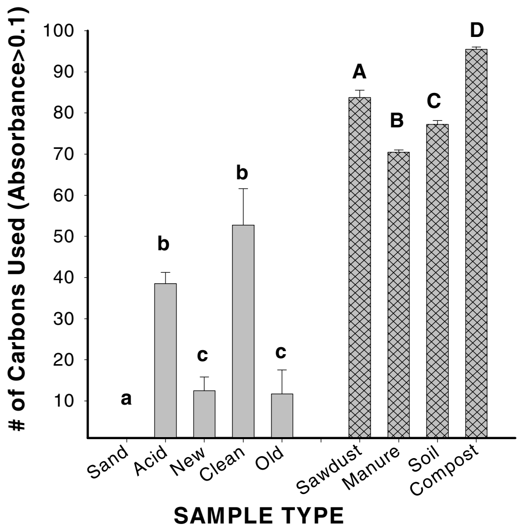


Figure 6. Number of carbons used for all samples. Lowercase letters designate substrate treatment differences (p=0.003), and uppercase letters designate organic matter treatment differences (p=0.003). n=4. Error bars represent ± SE.

The number of carbons used was lower in the substrates than in the organic matter types (Figure 6). The new and old tailings showed lower carbon-utilization diversity than the acid tailings and cleaned tailings. All substrate measurements had large variances associated with the data. The compost had the highest number of carbons used, with almost all 95 substrates showing significant activity (Figure 6). The other organic matter sources also showed high carbon-utilization diversity with manure being the lowest, followed by soil, and then sawdust. It is interesting to note the relatively high metabolic diversity of the sawdust, in spite of relatively low plate counts.

AWCD Across Time.

Organic amendments, in general, had higher levels of microbial activity, as measured by AWCD of Biolog plates (Figure 7). Compost showed the highest level of activity, a pattern that was established within 48 hours, and consistent across time. Soil and sawdust also showed higher levels of activity than the substrates. The response curve for manure was unique, in that microbial activity was initially very low, but after 300 hours, which is longer than we usually monitor Biolog plates, activity on manure substrates was equivalent to that of soil and sawdust. Substrates were consistently low in microbial activity, but the clean tailings treatment showed higher activity than the other substrates.

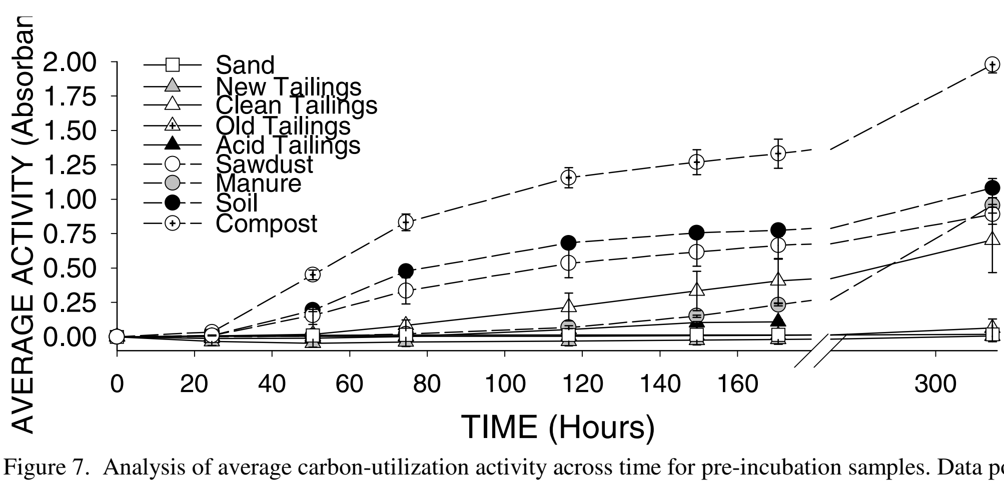


Figure 7. Analysis of average carbon-utilization activity across time for pre-incubation samples. Data points represent the mean +/- SE. n=4.

DISCUSSION AND CONCLUSIONS

Substrate Differences

When considering the inoculation potential of organic matter sources for mine reclamation, it is important to understand how various types of mine tailings can differ geochemically and biologically. The tailings substrates measured in this study varied in pH, moisture content, and microbial characteristics, all of which could affect the survival and composition of the organic matter introduced microbial communities.

The silica sand measurements were below detection limits for microbial measurements and soil moisture. The acid tailings samples were interesting because soil moisture, and especially pH, were low. The significantly higher fungal numbers in the acid tailings are typical of dry, acidic, metal-rich soils. The new and clean tailings generally had intermediate values for the parameters measured. The clean tailings had slightly higher carbon-utilization activity and diversity, but those measurements showed a large variance. Both had higher moisture content and pH than the other substrates. The old tailings had higher plate counts than other substrates, and was the only substrate containing detectable actinomycete bacteria. Those actinomycetes could have been associated with the plant matter observed during soil processing.

It is likely that various organic matter microbial communities would respond differently to these different soil environments. The next phase of this research will consider how the microbial communities associated with each of the organic matter sources changes after incubation with each of the substrate types.

Organic Matter Differences

The organic matter sources varied significantly in their microbial communities and soil measurements. The sawdust had the lowest moisture content, and lower pH than the manure. The sawdust also had the lowest heterotrophic bacteria, actinomycete, and fungal plate counts. In spite of these low counts the sawdust microbial community showed higher carbon utilization diversity than the manure and soil and comparable carbon-utilization activity.

Topsoil samples had moderate moisture and pH measurements. The soil had high heterotrophic bacteria, actinomycete, and fungal plate counts and carbon-utilization activity measurements, second only to compost. The soil was one of only two organic matter types that contained detectable actinomycete colonies.

The manure, which had the highest moisture content and pH, showed moderate microbial community measurements. Heterotrophic bacteria and fungi were lower than compost and soil, and there were no detectable actinomycetes. Manure showed the lowest carbon-utilization diversity and activity, until the final reading after almost two weeks of incubation.

The compost samples had the most abundant and diverse microbial community. While soil moisture and pH were moderate, the compost had the highest heterotrophic, actinomycete, and fungal plate counts. Both carbon-utilization diversity and activity were significantly higher than the other organic matter sources.

Conclusions

This study suggests that different organic matter amendments can potentially alter soil microbial communities when used as tailings amendments. It should be emphasized that microbial inoculation potential is only one of many important factors that should be considered when selecting an organic matter amendment. Other contributions of organic matter addition include effects on soil structure and bulk density, long- and short-term nutrient quality, and effects on desired plant species.

This research provides important insights to microbial changes with organic matter additions, but its applicability is limited by the fact that these are baseline measurements. The next phase of this research project will examine how microbial communities of organic matter additions change after incubation with the various mine tailings substrates. This work also should be repeated in a more realistic field setting to include influences such as influx of microbes through abiotic and biotic vectors, temperature fluctuations, and natural wetting and drying cycles.

These results underscore the importance of further studies examining effects of different organic matter types on reclaimed microbial communities. Further studies should go beyond microbial community characterization to examine soil ecosystem functions, such as organic matter degradation, nitrogen fixation, and soil formation. Such studies can result in a better understanding of ecological soil restoration, which will lead to more successful mine revegetation and restoration.

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ABSTRACT

EVALUATING ORGANIC AMENDMENTS FOR REVEGETATION

PART 1: ORGANIC CONTENT AND COST

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Organic soil amendments may benefit reclamation in many ways, especially where biologically inert coversoils must be revegetated. A good compost usually is the best organic amendment. This information is needed to calculate the amount of amendment needed:

Target organic concentration of soil, minus existing concentration.
Dry bulk density of amendment.
Organic matter as a percent of amendment (dry weight basis).

With this information and measured or assumed bulk density of the target soil, amendment quantities can be calculated. The cost for three commercial composts required to bring the organic content of a given volume of soil (e.g., 6-inch-acre slice of coversoil) to the target organic content is compared without the cost of distributing and incorporating amendments. Calculations are explained. Heterogeneity of organic amendments and inaccurate laboratory analysis are the major limitations to reliable amendment characterization.

Key Words: organic matter content, compost, bulk density, percent dry matter.

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INTRODUCTION

Organic amendments may be prescribed in revegetation to improve the physical condition of the soil, to provide nutrient and carbon pools that otherwise would take decades to accumulate, to attenuate metals, to fertilize the soil, and to provide useful soil microorganisms populations. A good organic amendment can significantly advance the development of biologically inert coversoils and prevent plant diseases, which bears directly on vegetational development.

The first part of this paper provides criteria and evaluates three compost products in terms of cost per unit of organic matter (OM). In the second part, evaluation continues considering fertility and the abundance and diversity of soil microorganisms. Certain commercial compost properties have been changed to prevent identification by means of advertised characteristics.

COMMON ORGANIC AMENDMENTS

In the western United States, the most commonly used organic amendments are compost, bovine manure, and wood waste. Of these, a good compost is the best organic amendment for revegetation. Composting is a process that uses microbial activity to degrade raw organic materials, yielding a lesser quantity of relatively stable amendment. Composting stimulates the decomposition of OM by providing the proper ratio of high-N material, green leafy material, and wide C:N material, often combined with microbiological inocula. Under near-optimal conditions of moisture, temperature, and aeration, decomposition is accelerated. A 3-day sustained temperature of 57^{o} to 68^{o} C (135^{o} to 155^{o} F) kills weed seed and undesirable pathogens. In a mature compost, the food for bacteria, actinomycetes, and fungi is nearly exhausted. As temperature drops, the microbial grazers/predators necessary for nutrient cycling reestablish. Compost is relatively rich in humic materials that have long residence times in soils.

The problem with the wide C:N (>100) amendments such as sawdust is that they consist largely of decomposition-resistant substrates to which few microorganisms are adapted. When carbon does become available to soil microorganisms, it cannot be assimilated except in ratio to N and other nutrients. For these and environmental reasons, decomposition is slow and little N is available to vascular plants. Nitrate levels in soils thus amended and planted are usually near zero during the growing season. Thus, amending soils with decomposition-resistant, wide C:N products is a long-term nutrient impoverishment strategy best applied to soils considered excessively fertile.

