Applied and Environmental Soil Science

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Guest Editors: Giuseppe Corti, David C. Weindorf, Maria J. Fernández Sanjurjo, and Horea Cacovean

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Editorial Board

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Editorial

Use of Waste Materials to Improve Soil Fertility and Increase Crop Quality and Quantity

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The special issue received 21 papers from many countries. Of those, after a peer review, 13 were considered valuable for publication. Among the published papers, the use of broiler litter, stubble, mustard and flax meal cake, tannery land plaster, swine manure, and biosolids in cultivated soils was presented. Also, the use of compost to improve quality of urban soils, as well as inoculation of earthworms and application of biosolids and organic and inorganic residue to recover mine tails was included.

Specifically, S. L. Dillard et al. studied productivity and nutritive quality of Johnsongrass (*Sorghum halepense*) for forage after the soil had been treated with broiler litter and a commercial fertilizer made of ammonium nitrate and diammonium phosphate. Results indicated that broiler litter supported productivity and nutritive quality of the forage comparably to those obtained with commercial fertilizer when a supplement of P and N was applied.

G. B. Huang et al. assessed the role of stubble removal or retention and of soil management (no tillage and tillage consisting of 3 ploughing and 2 harrowing per year) on soil fertility in a loessic dryland cropping system dominated by alkaline soils mostly cultivated by wheat (*Triticum aestivum* L.) and pea (*Pisum sativum* L.). Other things being equal, after eight years of experimentation, the retention of stubble was able to improve the content of organic matter and the soil nutrient status and increase the grain yields too.

T. P. McGonigle et al. reported on the use of textile manufacturing wastewater sludge in greenhouse experiments using a silt loam soil, a *gleyed melanic Brunisol* according to the Canadian system of Soil Classification. The major focus of this paper is that sludge, even though not fragmented, may rapidly provide N in inorganic forms. Because of this, the use of sludge may substitute part of the N fertilizers needed for cereal cultivation.

Substituting fossil fuel cultivations generated the problem of eliminating meals and cakes derived from extraction of oil from seeds. A. S. Wang et al. considered the impact of biofuel oilseed meal application on some soil properties of a *fine-silty, mixed, superactive, thermic Udifluventic Haplustept* (USDA). The use of mustard and flax cakes may help in reducing fertilizer requirements, but there are also some risks derived from their use that are illustrated in the paper.

C. Giacometti et al. studied an animal-derived material, a by-product of leather shaving (TLP). Together with nutritive elements, this matter has a certain content of Cr(III), which may negatively impact soil biochemical properties. Using samples from a *Typic Udifluvent* (USDA), mesocosms were prepared with different amounts of TLP and incubated. Results indicated that TLP is a good source of available N and that extractable Cr(III) is rapidly reduced because of precipitation, but the effect of repeated applications must be further tested.

In the paper of S. Amini et al., the effect of paper-mill sludge management on soil available nutrients and wheat grain production was tested. The authors worked on a loess soil from North-eastern Iran and evaluated the effect of the sludge used as mulching or incorporated into the soil by mechanical works. Results indicated that sludge mulching was able to improve physicochemical soil properties better than incorporation, and also yield production improved.

S. King et al. studied the very cogent matter of recycling swine manure. In this case the manure was submitted to an anaerobic digestion method known as ISPAD. The authors tested the validity of the ISPAD procedure in reducing ammonia volatilization *versus* conventional (untreated or stored in open tank) swine manure treatment when they are applied to soil. Manures were mixed to five experimental soil substrates and maintained into wind tunnels. Results indicated that the ISPAD procedure was able to reduce ammonia volatilization when manure is applied to soil and that plant available N is higher with respect to manure when conventionally treated.

A. A. Farooque et al. presented another particular treatment (the N-Viro) applied on sewage sludge disposal to produce biosolids used as fertilizers. In this paper the effect of these biosolids, together with irrigation, was evaluated on the availability of soil nutrients, plant nutrient uptake, and production of *Vaccinium angustifolium* growing on an *Orthic HumoFerric Podzol*. The results show that biosolids applied were comparable to the inorganic fertilizers used in the experiments.