Uncomposted manure immediately boosts fertility, but the effects don't persist. Manure is an active, nitrogen-rich amendment that decomposes rather quickly, mineralizing N. Raw wastes lose their OM and degrade in a few years (Cole 1998). Since manure decomposition is bacteria-dominated, it will "burn up" quickly; aerobic bacteria may fix only 5-10% of the carbon from a substrate. Manure that hasn't been composted is a potential weed source, always an important consideration, but the focus here is fertility and microbiological aspects. Fresh manure may be almost 8% N, but 1.5-3.5% N is a reasonable estimate for generic manure (Eck and Stewart 1995, Table 1, p. 172). However, the same authors cautioned that bovine manures that have been aged by cycles of wetting and drying and leached by rainwater may lose much of their value as fertilizer. Phosphorus and potassium do not volatilize and are less subject to leaching than nitrogen.

SAMPLING AND ANALYZING AMENDMENTS

It's a challenge to accurately characterize the "as sold" condition of organic amendments due to inherent heterogeneity. Composite sampling is necessary. In composite sampling, numerous subsamples are combined and thoroughly mixed before analysis to attempt to get representative values while minimizing laboratory costs. Any static pile, whether it be compost or manure or even wood waste, will vary. Depth or nearness to the surface determines oxygen availability and temperature. Unless aerated, deeper portions may become anaerobic while exterior portions may not fully compost. The degree of initial mixing also plays a role. Amendments that are regularly turned for aeration are more homogeneous.

Samples should be taken from screened amendment or following the final mixing step before load out. Take a composite sample consisting of 10 subsamples from different portions of the pile at different depths. The temperature inside the pile should not differ greatly from near the surface. Thoroughly mix the subsamples for analysis. Better yet, take several composite samples and calculate the variance as well as the mean for important parameters. Due to cost, this is rarely done, but it allows putting a confidence interval on important amendment characteristics.

Composite samples from a low-nutrient, low-salinity compost of the type discussed here were submitted to six laboratories for analysis of 10 parameters (Granatstein, 1997). Of the parameters mentioned in this paper, pH, OM, and C:N ratio values were consistent across laboratories. Less reliable were measurements of total K, available P, nitrate, and EC. An example would be a nitrate mean of 486 ppm with a coefficient of variation (CV) of 35% but individual values ranging from 324 to 800 ppm. The least reliable parameter was ammonium, with values ranging from 17 to 3100 ppm and a CV of 150% – virtually useless data.

ORGANIC MATTER CONTENT

Organic matter refers to compounds formed by living organisms and the organisms themselves, most of which contain carbon in cell walls and other compounds. Plant tissues are approximately 40-50% carbon on a dry weight basis. In soils, OM originates as plant materials on and within the soil, animal tissues and excretory products, and soil organisms. Water-soluble compounds are the most easily used (labile) forms of OM for soil microflora and lignin and some aromatic compounds are among the most resistant to decomposition (recalcitrant). Cellulose, the most common polysaccharide in the nature, is intermediary: about six months to decompose under good conditions. Hemicellulose is somewhat more readily decomposed.

The organic content of compost depends on the feedstock, the amount of mineral material present in or added to the compost (e.g., dirt introduced in moving or mixing compost), and the compost age. The less the mineral content, the higher the organic content. The older the compost, the lower but more stable the organic fraction, because microbial decomposition produces CO_2, depleting the organic C pool. The same reactions lower the C:N ratio.

The three evaluated compost products combine wood products with sewage sludge, garden wastes, and some incidental dirt. One product is a volumetric blend of approximately 63% wood waste, 25% dewatered sewage sludge, 8% yard waste, and 4% barnyard manure. If you wash any of these compost products with a medium-pressure stream of water, you will find lots of distinguishable wood pieces. The wood constituent provides intermediate and recalcitrant forms of carbon, whereas the sewage sludge provides nutrients and more labile forms of carbon and nitrogen (and sometimes biological contaminants). If properly composted, vascular plant propagules and human pathogens will be killed in thermophilic decomposition, but a healthy microflora will endure or reestablish.

There are several ways to quantify the OM content of composts. Those skeptical of advertising claims should get their own data. The best technique is to oxidize the OM in a LECO furnace (Nelson and Sommers 1996, pp. 975-976), analyzing for CO_2. For composts lacking carbonates, the procedure is straightforward. For the best comparisons, have a single laboratory determine the OM content of all amendments being evaluated.

A more commonly available procedure is to ignite the sample at high temperature in a muffle furnace (loss on ignition test or LOI). Temperature is a major variable. Heating to less than about 375^{o} C, especially when coupled with short times, results in incomplete removal of OM. Temperatures above about 500^{o} C may remove some constituent other than OM. In discussing loss on ignition procedures, Nelson and Sommers (1996) concluded that "...ignition of soils at 400 to 450^{o} C will remove all organic matter and cause minimal dehydroxylation of clay minerals." **They cite and recommend heating to 400^{o} C for 16 hours.** High temperatures such as 750^{o} C will result in overestimates.

The Walkley-Black technique determines organic carbon, but it is not well-suited to the high organic content of organic amendments. In any method, the empirical constants used to extrapolate from organic carbon to OM should be appropriate for the organic amendment.

The OM content for dry samples of the three compost products ranged from 55% for Compost A to 70% for Compost C, with Compost B in the middle at about 60% (Table 1).

EVALUATING COST

Evaluating cost is easy once you have the information in the proper units, which is the cost of delivered amendment it will take to bring a given volume of soil to the target OM content. In this comparison, the target will be 1% organic content in a six-inch-acre slice of coversoil. Although not reported here, the cost of distributing and incorporating amendments must be included.

Target OM concentrations are likely to range from 0.5 to 3%, with cost playing a major role. Turning deep borrow material into an approximation of a mature soil with organic amendment is impractical. It takes many pounds of OM to eventually get one pound of humus in the soil. In coarse soils, the high rate of gas exchange will result in quicker oxidation of OM than would occur in fine-textured soils. In contrast, clays will retain carbon and suppress decomposition. A bacteria-dominated decomposer population will fix less carbon (put out more CO_2) than a fungi-dominated one.

Table 1. Compost Properties.

	Wet Bulk Density (lbs./cy)	Dry Matter (%)	Dry Bulk Denstiy (lbs./cy)	Organic Matter (%)	Organic Matter (lbs./cy)	NO_3 (ppm)	P (Olsen,ppm)
Compost A	830	55	456	55	251	170	240
Compost B	1030	45	463	60	278	900	450
Compost C	800	40	320	70	224	2000	1200

Information needed for a cost evaluation:

Delivered cost per cubic yard of amendment,
Weight of dry matter in one cubic yard, as delivered, and
Organic content on a dry weight basis.

To determine bulk density for a sample, calculate the percentage of dry matter [dry weight divided by wet weight] X 100, or the dry bulk density, which is the ratio of the mass of dried organic amendment to its volume. To determine this, you need a metal container such as a three-pound coffee can. Weigh the empty can and figure out the volume in English units such as pounds/cu. ft. Fill it with amendment and thump it down on the floor until it settles, then top it off and thump it a little more. Stop when you have a reasonably constant volume for the full container. Weigh it.

Heat it at 105^{o} C, preferably with circulating air to carry off the moisture. This will take less time if you spread it on a cake pan, but it still could take 24 hours. Don't take a high T shortcut and risk charring the amendment. When the weight doesn't change with continued drying, it's oven-dry. Record the dry weight.

Wet bulk density is the weight of the can full of amendment, minus the can weight, divided by the volume of the can. For composts produced outdoors, wet bulk density is influenced by weather.

Dry bulk density is the weight of the amendment after drying, divided by the volume of the can. Particle size plays an important role in determining bulk density for a specified feedstock.

Percent dry matter on a weight basis is [weight of the dry amendment divided by the weight of the wet amendment] X 100. You can multiply the % dry matter by the wet bulk density to get the dry bulk density.

Among composts evaluated, Compost A was more than half dry matter, whereas Composts B and C contained more moisture (Table 1). Composts A and C had similar bulk densities of about 800 pounds/cubic yard, but the bulk density of Compost B was about 1,000 pounds/cubic yard.

Multiplying percent dry weight X percent OM X wet bulk density in pounds/cubic yard gives the pounds of OM on a dry weight basis per cubic yard of compost. Divide this number into 20,400 pounds (1% the mass of a six-inch-acre slice of soil assuming a bulk density of 1.5 g/cc) to determine the number of cubic yards of compost that must be added to a six-inch-acre slice of soil to bring the OM content to one 1% (first column, Table 2). Use a different bulk density for the coversoil if appropriate. The relationship is linear, so for 2% OM in the upper six inches or 1% OM in a one-foot-acre slice, double the amount of compost added.

The relative costs of the three compost products excluding shipping are summarized in Table 2. While not included, the cost of spreading and incorporating compost is pretty much a linear function of volume, so it would be highest for Compost C and least for Compost B. Composts A & B are the best values considering only the cost/unit OM.

CONCLUSIONS

Calculating the needed quantity of organic amendment begins with a target OM concentration for the soil or coversoil. To calculate the amount of organic amendment needed to bring the soil to the target concentration, determine the dry bulk density and organic matter content of the product. Calculate the weight (mass) of organic matter per dry cubic yard and divide into the desired percentage of a mass of soil. At 1.5 g/cc, a six-inch-acre slice of soil weighs about two million pounds. One percent of that is 20,000 pounds, which is the amount of organic matter needed to bring one six-inch-acre slice of soil to 1%.

In the comparison of three commercial composts reported here, between 73 and 91 cubic yards of amendment would be required to bring the coversoil concentration to 1%/six-inch-acre slice. Cost without application ranged from $970 to $1,820.

Compost properties can change, e.g., as a result of differences in raw materials and composting procedures. Test fresh samples immediately before ordering, using composite sampling and, if possible, replicates. By sharing your test results with manufacturers, you will provide them with useful information and emphasize to them the importance of providing a high-quality product.

Other considerations for evaluating amendments are discussed in Part 2.

Table 2. Compost Cost, Application Rates, and Resulting Fertility.

	CuYd of Compost / 6"- Acre Slice*[1] For 1% OM	Compost Cost / CuYd (FOB)	Compost Cost / 6"- Acre Slice *[2]	Resulting N conc. (ppm) *[3]	Effective N conc. with mulch (ppm)*[4]	Resulting P conc. (ppm)*[3]
Compost A	81	$12 - $14	$972 - $1,134	3	0	4
Compost B (3/8" minus)	73	$13.70 - $15.20	$1,000 - $1,110	15	0	7
Compost C	91	$20	$1,820	28	14	17

*[1] Volume of compost needed to bring the organic matter content of the coversoil (1.5g/cc) to 1%.

*[2] Compost cost per 6"-acre slice, not counting application and incorporation.

*[3] The concentraions of nitrogen and phosphorus in a 6"-acre slice after amendment, assuming no nitrogen or phosphorous prior to amendment.

*[4] Based on applying 1.5T/acre mulch, assuming soil microbes immobilize 10 lbs./acre nitrogen per 1000 lbs. of mulch.

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2000 Billings Land Reclamation Symposium

ABSTRACT

EVALUATING ORGANIC AMENDMENTS FOR REVEGETATION

PART 2: FERTILITY AND MICROBIOLOGY

R. A. Prodgers[1]

The type and application rate of amendment must complement other aspects of the revegetation plan. Fertility concerns include plant nutrition, nitrogen fixation, and mycorrhizae establishment. Short-term fertility concerns include nutritional requirements of vascular plants and the desirability of establishing plants in a fertility regime similar to long-term conditions, ruderal-controlling strategies, nitrate levels that complement nitrogen fixation, and phosphorus concentrations that don't inhibit mycorrhizae establishment. The unamended fertility of the soil must also be considered. Focusing solely on the C:N:P ratio ignores the fact that recalcitrant carbon-rich residues will persist for years. The major midterm nutritional concern is the nutrient-immobilizing tendency of amendments once labile constituents have decomposed. For successful revegetation, decomposition must complement primary productivity. A good compost should provide adequate densities of bacteria, fungi, protozoa, and beneficial nematodes to establish a functioning soil food web and assure nutrient cycling. Microbiological targets include more than 100 ug/g total bacteria and fungi (each) and at least 30,000 protozoa/gdy will. Bacterial dominance would be normal for a grassland soil. No root-feeding nematodes should be tolerated in amendments.

Key Words: organic matter, soil fertility targets, nitrogen fixation, mycorrhizae establishment, C:N:P ratios, soil microbiology.

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INTRODUCTION

Organic amendments are often evaluated solely based on cost and organic content or C:N ratio. From an ecological perspective, other attributes are equally important. The suitability of a given product depends on other details of the revegetation plan. An organic amendment may complement one revegetation strategy but conflict with another.

This paper provides criteria and evaluates three compost products in terms of fertility and the abundance and diversity of soil microorganisms. Certain commercial compost properties have been changed to prevent identification by means of advertised characteristics.

COMPOST AND PLANT NUTRITION

Abundant essential nutrients make plants grow big and fast. Nonecologists typically are impressed when target areas turn green fast, but in a seminatural system high fertility isn't necessarily better – especially if temporary. The response of native prairie plants to fertilization is variable, with some species benefitting from all reasonable fertilization rates and others responding positively to light fertilization but negatively to higher levels of fertilization (Anonymous 1975). Others hardly respond at all. In mixed plant communities, fertility is a powerful selective force. High initial fertility selects for heavy nutrient users, but these same species might be poorly adapted to conditions after fertilization ceases or the mineralized nutrients in compost become depleted. At the same time, temporarily fertile soils select against species well-adapted to a low fertility regime. This sets the stage for declining plant performance and sometimes weed proliferation or invasion.

High levels of mineralized N also can inhibit *Rhizobium* establishment for nitrogen fixation and depress populations of chemoautotrophic bacteria responsible for nitrification, but the latter process will reestablish as nitrate levels decline. A coversoil concentration of 12 to 15 ppm nitrate would be the upper desirable concentration, but nodule formation will be greater at nitrate levels <5 ppm. Vascular plants are likely to quickly immobilize up to 15 ppm nitrate supplied by the compost.

Short-Term Nutrition Concerns (Mineralized Nutrients)

High but declining mineralized nitrogen concentrations are undesirable for three reasons. First, the critical plant establishment dynamic should occur in a fertility regime similar to the long-term condition, not in an environment that favors copious nutrient users. Second, nodule formation for nitrogen fixation is greatly diminished by high nitrate levels, and the challenge of revegetating biologically inert coversoils often is best addressed with a combination of organic amendment and nitrogen fixation. Third, high nitrogen fertility promotes some types of weeds.

The mineralized N (ammonium, nitrate, and nitrite) and P (Olsen) concentrations in the amendment are determined with fair precision using standard agronomic tests. Determine nitrate (plus ammonium and nitrite, but these should be scarce at neutral to alkaline pH if nitrification is ongoing) and Olsen P in ppm (or mg/kg, etc.). **This will be on a dry weight basis for the amendment, not just the organic matter.** It's easy to consider only the organic content, since this is what we used to evaluate cost, and underestimate the effect of amendment on initial fertility.

Remember: a six-inch-acre slice weighs around two million pounds. (At 1.5 g/cc, it would weigh 2.04 X 10^6 pounds, or you can adjust for known soil bulk density.) At 50% organic content, it takes 40,000 pounds of dry matter in the amendment to bring the organic content of a six-inch-acre slice to 1% OM.

One easy way to calculate the effect of N or P additions, whether from compost or mineral, is based on the fact that 10 ppm (nitrate, P, whatever) in a six-inch-acre slice weighs about 20 pounds and all relationships are linear. For example, adding 12 pounds of nitrate would raise the concentration by 6 ppm.

Although a nitrate target of around 5 ppm might seem desirable, field trials often show soil that should have about 5 ppm nitrate actually drop to <1 ppm in a few weeks as plants establish. In other words, vascular plants quickly immobilize the nitrate. Thus, a target of 12-15 ppm nitrate may be appropriate even for nitrogen fixation. Without nitrogen fixation, incremental nitrogen fertilization may be desirable during the first few growing seasons.

There is one more consideration regarding nitrate. If the revegetation plan calls for organic mulching with a wide C:N product such as straw, its decomposition will immobilize more N. Decomposition of a substrate such as straw will be sped by having more N in the soil, but as mentioned earlier, the initial hydrolysis of lignin and other recalcitrant compounds limits the rate of decomposition. It's complicated and not completely predictable (straw in air vs. straw in soil, etc.), but a "rule of thumb" is to allow 10 pounds of nitrogen for each 1,000 pounds of mulch. If the mulch was half carbon and had a C:N ratio of 100:1, adding 10 pounds of N would change the ratio to 33:1. Thus the mulch acts less as a nitrogen sink. A typical heavy application of mulch might be 1.5 tons/acre, requiring 30 pounds of N/acre.

As Table 2 (Part 1) indicates, the relatively infertile Compost A could be applied in conjunction with nitrogen fertilizer even without mulch, and B would need some nitrogen fertilizer if used with 1.5 tons straw/acre. Alternatively, one could say that enough of Compost A could be added to bring the upper six inches to 3% OM without making the coversoil too fertile for nitrogen fixation. If nitrogen fixation is desired and nitrate-loving weeds are not, Compost C provides excessive fertility even at a 1% OM application rate. These calculations were for biologically inert coversoils initially containing no nitrate.

Elevated levels of P inhibit establishment of the fungal component of vesicular-arbuscular mycorrhizae (VAM), thus conflicting with plant nutrition strategies that rely on their establishment. VAM are especially adept at securing P from phosphorus-poor soils (St. John 1996). For VAM to establish, P must be sparingly available, on the order of 10 ppm (St. John pers. comm., 1998), although the inhibitory concentration depends on circumstances.

Olsen P in the composts ranged from 240 ppm for Compost A up to 1,200 ppm for Compost C

(Table 1, Part 1). The last column in Table 2 (Part 1) indicates that one could add enough Compost A to bring the OM content of a six-inch-acre slice to almost 2% while P remained low enough for VAM to establish. For Compost B, only enough compost could be added to bring the OM content of a six-inch-acre slice to 1% if VAM establishment is desired. However quickly it would grow plants, the wonderfully rich Compost C is at the upper threshold for VAM to establish even with no available P present in the unamended coversoil.

The above assessment assumes that P is absent from the soil or coversoil before amendment. If P already is present at >15 ppm before amendment, VAM in conjunction with compost may not be an option. In that case, there is no downside to abundant mineral P. A straw mulch (C:P ratio >300) favors immobilization. Mulch will immobilize P in ratio to N. The nitrogen mineralized is eight to 15 times the amount of phosphate made available, often generalized as 10:1. Therefore, if 1.5 tons/acre of straw mulch will immobilize 30 pounds of N, it will also immobilize roughly three pounds P (1.5 ppm in a 6-inch-acre slice). Of course, the establishment of revegetation will immobilize some of the free P, and it readily forms insoluble compounds in low pH soils. The net effect is best assessed by determining the P concentration in similar soils with two-year-old revegetation.

Summing up so far, rather infertile compost may be desirable where nitrogen fixation and VAM are part of revegetation prescriptions. High nitrogen levels can promote weeds, inhibit nitrogen fixation, and select for vascular plant species ill-suited to a lower long-term fertility regime. High plant-available phosphorus concentrations may conflict with optimal VAM establishment. The reason is that P may already be near or above optimal levels in soils/coversoil prior to amendment.

Midterm Nutritional Concerns

Much more difficult to evaluate is how fertility will change after the coversoil is amended and vegetated, when vascular plants are immobilizing nutrients and the labile constituents of plant and microbial litter are simultaneously being decomposed, etc. In the OM and microflora, nitrogen mineralization and immobilization can be thought of as opposing processes occurring simultaneously at rates determined by the substrate types involved and the abundance and diversity of soil microorganisms. This is related to the C:N:P ratio of the amendment and also by how easily degraded the compost constituents are. Only the net effect is measured.

Midterm fertility can be evaluated empirically. For a labile, nutrient-rich amendment such as bovine manure, evaluate the C:N:P ratio and estimate the decomposition rate. Unless it has long been weathered, manure is likely to bring N and P concentrations above target levels and elevate K even more, but too much K probably won't become a problem when a single application of manure is applied unless the unamended coversoil is already saline.

Wood-based compost tends toward nutrient impoverishment once the labile constituents have been immobilized in the soil-plant system. About 42 pounds/acre of mineral N was immobilized just a few months after application in first-year, compost-amended and mulched Butte Hill revegetation. A soil-plant system that immobilizes added nutrients is desirable, within limits. Absent nitrogen fixation, incremental annual fertilization may be desirable for a few years until mineralization and immobilization equilibrate.