R. E. Zartman et al. studied the effect of biosolids from waste-water treatment facilities on physical properties of *fine, mixed, superactive, thermic Ustic Calciargids* and *loamy-skeletal, mixed, superactive thermic Ustic Haplocalcids* submitted to artificial rainfall in order to improve vegetal cover and reduce erosion. The results obtained inform us that application of biosolids have achieved the goals and promoted the biosolids as environmental care product even for arid lands.

R. Pini et al. considered improving physical properties of poor quality urban soils with the application of compost derived from source-separated municipal solid waste so to increase the probability of success in establishing flowers. The soils used were those of traffic island, which were mainly made of reconstructed soil, and the plants used were wildflowers. After seven months from the compost application, the soils displayed improved physical conditions and also demonstrated a higher production of flowers for a longer time.

The paper of M. Rutheford and J. M. Arocena is one of the papers presented on the recovery of mine tailings. The aim was pursued working with materials from a Canadian non-acid mine tailing, which was amended with pulp sludge or pine sawdust plus biosolids, both enriched with earthworms of the species *Dendrobaena veneta*, an annelid. Results indicated that the addition of organics together with earthworms improved physicochemical properties of the mine tailing material that may favour plant establishment.

Also the paper of T. P. McGeehan concerns recovering mine tailings from Idaho (USA) by using seven waste-based and organic soil amendments in order to investigate the impact on soil-plant relationships. All the materials used improved soil fertility and were successful in establishing plant cover, even though biosolid products and waste log plus urea gave the best results.

Finally, the paper of C. Santibañez et al. is the third one to face the problem of recovering mine tailings. The work was made on tailings from a Chilean Cu mine. Several types of organic and mine waste materials were added to assess their efficacy on phytostabilization of the tailings. Results indicated that both hard-rock mine wastes and organic materials are adequate for improving chemical and biological properties of tailings, but excess of organic distribution must be avoided to prevent Cu mobilization.

All the results shown by the papers published in this issue are valuable for many viewpoints, including for law makers of any country, who may take the opportunity of this open issue to improve the environment and the economy of some farms where the materials here studied are produced or may be used.

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Research Article

Compost and Wildflowers for the Management of Urban Derelict Soils

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The aim of this study was to verify whether the use of source-separated municipal waste compost could improve the physical quality of urban soils and create better conditions for their management when planted with herbaceous species. A sandy soil in traffic islands was tilled to a depth of 10 cm, and half of the surface was treated with compost (3 kg/m^2). A mixture of 25 herbaceous annuals was then sown in the entire area. Organic carbon content and physical characteristics were determined at different times in the soil treated and not treated with compost. The vegetation was monitored in terms of its growth and flowering. The compost-treated soil showed an increase in organic carbon content. Total porosity increased with time in the compost-treated soil, due to a higher volume of transmission pores, which play a role in water movement. Soil aggregate stability also improved in the compost-treated soil. The duration of flowering of the individual species and the overall quantity of flowers were greater in the compost-treated soil.

1. Introduction

Soil is one of the limiting factors for the successful cultivation of plants in towns and cities [1]. The poor quality of soils in urban areas is due to their chemical and biological properties but more often it is due to physical properties. Often urban soils are lacking in structure and humus, leading to asphyctic conditions, compaction, and erosion. These poor physical properties reduce the water available for plants and the penetrability of soils by the roots. Moreover, urban soils can be contaminated by several anthropogenic materials (bricks, concrete, and plastic), which can greatly reduce the volume available for plant roots. The use of source-separated municipal solid waste compost could provide a good solution for increasing the organic matter content in urban soils and for improving their physical properties. In Italy, the production of such compost is rapidly increasing due to the efforts of municipalities to achieve a better selection of organic solid waste and to improve composting processes. So using this compost in vast and heterogeneous sets of urban soils could also help to create a high-value market for locally produced compost, as pointed out by Cogger [2]. While compost effects on agricultural soils have been widely studied, less information is available on urban soils, though interest and research are increasing [3]. In particular one possible effective use of quality compost is in urban derelict soils. These kind of soils, found in roadsides, roundabouts, and filling soils, are generally highly compacted in subsoils, particularly poor in organic matter and structure, and are polluted by traffic [4]. Thus, they are often unsuitable for growing any ornamental vegetation. These soils could benefit from being treated with compost, thus improving their structure and water-holding capacity and making them suitable for growing some ornamental vegetation [5]. They are planted generally with grasses selected for rapid growth and effectiveness in terms of erosion control [6]. These species provide a monotonous landscape and require a great deal of water plus frequent cutting, which involve high costs in terms of maintenance. However, many herbaceous plants, native to the Mediterranean climate and which tolerate drought conditions, have the potential to be used for low-maintenance landscaping [7]. Over the last few decades, interest in using flowering plants (wildflowers) for landscape purposes has increased [8]. In fact, these plants enable derelict soils to vegetate, and when sown in mixture they enhance wildlife and look colorful and attractive. The