Wood waste is a strong nutrient impoverishment amendment that may bring N and P below 1 ppm and, in conjunction with growing plants, keep it there. This type of amendment is appropriate only when nutrient impoverishment is desired.

Summing up, low to moderate amounts of bovine manure have a low residence time; consequently midterm fertility is much diminished from the initial condition. If considering compost composed of wood waste, sewage sludge, and garden waste, the midterm nutritional consequences tend toward nutritional impoverishment because the recalcitrant substrates endure while the labile ones disappear quickly. At the same time, the C:N ratio declines naturally through time. A slight nutritional deficit is compatible with nitrogen fixation or incremental mineral fertilization, which may be necessary during the establishment phase. Wood wastes tend to bring about nutrient impoverishment in the short- and midterm. Fertility should approximate long-term conditions.

The C:N Ratio

The ratio of carbon to nitrogen is often given too much weight in evaluating organic amendments. Since humus typically has a C:N ratio between 5:1 and 15:1, a similar ratio sometimes is specified for compost. The guiding principle is that nitrogen will be conserved while carbon is lost as CO_2, so the ratio narrows through time until an equilibrium is reached. This is despite the fact that some N may be lost through leaching of nitrate/ ammonium, through denitrification (N_2), and through volatilization (NH_3).

Of course, as the C:N ratio narrows, C will be found in increasingly recalcitrant forms. Decomposition of these gigantic polysaccharides is limited by lack of enzymes capable of their initial hydrolysis. (Otherwise, adding mineral N to mature compost, straw, or wood waste would result in the dramatic decomposition of carbon substrates.) These exoenzymes, often originating with fungi, usually are rare in grassland soils and are absent from biologically inert coversoils. When carbon becomes available to soil microorganisms, it cannot be assimilated except in ratio to N and other nutrients. For these and environmental reasons, decomposition is slow. When mineralized, most nutrients rarely persist in inorganic form long enough for plants to access them because the microflora assimilates essentially all the nitrogen contained in protein-poor residues. The apparent retarding influence of carbohydrates on the production of ammonium from proteins and amino acids may be attributed to an assimilation of ammonium by the additional organisms appearing in the decomposition of the carbohydrate (Alexander 1991, Chapter 15).

Thus the C:N ratio reveals only part of the picture of N availability. In practice, as C becomes recalcitrant, substrates such as wood chips act more as bulking agents than as N sinks. Further decomposition is limited by critical enzymes, not just the quantity of available N. If mineral N is applied to amended coversoil, vascular plants will get most of the nitrogen, not the microflora.

For a producer, the C:N ratio can be altered only by changing the original feedstock (more N) or by letting the compost sit longer while the ratio narrows. But as carbon is respired, the physical quantity of compost decreases. The producer is much more likely to apply relatively inexpensive mineral fertilizer. Now we have a C:N ratio near "equilibrium," but it consists of mineral N and a pool of recalcitrant C. The initial justification for a narrow ratio has been stood on its head. Nitrate levels of amended soils become elevated in the plant establishment phase and infertile later, a subject already discussed.

MICROBIOLOGICAL CONSIDERATIONS

In amending coversoils originating as deep (>1 m) borrow material, the microflora in compost is among its most important potential long-term contributions. Microbial enzymes are key agents of the decomposition and transformation of organic substrates. Without them, there will be no nutrient cycling. The heterogeneity of organic constituents and important environmental conditions such as temperature, reactivity, fertility, and moisture largely determine which class and even which species of soil organisms are most active for a given substrate and environment. Each species has a complex of enzymes that is effective on a fixed array of chemical compounds, but not others.

A good organic amendment for biologically inert coversoils should provide an incipient "soil food web." The idea is to provide a diverse set of beneficial microorganisms, the best-adapted of which will establish in the coversoil, feeding off the organic amendment first and plant residues as they become available. Having the proper microorganisms in the soil is important to prevent disease in vascular plants as well as for nutrient cycling. Soil organisms hold nutrients in the soil. Without bacteria and fungi in the soil, most applied fertilizer will wash through the soil, which explains why Reeder (1990) found that fertilized spoil was ineffective in providing N to vascular plants.

Nutrient cycling cannot occur without several groups of soil microorganisms. Soils contain five major groups: bacteria, actinomycetes, fungi, algae, and protozoa. In adequately aerated soils, bacteria and fungi dominate, whereas bacteria alone account for most biologic activity where oxygen is strongly deficient (e.g., wetlands). Actinomycetes are important in the composting process, especially at elevated temperature. Compost is not expected to contain a significant number of mycorrhizal fungi.

In evaluating compost, focus on total (as opposed to active) bacteria and fungi, protozoa and nematode density, and the ratio of bacteria to fungi. In grasslands, a bacterial dominance is normal, but the fungal component remains important. The optimal ratio of fungi to bacteria changes with soil type, but, in general, grassland soils have bacterial dominance, whereas soils of woody plants have fungal dominance. Units for bacteria and fungi are ug/g dry compost.

Bacteria are the major decomposers of alkaline soils and quickest to respond to changes in environmental conditions and substrate. They are most effective on labile compounds, and their populations can build very quickly when food is available. Bacteria produce "slime layers" around their bodies, which they use to glue themselves to surfaces. This prevents them from being washed out of the soil and is a major agent of microaggregation of soil particles. This slime layer is most often made of alkaline materials, which may cause soil to become more alkaline. In alkaline environments, maintained by the slime layers and secondary metabolites that bacteria produce, nitrifying bacteria thrive and convert ammonium to nitrate quite rapidly. Nitrate is the preferred form of N for most grasses. Thus, these plants grow best in bacterial-dominated soils.

Composts A and C had the same amounts of active and total bacteria, with Compost B lagging.

Fungi: Fungi are important decomposers of intermediate and recalcitrant forms of carbon (including otherwise toxic compounds), especially in acid soils. Fungi make organic acids as their waste products, lowering the pH of initially neutral substrates. Because fungi maintain soil pH on the acidic side, and indeed, beneficial fungi appear to buffer soil pH between a pH of 5.5 and 6.5, nitrifying bacteria are excluded from the food web in strongly fungal-dominated soils. Fungi retain N in the soil, compete with some pathogens, and promote soil aggregation. Fungi grow as long threads which bind the microaggregates together into macroaggregates. Aerobic bacteria may fix only 5-10% of the carbon from a substrate compared to 30-40% by fungi, so OM accumulates more rapidly in fungal-dominated soils.

Total and active fungi were highest in Compost A. Total fungi biomass in Composts B and C was very low, suggesting they may have become anaerobic during the composting process.

Protozoa are primitive, single-celled animals. Bacteria are eaten by protozoa and bacterial-feeding nematodes, releasing ammonium into the soil. Protozoa are essential for nutrient cycling. Without protozoa, nutrients become immobilized in bacteria for too long and vascular plants suffer. Straw and hay (as in mulch) can provide a good inoculant. Among composts, products A and C were excellent sources, whereas Compost B lacked protozoa.

Nematodes are unsegmented worms; all terrestrial species are microscopic. Fungi are eaten by fungal-feeding nematodes, a few species of large amoebae, and fungal-feeding microarthropods. Nematodes can be common in compost but may not be critical in local grassland soils. Check a healthy local soil to see how important nematodes are locally.

Composts A and C contained some bacteria-feeding nematodes, and Compost A also contained a fungi-feeding species of nematode. (Compost C had virtually no fungi, hence no fungi-feeding nematodes.) Compost B not only had few desirable nematodes, but it also contained a root-feeding nematode -- good compost shouldn't contain any root-feeding nematodes. Zero.

Summarizing microbiological factors, Compost A was superior, Compost C was good in some respects but lacked fungi, and Compost B was inferior in all respects.

The following densities are characteristic of good compost:

1. Total Bacteria: >100 ug/g (ug/gram dry weight [gdy] of compost).

2. Total Fungi: >100 ug/g (for compost with a strong wood component).

3. Protozoa: >30,000 individuals per gdy.

4. Nematodes: Zero root-feeding nematodes. A specified minimum density of beneficial nematodes may be appropriate for some coversoils, depending upon the indigenous density in native soils.

In writing technical standards for compost, fungi density might be lowered to 50 ug/g for grassland soils. A minimum protozoa density might be 10,000 per gdy.

CONCLUSIONS

It is important to tie compost requirements to other aspects of the revegetation plan, including anticipated temporal developments. Compost can be a big budget item, and should provide commensurate benefits to both plants and soils.

Nutritional effects of amendments must not exceed mineralized nutrient targets in coversoils, but nitrate levels can be above targets because establishing vascular plants will immobilize it quickly. Below-target macronutrient concentrations are less of a problem than excesses.

Unless decomposition complements primary productivity, revegetation will eventually stagnate no matter how good it looks initially. A good amendment should initiate a healthy soil food web in biologically inert coversoil. Where a functioning soil food web exists, OM acts more like food for existing microorganisms. Soil microbes are agents of soil aggregation in addition to their direct effects on plant performance.

It is important to recognize that no reasonable amount of compost will make marginal soil materials into good ones. Although a good organic amendment will advance the development of biologically inert coversoils, organic amendments are not humus. However, a proper compost with incipient soil food web can greatly advance soil development and enhance the performance of coversoil as a plant growth medium

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SOIL AND WATER QUALITY ASSOCIATED WITH LAND APPLICATION OF BIOSOLIDS FOR SURFACE MINE RECLAMATION: FOUR CASE STUDIES

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ABSTRACT

Land application of biosolids (municipal and industrial wastewater treatment plant solids) was employed as a beneficial-use soil amendment for reclamation of four surface coal mines in Pennsylvania. Soil and water quality data collected for regulatory reporting before, during, and after biosolids application were analyzed to assess the effects of application on site soils and surrounding receiving water points. The number of background water samples included in the regulatory sampling program was found to be insufficient to make comparisons between pre- and post-application water quality conditions. Soil sampling data from the biosolids application areas indicate that phosphate levels are relatively constant for the first four years following application, while potash and magnesium levels decrease by approximately 15 percent per year, and cation exchange capacity decreases by approximately 9 percent per year. Although declining trends were observed for the latter parameters, post-application levels of all four parameters were significantly higher than pre-application levels, and the addition of biosolids to severely disturbed soils benefits soil quality in the short term and promotes long-term improvements in soil fertility. Additional data not included in the regulatory monitoring program for these sites would be required to fully evaluate the success of biosolids application with regards to water and soil quality.

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INTRODUCTION

Restoration of acceptable vegetative cover on reclaimed mine lands can be difficult because post-mining soils often have a less developed soil structure and reduced organic matter and plant-available nutrient contents than the original in-situ soils. Bulk stripping of soil tends to mix organic topsoil and mineral subsoil horizons, resulting in a new media on which it can be difficult to establish and maintain vegetative cover. Segregation of soil horizons during site clearing and replacement of horizons in their original order is impractical, and it is still inevitable that productive soil structure and properties will be lost. Additionally, on many mine sites, thin soils or poor initial soil quality precludes stripping and replacement, necessitating the use of a manufactured soil substitute created from crushed spoil and various amendments. A common solution in Pennsylvania has been the addition of organic material and mineral fertilizer to either the stockpiled soils or crushed spoil to create a material capable of supporting seed germination and establishing initial plant cover.

One class of organic amendments being increasingly used for mine reclamation is biosolids, which are municipal and industrial wastewater treatment plant byproducts that possess a high organic-carbon content, contain valuable plant-available nutrients, and have a high water holding capacity. Municipal sewage sludge is probably the most common biosolid used for mine reclamation, but many industrial and food processing operations can yield similar materials. These materials are often disposed of as a solid waste, but biosolids are now widely recognized by regulators as a beneficial soil amendment and are increasingly being used in agricultural and mine reclamation activities. Biosolids offer the double advantages of superior vegetative establishment on disturbed soils for the mine operator, and reduction of landfill disposal costs for the biosolids generator.

The Soil Foodweb

In healthy soil, there are typically millions of microscopic organisms that live in the soil and around the roots of plants. The environment that these organisms inhabit and the ways in which they interact with each other and with plants is called the soil *foodweb*. There is an optimum balance of soil air, water, nutrients, and different microorganisms that allows plants to establish and thrive. Healthy soil should have a developed structure with voids for soil air and water, a balanced supply of plant-available macro- and micro-nutrients, as well as contain beneficial species of bacteria, fungi, nematodes, and protozoa, which are often missing in severely disturbed mine soils.

Developing the proper physical, chemical, and biological environments in the root zone can yield substantial benefits to mine soil reclamation projects. A balanced soil foodweb will: (1) suppress disease-causing and pest organisms; (2) retain nitrogen and other nutrients, such as calcium, iron, potassium, and phosphorus; (3) make nutrients available for plant growth at the times and rates that plants require; (4) decompose plant residues rapidly; and (5) produce good soil structure, improving water infiltration, oxygen diffusion, and water-holding capacity.

Disease suppression requires specific species of soil bacteria and fungi that compete with, inhibit, and parasitize disease-causing organisms. A plant uses a minimum of 25% of its fixed energy each year to feed beneficial organisms in the volume of soil around its roots. Pesticides and intensive earthmoving activities (bare soil for long periods) significantly reduce the diversity and amount of these beneficial organisms. In order to reestablish a viable soil foodweb, these organisms must be reintroduced through inoculation or through feeding with the right kinds of materials. A healthy soil contains a broad diversity of microbial types and most often contains species that consume, inhibit, or suppress the kinds of fungi that cause root rots and the kinds of nematodes that attack roots.

Nitrogen and other nutrients can leach out and be lost unless they are contained in less mobile forms in the soil until required for plant growth. Nutrient retention can occur when bacteria and fungi multiply in an organic-enriched environment and increase their populations in the soil. When bacteria and fungi multiply, they gather nitrates, ammonium, and other forms of nitrogen from the soil and convert it into protein in their bodies. Nitrogen in this form does not leach easily and is not lost as a gas. Products and cultural practices, such as biosolids applications that stimulate a "bloom" of bacteria or fungi reproductive growth, can be used as tools to achieve nutrient retention. When this function is working in the soil, lower rates of nitrogen and phosphorus can be applied with no reduction in crop yield. Organic amendments such as biosolids help retain the soils' N, P, S, Ca, K, Fe, etc, for plant use, and make these parameters less likely to leach into surface or groundwater resources.

Plant residues will only decompose if certain species of fungi and bacteria, the "decomposers," digest them and allow the nutrient recycling processes to occur. The ideal decomposition process forms large amounts of humus. The decay function breaks down plant residues and converts the food energy in fresh organic matter into biological forms that feed other soil organisms that perform different indispensable functions.

In order to maintain a well-aggregated soil structure (i.e., to improve or maintain good tilth), the organisms that glue, bind, and engineer soil structure and soil pores must be present. Good tilth or good soil structure allows optimum infiltration of air, water, and roots. Aggregates will not form unless sand, silt, and clay particles are "glued" together by the gums and gels that many species of soil bacteria produce. These aggregates are further strengthened against collapse by species of beneficial fungi that grow throughout the aggregate and physically bind it. The large pore spaces holding "reservoirs" of water must be built by larger organisms: microarthropods, earthworms, beetle larvae, enchytraeids, etc. The better the set of soil organisms producing resilient structure, the more "strength" a soil will have to resist damage, such as by vehicle passage.

Site Backgrounds

The four biosolids application sites, referenced herein as the PS, MS, SM, and PAC sites, were surface bituminous coal mines located in central Pennsylvania. The mines were situated on ridge crests above incised stream valleys, and discharged through erosion and sedimentation controls during the application periods. Reclamation typically progressed in phases, with biosolids application occurring following regrading of mine spoils and, when employed, replacement of stockpiled soils. Biosolids and fertilizer were applied one time on each regraded area, incorporated, and then seeded.

The applied biosolids were a "mine mix" consisting by dry weight of approximately 57% wood chips and 43% municipal sludge cake obtained from the Philadelphia Water Department (wastewater treatment solids). The mine mix was applied to achieve 60 dry tons of sludge per acre, equivalent to 332 wet tons as received when accounting for the moisture content of the mix material. Spreading occurred between April 15 and September 15 using conventional tractor-towed agricultural manure spreaders. After initial spreading, the mine mix was incorporated into the upper 6 to 12 inches of the regraded spoil surface using a chisel plow. Lime additions and any needed N-P-K fertilizers were applied on a site-specific basis as determined by nutrient analysis of the stockpiled soil or spoil.

Between 1993 and 1998, Gannett Fleming monitored soil and water quality on the four sites to satisfy land application permit reporting requirements administered by the Pennsylvania Department of Environmental Protection (PADEP). Following completion of the monitoring programs, Gannett Fleming examined these data sets to quantitatively evaluate the impact of the four application projects on water quality and the regulatory-selected soil quality parameters of nutrient levels and cation exchange capacity (CEC).

METHODS

Water quality monitoring points surrounding the application sites were selected in accordance with PADEP requirements to represent upstream and downstream conditions in receiving streams, downgradient groundwater discharges, and private water supplies. Sample parameters included a wide range of physical and chemical parameters, including nutrients and EPA priority pollutant heavy metals. The PADEP monitoring schedule for the application sites included collection of two background samples prior to biosolids application, and quarterly sample collection concurrent with and following biosolids application until the PADEP released the sites from further sampling. The water sampling duration ranged from 1 to 2 years following application.

Soil samples for PADEP reporting were collected from representative plots within the application areas of each mine site, with the number of samples varying depending on the size of the site. Samples were collected from the upper 6 to 8 inches of the soil surface by grab methods. Laboratory analyses of soil macro- and micro-nutrient levels and exchangeable cations were performed by the Agricultural Analytical Services Laboratory (AASL) of the Pennsylvania State University. The AASL provided results in the form of recommendations for liming and nutrient amendments for each sample based on the determined background soil levels, type of desired cover crop, and regional climatic conditions. In this study, initial pre-biosolids application samples were collected on three of the four sites, with no background sample required for the SM site. Additional samples were then collected on all four sites annually following biosolids application until the PADEP released the sites from further sampling. The duration of soil sampling ranged from 3 to 4 years following application.

RESULTS

The following presents the findings of the water and soil quality analyses for the four sites in composite, with discussion of the specific factors that may influence the results observed for each of the parameters considered.

Water Quality

After an analytical review of the water quality sampling programs for the four application sites, it was determined that the data sets were insufficient in size to reliably determine differences in water quality conditions due to biosolids application. All data in this study were collected solely for regulatory compliance purposes, which did not lend itself to a proper statistical design and analysis (treatment vs. effect). The PADEP required only two initial background samples and this proved inadequate to quantify baseline water quality conditions that existed prior to biosolids application. Valid analysis would require a minimum of one year of pre-application water quality baseline data, coupled with control samples from an area with no biosolids amendment or solely conventional mineral fertilizer amendment. No significant isolation of treatment vs. effect can be drawn, so the water quality analysis is not presented in this paper.

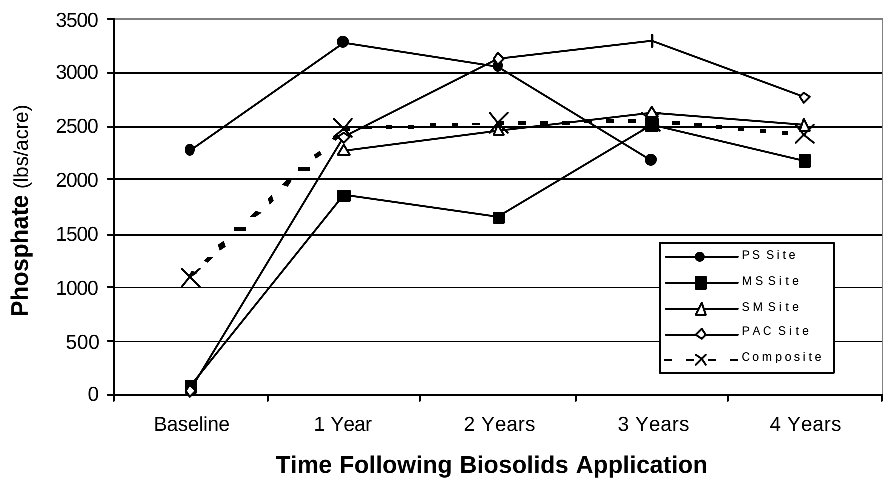
Soil Quality

The soils data sets were similarly shaped by the regulatory requirement of one round of background samples. However, these have been found more relevant because the post-application information covers a longer period than the water quality sampling, and the reported soil parameters are not expected to be as heavily influenced by seasonal fluctuations. Overall trends in soil parameters were found to be reasonably consistent over time and between separate sites. The results discussed here are for the soil macro- and micro-nutrient levels of phosphate, potash, and magnesium nutrient levels and overall soil CEC. These parameter trends are presented in Figures 1 – 4, respectively, as the composite trends for all the samples of each individual site and as a composite of the sampling data for all four sites.