Table 1: Compost characteristics and corresponding values requested by Italian law.

	compost	Italian law
Water content %	21.6	<50
pH	7.15	6.0–8.5
Organic C%	30.1	>25
Total N%	1.68	—
Organic N% of tot N	88.1	—
C/N	17.9	<25

establishment of a wildflower meadow leads to a reduction in management costs due to the absence of fertilization and irrigation and to the low level of other management practice [9].

The aim of this study was to see whether the use of source-separated municipal solid waste compost could improve the physical quality of an urban derelict soil and create better conditions for its management when planted with herbaceous species.

2. Materials and Methods

2.1. Study Site. The study was carried out during a growing season (November to June) in Livorno, a Tuscan town on the Mediterranean coast, where the maximum and the minimum temperature recorded in the experimental period were 33.0°C and 1.9°C, the coldest month being February (mean temp. 12.4°C) and the warmest month being June (mean temperature 22.6°C), with an average relative humidity of 74.7% and a total precipitation of 367 L/m^2.

The field site was a group of three traffic islands of different shape measuring, respectively, 160 m^2 (island A), 120 m^2 (island B), and 280 m^2 (island C). They were very close to each other and presented homogeneous soil properties [10]. The soil contained 13% clay, 10% silt, and 77% sand. The pH was 8.4 and the CEC was 19 cmol kg^{-1}. The organic carbon content was 1.2% and the total nitrogen was 0.11%. The soil was structurally poor, dense and difficult to penetrate for air, water, and roots [10]. A scarce and weak weed vegetation was present.

Management of the Study Site. In November, the traffic islands were glyphosate weeded. At the end of the same month, they were tilled to a depth of 10 cm, then in half of the area, ("Compost Treated soil", CT hereafter), a source-separated municipal solid waste compost was incorporated into the soil at the rate of 3 kg/m^2, usually indicated for herbaceous and horticultural plants. The other half was left untreated ("No Compost Treated soil", NCT hereafter). The compost derived from a windrow-composting process with forced aeration, where temperature was higher than 60°C. The compost complied with the Italian law n. 217/2006, also in terms of absence of pathogens and low heavy metal content, for its safe use as fertilizer. Some selected characteristics of the compost are showed in Table 1. A mixture of 25 herbaceous annuals was sown by hand broadcasting in the whole area at the rate of 5 g/m^2. The species were native and exotic, each of them flowering in a different period from early spring to late summer. The percentage of each species in the mixture was determined after a preliminary investigation on the germination capacity, the field assessment, and the morphological characteristics. The seeding was not irrigated and no irrigation was performed during the experimental period.

2.2. Analytical Determinations. Six soil composite samples (three subsamples) per treatment were taken in the 0–10 cm layer in December, February, May, and July. C content was determined with dry combustion by using a Leco CHN Analyzer. Bulk density was determined using the core method [11]. Wet sieving was used to determine the stability of soil aggregate in water. Air-dried aggregates (1-2 mm) of soil were placed in 0.25 mm mesh sieves and moistened by the water rising by capillarity from a layer of wet sand, then immersed in deionized water and shaken at the rate of 60 rpm. A Water Stability Index (WSI) has been defined as: WSI $= 100(1 - A/B)$, where A and B are the weights of aggregates passing through the sieve after 5 and 60 minutes, respectively [12]. Measurements of porosity and pore size distribution in the range 0.002–160 μm were made with mercury intrusion porosimetry on undisturbed aggregates of about 2 g [13]. The volume of pores, measured as mercury intruded, was determined by means of the Washburn equation $p = -4\gamma\cos\theta/d$, where p is the pressure required to force the mercury into the pores of diameter d, γ is the surface tension of the mercury (480 dyne/cm), and θ is the contact angle between the mercury and the soil surface (140°).