Phosphate

Phosphate is not a highly mobile parameter (as compared to nitrate) and is normally only lost through erosion and plant uptake. In Figure 1, phosphate levels on the individual biosolids application sites varied between 1,650 and 3,280 pounds per acre (lbs/acre) in the years following application. The composite trend for all site data indicates little variation with time from an average of about 2,500 lbs/acre. None of the composite averages for years following application are significantly different from preceding post-application years. These data indicate that a good cover crop has been established and that erosion has not removed this plant-essential macro-nutrient provided by the biosolids application. Due to the large initial phosphate application rate, it is likely that any reduction in soil levels due to plant uptake has been overshadowed during these first four years.

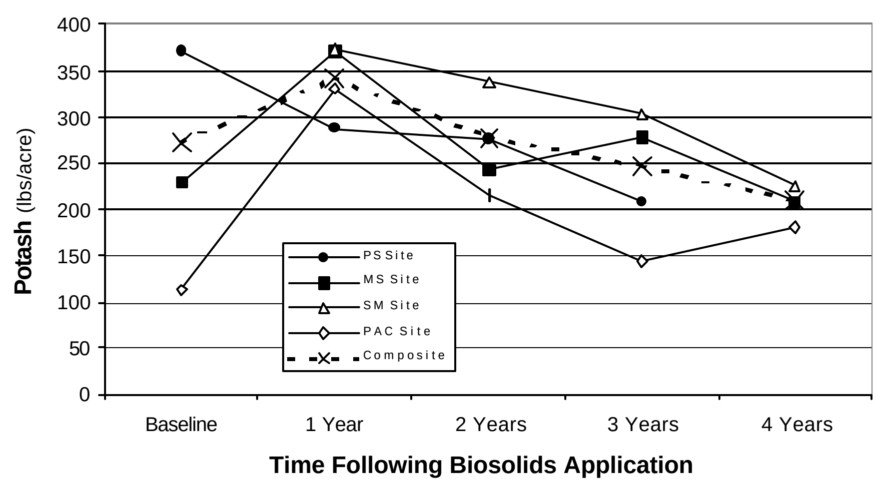
Figure 1. Phosphate Nutrient Levels with Time



Potash

Potash levels shown by Figure 2 range from a high of 373 lbs/acre to a low of 144 lbs/acre in a relatively consistent diminishing trend for the individual sites. This is apparent in the composite trend as well, with potash levels decreasing by approximately 15 percent each year following biosolids application. These decreases are highly significant between Year 1 and Year 2, and significant between Year 2 and Year 3, but not significant between Year 3 and Year 4. These decreases in potash levels are in agreement with the typical plant uptake rate of 200 lbs/acre for the cover crop of cool season grasses. The leveling off of potash removal between Years 3 and 4 may indicate the beginning of nutrient cycling in the newly established biosolids-amended mine soil.

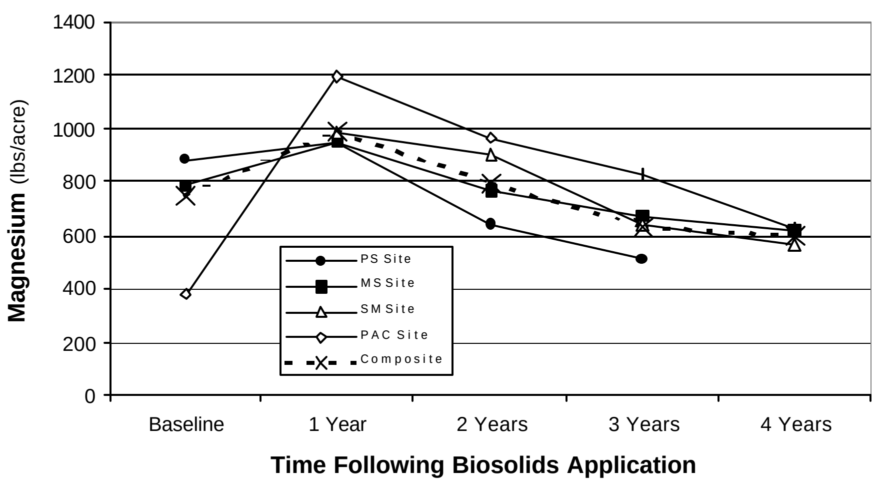
Figure 2. Potash Nutrient Levels with Time



Magnesium

Trends for magnesium levels, shown by Figure 3, are similar to those for potash and range from a high of 1,195 lbs/acre to a low of 507 lbs/acre. The average decrease of the composite trend is also about 15 percent per year following biosolids application and appears to begin an asymptotic reduction in change in Year 4. Decreases in magnesium levels are highly significant between Year 1 and Year 2, and between Year 2 and Year 3, but are not significant between Year 3 and Year 4. Soil magnesium is a moderately leachable nutrient and greater amounts are often found in the subsoil than in upper parts of the profile. The initial decreases in soil magnesium are likely due to plant removal and may indicate that the more mobile forms of magnesium (primary, acid soluble, and exchangeable) have moved below sampling depth, leaving mainly the organically-complexed material in the top horizon.

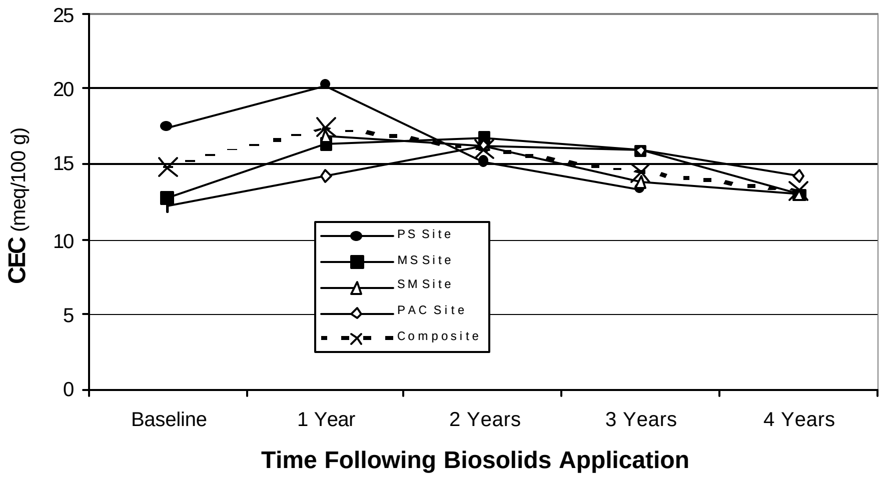
Figure 3. Magnesium Nutrient Levels with Time



Cation Exchange Capacity

CEC trends between the four sites are relatively well grouped on Figure 4, ranging from a high of 20.2 meq/100g to a low of 12.9 meq/100g for the individual sites. The composite trend is consistent over time and averages a decrease of approximately 9 percent per year following biosolids application. The decrease between Year 2 and Year 3 is highly significant, while the decreases between Year 1 and Year 2, and between Year 3 and Year 4, are just under the threshold of significance. CEC can vary depending on soil pH, and broad trends in the soil data suggest that in the initially acidic soil samples, pH rose as expected after the lime and biosolids application. The soil pH and CEC decreased over time in these same samples, and this is attributed to acid deposition (rain) and the formation of organic acids. In the initially acidic and low CEC level pre-application soils, biosolids and lime raised the CEC significantly.

Figure 4. Cation Exchange Capacity with Time



DISCUSSION

Regulations governing the land application of biosolids stress that these materials should pose no greater risk to the environment and the public health than conventional reclamation materials (mineral fertilizers and lime). With this as the guiding principal, the required regulatory sampling is heavily slanted towards the more easily understood and commonly measured soil chemistry parameters. This study found that this type of testing stresses only the "chemical" fertilizer benefits of biosolids, and provides a poor evaluation for this proven reclamation method. Of the three primary soil quality factors - physical, chemical, and biological - the least measurable benefits from biosolids applications are related to soil chemistry. The greatest benefit of biosolids is in helping rebuild soil structure and restock the soil foodweb for beneficial biological organisms.

Biosolids-amended soils by definition have a higher organic matter content and, therefore, increased water holding capacity, tilth (porosity), nutrient holding capacity, buffering capacity, availability of soil minerals, activity of soil microorganisms, and both short- and long-term improvement in soil fertility. The authors have worked with biosolids in numerous reclamation and agricultural settings and found their use to be highly beneficial in establishing and maintaining plant growth in harsh conditions. In our studies provided to regulators, yields of plant biomass and health evaluations of plants cultivated in biosolids-amended soil were always significantly higher than the control samples (untreated soil). Laboratory surface and groundwater water quality assessments showed that biosolids were a clean, low-impacting source of plant nutrients, and have lower non-point source pollution potential than commercial fertilizers. Nitrogen and phosphorous content of the surface runoff and groundwater recharge from biosolids-amended soils were less than that of fertilizer-amended soils, even in the extreme case where the total nitrogen application rate was 10 times greater for the biosolids than for the mineral fertilizer.

CONCLUSIONS

The trends in soil nutrient content and CEC observed on the four biosolids application sites are consistent with establishment of viable soil foodwebs within the reclamation soils. Biosolids are an effective means of improving soil structure and nutrient content on mine reclamation sites. However, current regulatory monitoring programs may not provide sufficient background information to reliably assess the benefits and impacts of biosolids applications, particularly with regard to water quality and the physical and biological properties of reclamation soils.

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ALKALINE INDUSTRIAL BY-PRODUCTS AS MINE WASTE AMENDMENTS*

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ABSTRACT

A great amount of neutralizing chemicals are necessary to remediate acidic mine wastes in the Butte and upper Clark Fork River Basin, Montana. A potentially important alternative to commercial-grade lime used to treat acidic/metalliferous mine tailings exists in recycling industrial by-products such as kiln dust from the production of cement and lime. A greenhouse experiment was conducted to test the efficacy of industrial by-product lime materials vs. commercial liming products as alkaline amendments for acidic/metalliferous tailings from the Butte-Anaconda site. Industrial by-products tested were lime kiln dust from the Continental Lime facility near Townsend, MT and Di-Cal, a product resulting from the production of magnesium from dolomite, from Northwest Alloys in Addy, Washington. A completely random experimental design was instituted with four replications of each treatment. The substrate used was tailings from the Opportunity impoundment in Anaconda, Montana. Treatments tested were: greenhouse potting soil control, unamended tailings; tailings amended with commercial grade calcium carbonate and calcium oxide; tailings amended with Townsend lime kiln dust; and, tailings amended with Di-Cal. Two plant species were planted, *Thinopyrum intermedium* (Intermediate wheatgrass) and *Elymus trachycaulus* (Slender wheatgrass). All treatments received an equal fertilizer and water application.

Results indicate that Di-Cal, although an excellent neutralizing amendment, has unknown chemical constituents that significantly reduce plant growth. Based on these data, tailings treated with the industrial by-product Townsend lime kiln dust produced plant growth comparable to that attained with commercial lime.

However, in the Montana Limestone commercial lime amended tailings, some of the CaO had not been sufficiently carbonated to $CaCO_3$ indicated by a root zone pH of 9 during the plant growth period. Therefore, the lower nutrient availability and potential caustic effects on plant roots may have emanated in a lowering of the plant growth characteristics of the commercial lime treated tailings. So if the pH had been <8.5 in the Montana Limestone commercial lime, the plant productivity may have been greater.

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INTRODUCTION

Throughout the United States, certain agricultural or mining practices have left large areas of land too acidic to support normal plant growth. The standard method for treating these acid soils relies on commercial-grade lime, which is very expensive, often prohibitively so in the Northern Plains and other areas of the United States. This experiment evaluated an alternative approach one that is both lower cost and environmentally attractive. The new method recycles alkaline industrial by-products that are presently disposed of in landfills, using these inexpensive by-products to chemically treat soil acidity.

Because there is little or no market for these alkaline by-products, which are now simply placed in landfills, they can be acquired at little or no cost. An added benefit is that utilization of these by-products results in removal of a potential industrial waste stream. This potentially valuable resource is being lost to landfills. The experiment outlined here investigated the use of alkaline industrial by-products to treat soil acidity in waste materials resulting from mining and evaluated this methods efficacy for facilitating good plant growth.

The Butte-Anaconda area of Montana has vast tailings deposits in the landscape that have elevated metal concentrations (Cu, Zn, Pb, Cd) and are highly acidic, with pH values typically ranging from 2.4 to 4.5. In the soil solution, these metals are soluble at pH levels lower than 5.5, a result that is phytotoxic to plant growth, and the tailing impoundments are devoid of vegetation. In Anaconda, Montana, alone, two tailing impoundments cover 3,800 acres of land.

The technology at present is to treat the acidity and heavy metal contamination with commercial-grade lime, such as calcium carbonate ($CaCO_3$), calcium oxide (CaO), or calcium hydroxide (CaOH). Greenhouse experiments and field tests have demonstrated that using these amendments will raise the soil pH level to a suitable range (6.5-8.5), which has enabled good plant growth. Raising the soils pH level precipitated the heavy metals and removed these contaminants from the soil solution, thereby removing the phytotoxic or inhibitory effects on plant growth.

The problem with this method is that treating such vast areas of land requires large amounts of expensive commercial-grade lime. Added to this tremendous cost are the costs of amendment incorporation, labor, seed, fertilizer, mulch application, etc. needed for successful re-vegetation. Utilization of alkaline industrial by-products to treat acid materials has potential to notably decrease land reclamation costs. However, these by-products may contain additional contaminants arising from 1) the ore body used, 2) from the industrial process of re-circulating the product through a kiln (which concentrates undesirable compounds), or 3) from the type of fuel used to fire the kiln. Preliminary research shows that some by-products can be enriched in heavy metals and in certain cases are at higher concentration levels than those at the sites to be treated (Dollhopf 1996ab). Therefore, plant growth must be tested.

MATERIALS AND METHODS

A bulk composite waste sample (661 liters) was collected from the Opportunity tailings impoundment (Pond D) located adjacent to Anaconda, MT. A specific pit location within Pond D has been used in past greenhouse investigations and the physicochemical characteristics of this material were known. These tailings are acidic (pH 2.2) and had an electrical conductivity of 16.9 dS/m. The total concentration of zinc was 470 ppm and copper was 410 pmm. The tailings had enriched levels of other metals and arsenic as well.

Approximately 23 kg of calcium carbonate (≤ 60 mesh) and calcium oxide (≤60 mesh) were acquired from Montana Limestone. A 44-liter bulk sample of lime kiln dust (LKD) was acquired from Continental Limestone, Townsend, MT. A 44-liter bulk sample of Di-Cal (a by-product resulting from the production of magnesium from dolomite) was acquired from Northwest Alloys in Addy, Washington.

The total lime requirement of the Opportunity tailings was calculated to be 17.4 g $CaCO_3$/200 g waste using an assessment of the potential acidity with acid-base accounting, and active acidity using the SMP test (Sobek et al. 1978 and McLean 1982). The lime requirement for the commercial limestone treatment was met using a 60% $CaCO_3$/40% CaO mixture. The physiochemical characteristics of the lime materials used are listed in Table 1:

Table 1. Physiochemical characteristics of the substrate treatments.

	Calcium		Gravimetric
Montana Limestone $CaCO_3$	94%	100	0
Montana Limestone CaO	105%	100	0
Townsend lime kiln dust	89.5%	100	0
Di-Cal	85.5%	100	0

Mixed substrates of tailings and lime materials that exhibited a saturated paste extract of >8.5 s.u. were purged with CO_2 gas and dry ice to transform oxides and hydroxides of calcium into carbonates, which resulted in a soil pH of < 8.5. This was verified with a saturated paste extract pH measurement prior to seeding. The substrate pH values before seeding and at the time the plants were harvested are listed in Table 2.

Table 2. Substrate treatment pH values before seeding and at the time the plants were harvested.

Substrate Treatments	pH (s.u.) prior to	pH (s.u.) post-
Unamended Opportunity tailings	2.19	3.57
Montana Limestone $CaCO_3$ and CaO	7.58	**9.0**
Townsend lime kiln dust	7.49	8.03
Di-Cal	7.65	7.64

These greenhouse growth trials were conducted in the Montana State University Plant

Growth Center. A completely random experimental design was instituted with four replications of each treatment. The study used two plant growth substrates, the greenhouse potting soil (optimal control) and Opportunity tailings. Each of the replications for all the controls and treatments received a fertilizer application of 377.0 kg/ha-Nitrogen, 280.3 kg/ha-phosphorous, and 103.7kg-ha potassium.

Two plant species were used as indicators of the effectiveness of the treatments in amending acidic-metalliferous waste: *Thinopyrum intermedium* (Intermediate wheatgrass, var. Tegmar) and *Elymus trachycaulus* (Slender wheatgrass, var. Pryor).

Each plant growth container consisted of a PVC pipe, 10.16 cm in diameter and 61 cm long. Substrates identified in Table 3 were prepared, and then used to fill each cylinder. Ten seeds per cylinder were planted 1.27 cm deep. Each cylinder was watered daily. After germination and emergence, plants were culled to five per cylinder. The growth period was 90 days. Greenhouse conditions were 14 hours of light per day with a daytime temperature of 21^{o} C and a night-time temperature of 18 ^{o} C.

Table 3. Plant growth study treatments. Two plant species (Intermediate wheatgrass and Slender wheatgrass) were evaluated in 5 different substrate treatments and the experiment was replicated four times. This effort resulted in 40 growth cylinders.

Substrate	Treatment
Greenhouse potting soil	Control with NPK fertilizer
Opportunity tailings	NPK fertilizer
Opportunity tailings	Montana Limestone $CaCO_3$ and CaO with NPK fertilizer
Opportunity tailings	Townsend lime kiln dust with NPK fertilizer
Opportunity tailings	Di-Cal and NPK fertilizer

The following plant growth response variables were measured in each cylinder:

1. Above ground production - The above ground biomass of all plants after drying at 50 ^{o} C for 24 hours.
2. Height - Distance from the ground to the end of the longest leaf for each plant measured.
3. Maximum root length - The maximum root length in each growth cylinder measured in centimeters (cm).
4. Root distribution - The number of roots at 5 cm, 10cm, 20cm, and 30cm.
5. Vigor - Plant vigor characterized by a numerical value of 4 = robust; 3 = good; 2 = acceptable; 1 = poor; and 0 = dead. The greenhouse control provided a gauge for robust growth.

Data from plant growth response variables were applied to analysis of variance techniques and mean separation tests to ascertain whether significant differences are present at the 95% level of confidence (p = 0.05). Significant differences at were separated using the Student-Newman-Keuls method of pair-wise multiple comparison for equal size data sets. These analyses were made using SigmaStat (Jandel 1995).

GROWTH OF INTERMEDIATE WHEATGASS IN AMENDED TAILINGS

In the greenhouse potting soil, 82.5% of the Intermediate wheatgrass seeds germinated (Table 4). Conversely, no seeds germinated in the unamended tailings. In the Montana Limestone commercial lime, 70% of the seeds germinated and 57.5% and 60% of the seeds germinated in the Townsend lime kiln dust and Di-Cal respectively. There were no statistical differences between the lime treatments.

Above ground production of the Intermediate wheatgrass in the greenhouse potting soil averaged 9.3 grams and zero in the unamended tailing. Production in the tailings amended with Montana Limestone commercial lime was 1.9 grams (Table 4). The treatment amended with Townsend lime kiln dust produced 1.2 grams of above-ground biomass and the Di-Cal amended treatment produced 0.12 grams of plant material. In the amended tailings, the production was significantly less than the greenhouse potting soil. There was no significant difference between the tailings amended with commercial lime from Montana Limestone and the industrial by-product Townsend lime kiln dust. The Di-Cal amended tailings had significantly less production than the other lime materials.

Plant height in the Intermediate wheatgrass averaged 40.8 cm in the greenhouse potting soil, 38.3 cm in the tailings amended with Montana Limestone commercial lime, 38.3 cm in the Townsend lime kiln dust amended tailings, and 18.2 cm in the tailings amended with Di-Cal (Table 4). The unamended tailings had no plant growth. There were no statistical differences between the greenhouse potting soil, Montana limestone commercial lime, and the Townsend lime kiln dust industrial by-product. The Di-Cal amended tailings had significantly less plant height.

At the 5 cm and 10 cm soil depth, the Intermediate wheatgrass grown in greenhouse potting soil had significantly more fine roots (< 1mm diameter) than those grown in the Montana Limestone commercial lime (Table 4). The plants grown in the Townsend lime kiln dust amended tailings had significantly more fine roots than the plants grown in tailings treated with Di-Cal. There were no statistical differences in the number of coarse roots (1-2 mm and >2mm diameters) between any of the treatments. At the 20 cm soil depth, there were no statistical differences between the number of fine roots in the greenhouse potting soil plants and the plants grown in tailings amended with Montana limestone commercial lime and Townsend lime kiln dust. The plants grown in Di-Cal amended tailings had significantly less fine roots. At the 30 cm soil depth, there were no statistical differences between the number of fine roots in the plants grown in Montana Limestone commercial lime and those grown in Townsend lime kiln dust amended tailings, and significantly less fine roots in the greenhouse potting soil, Di-Cal, and unamended tailings plants. For the number of coarse roots at the 30 cm depth, there were no statistical differences between any of the treatments.

The greenhouse potting enabled significantly better plant vigor in the Intermediate wheatgrass compared to other treatments. The Montana Limestone commercial lime and the Townsend lime kiln dust amended tailings were significantly better than the tailings amended with Di-Cal (Table 4).

GROWTH OF SLENDER WHEATGRASS IN AMENDED TAILINGS

In the greenhouse potting soil, 65% of the Slender wheat grass seeds germinated (Table 5). Conversely, no seeds germinated in the unamended tailings. In the Montana Limestone commercial lime treatment, 65% of the seeds germinated. The Townsend lime kiln dust treatment had 75% seed germination. In the Di-Cal treatment, 67.5% of the seeds germinated. There were no statistical difference between germination in the greenhouse potting soil and all lime treatments.

Above-ground production of the Slender wheat grass in the greenhouse potting soil averaged 7.9 grams and zero in the unamended tailings. Production in the tailings amended with Montana Limestone was 0.41 grams (Table 5). The Townsend lime kiln dust amended tailings produced 1.45 grams of above-ground biomass and the Di-Cal treatment produced 0.06 grams of plant material. In the amended tailings, the production was significantly less than the greenhouse potting soil. There was no significant difference between the tailings amended with commercial lime from Montana Limestone and the industrial by-product Townsend lime kiln dust. The Di-Cal amended tailings had significantly less production than the other lime materials.

Plant height in the Slender wheat grass averaged 46.5 cm in the greenhouse potting soil, 26.3 cm in the tailings amended with Montana Limestone commercial lime, 46.4 cm in the Townsend lime kiln dust amended tailings, and 11.1 cm in the tailings amended with Di-Cal (Table 5). The unamended tailings had no plant growth. There were no statistical differences between the height of plants grown in greenhouse potting soil and Townsend lime kiln dust amended tailings. The Montana Limestone commercial lime had significantly less plant height than the Townsend lime kiln dust and greenhouse potting soil treatments, and the Di-Cal had significantly less than the commercial lime.

At the 5 cm and 10 cm soil depth, the Slender wheat grass grown in greenhouse potting soil had significantly more fine roots (< 1mm diameter) than those grown in all other treatments (Table 5). The plants grown in Montana Limestone commercial lime and Townsend lime kiln dust amended tailings had significantly more fine roots than the plants grown in tailings treated with Di-Cal. The unamended tailings had no plant growth and there was no significant difference between the number of plant roots in the unamended tailings and those amended with Di-Cal. There were no statistical differences in the number of coarse roots (1-2 mm and >2mm diameters) at the 5 cm and 10 cm soil depth in any of the treatments. At the 20 cm and 30 cm soil depth, the Slender wheat grass plants grown in greenhouse potting soil had significantly more fine roots. The plants grown in tailings amended with Townsend lime kiln dust had significantly more fine roots than those grown in the Montana Limestone commercial lime and Di-Cal amended tailing did. There were no statistical differences between the number of fine roots in the plants grown in the Montana Limestone commercial lime, Di-Cal and the unamended tailings. The number of coarse roots (both 1-2 mm and >2mm diameters) at the 20 cm soil depth showed no significant differences in any of the treatments.

The greenhouse potting enabled significantly better plant vigor compared to other treatments. The Montana Limestone commercial lime and the Townsend lime kiln dust amended tailings produced plant vigor that was significantly better than the vigor of the Slender wheatgrass grown in tailing amended with Di-Cal (Table 5).

CONCLUSION

In the Montana Limestone commercial lime amended tailings, some of the CaO had not been sufficiently carbonated to $CaCO_3$ as indicated by a root zone pH of 9 during the plant growth period. Therefore, the lower nutrient availability and potential caustic effects on plant roots may have emanated in a lowering of the plant growth characteristics of the commercial lime. So if the pH had been <8.5 in the Montana Limestone commercial lime, the plant productivity may have been greater.

The results of this study indicate that Di-Cal, although an excellent neutralizing amendment, has unknown chemical constituents that significantly reduced plant growth. Based on these data, it appears that the industrial by-product Townsend lime kiln dust produced plant growth comparable to that obtained with commercial lime.

These results were obtained with optimal plant growth conditions such as daily watering, fertilizer, and optimal lighting regime to facilitate maximum plant growth.

Table 4. Mean plant growth response variables for **Intermediate wheat grass** grown in greenhouse potting soil, unamended Opportunity tailings, and amended Opportunity tailings, Anaconda Montana (n=4). All treatments had nitrogen, phosphorus and potassium fertilizer added.

	TREATMENT AMENDMENTS				
Response Variable	**Greenhouse Potting Soil**	**Unamended Opportunity tailings**	**Opportunity tailings amended with Montana Limestone $CaCO_3$ and CaO**	**Opportunity tailings amended with Townsend Lime Kiln Dust**	**Opportunity tailings amended with Di-Cal**
Germination (percent out of 10 seeds planted)	82.5a[1]	0c	70b	57.5b	60b
Above ground production (g)	9.303a	0c	1.907b	1.185b	0.123c
Plant Height (cm)	40.775a	0c	38.305a	38.324a	18.162b
Maximum root depth (cm) up to 32.5 cm	30.75a[2]	0b	32.5a	32.5a	28c
Root distribution at 5 cm depth (<1mm diameter)	193.75a	0c	112.5b	85.0b	18.75c
Root distribution at 5 cm depth (1-2 mm diameter)	16a	0a	4.75a	8a	0.75a
Root distribution at 5 cm depth (>2 mm diameter)	0a	0a	0.25a	0a	0.5a
Root distribution at 10 cm depth (<1mm diameter)	150a	0c	82.5b	71.75b	8.75c
Root distribution at 10 cm depth (1-2 mm diameter)	3a	0a	1.5a	5.25a	0.25a
Root distribution at 10 cm depth (>2 mm diameter)	0a	0a	0.25a	0a	0.5a
Root distribution at 20 cm depth (<1 mm diameter)	57.5a	0b	58a	80a	4b
Root distribution at 20 cm depth (1-2 mm diameter)	0.25a	0a	0.5a	2.25a	0.25a
Root distribution at 20 cm depth (>2 mm diameter)	0a	0a	0.25a	0a	0.5a
Root distribution at 30 cm depth (<1mm diameter)	6.25b	0b	44.5a	68.7a	1.25b
Root distribution at 30 cm depth (1-2 mm diameter)	0a	0a	0a	1a	0a
Root distribution at 30 cm depth (>2 mm diameter)	0a	0a	0.25a	0a	0a
Plant Vigor 0 = dead 1 = poor 2 = acceptable 3 = good 4 = robust	4a	0d	2.75b	2.75b	1.25c

[1] Means followed by the same letter in the same row are not significantly different.
[2] Numbers reported in this row are medians and not means.

Table 5. Mean plant growth response variables for **Slender wheatgrass** grown in greenhouse potting soil, unamended Opportunity tailings, and amended Opportunity tailings, Anaconda Montana (n=4). All treatments had nitrogen, phosphorus and potassium fertilizer added.

	TREATMENT AMENDMENTS				
Response Variable	**(1) Greenhouse Potting Soil**	**(2) Unamended Opportunity tailings**	**(3) Opportunity tailings amended with commercial $CaCO_3$ and CaO**	**(4) Opportunity tailings amended with Townsend Lime Kiln Dust**	**(5) Opportunity tailings amended with Di-Cal**
Germination (percent out of 10 seeds planted)	65a	0.25b	65a	75a	67.5a
Above ground production (g)	7.9a[1]	0c	0.407d	1.447d	0.06c
Plant Height (cm)	46.485a	0d	26.265b	46.360a	11.060c
Maximum root depth (cm) up to 32.5 cm	33.75a	0b	32.5a	33.125a	22.625c
Root distribution at 5 cm depth (<1mm diameter)	212.5a	0c	71.25b	54.5b	19.5c
Root distribution at 5 cm depth (1-2 mm diameter)	5.5a	0a	3a	13.25a	0a
Root distribution at 5 cm depth (>2 mm diameter)	1.0a	0a	0a	0a	5.75a
Root distribution at 10 cm depth (<1mm diameter)	212.5a	0c	28.75b	52.75b	8.5c
Root distribution at 10 cm depth (1-2 mm diameter)	3.75a	0a	0.5a	5.0a	0a
Root distribution at 10 cm depth (>2 mm diameter)	1a	0a	0a	0a	2a
Root distribution at 20 cm depth (<1 mm diameter)	200a	0c	7.25c	46b	0.5c
Root distribution at 20 cm depth (1-2 mm diameter)	3.5a	0a	0.25a	0a	0a
Root distribution at 20 cm depth (>2 mm diameter)	1.0a	0a	0a	0a	0a
Root distribution at 30 cm depth (<1mm diameter)	200a	0c	4c	36.25b	0.5c
Root distribution at 30 cm depth (1-2 mm diameter)	3.25a	0a	0.25a	0a	0a
Root distribution at 30 cm depth (>2 mm diameter)	1a	0a	0a	0a	0a
Plant Vigor 0 = dead 1 = poor 2 = acceptable 3 = good 4 = robust	4a[2]	0d	2b	3b	1c

[1] Means followed by the same letter in the same row are not significantly different 0.

[2] Numbers reported in this row are medians and not means.

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