Every week, starting from the beginning of March, the quadrat method was used to detect and quantify the flowering of the species that had been sown. The number of flowering plants of each species was recorded in ten 1 m^2 quadrats for each treatment.

2.3. Statistical Analysis. All results are the means of six measurements. Analysis of variance was performed to test the effect of time and treatments on the parameters investigated. The means were compared by using least significant differences calculated at $P < 0.05$ (Newman-Keul's test).

3. Results and Discussion

3.1. Soil Characteristics. Levels and variations of organic C content in the two different areas and at different times are shown in Table 2. The two treatments had the same trend. Organic C content decreased in February, when the biological activities of the soil-plant system reached a minimum and did not produce new fresh organic matter, so that the catabolic processes affecting the organic matter became predominant. The organic C content raised in May, when the biological activities in the soil started again, due to the more favourable climatic conditions, and newly formed organic matter was present. In this picture, organic C content of CT was significantly greater than NCT on all sampling dates. In December, this was clearly due to the

Table 2: Values of organic carbon content, bulk density, and water stability index (WSI) in no compost treated (NCT) and compost-treated (CT) soil at different sampling dates.

	DEC	FEB	MAY	JUL
Organic C%				
NCT	1.36 aA†	0.81 bB	1.06 abA	1.18 abA
CT	2.07 aB	1.80 aA	2.80 bB	2.37 cB
WSI				
NCT	2.9 aA	2.4 aA	3.4 aA	6.3 bA
CT	3.0 aA	3.0 aA	8.6 bB	15.1 cB
Bulk density				
NCT	1.41 aA	1.50 aA	1.44 aA	1.42 aA
CT	1.46 aA	1.48 aA	1.30 bB	1.30 bB

† For each parameter values on the same row followed by the same lowercase letter are not significantly different; values on the same column followed by the same capital letter are not significantly different.

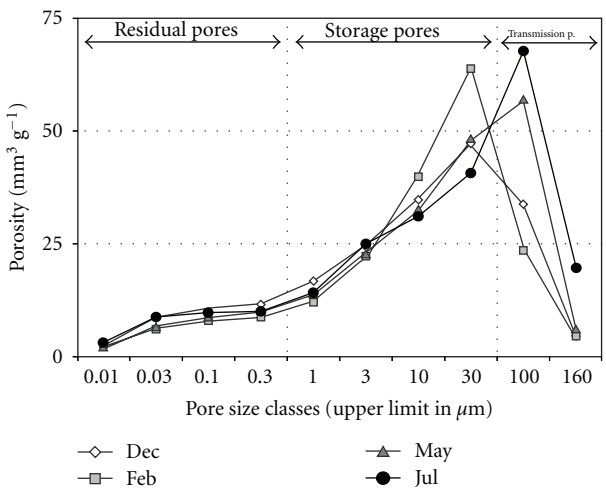

Figure 1: Pore size distribution of No compost-treated soil.

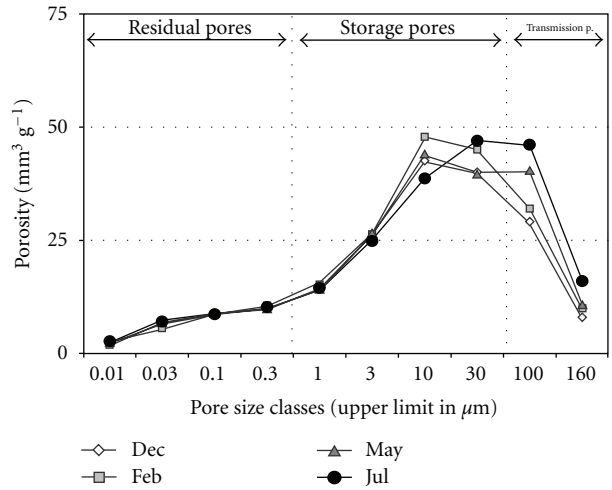

Figure 2: Pore size distribution of compost-treated soil.

Table 3: Values of transmission pores, storage pores and residual pores in no compost-treated (NCT) and compost-treated (CT) soil at different sampling dates (expressed as mm^3/g).

	DEC	FEB	MAY	JUL
Transmission pores				
NCT	38 aA†	28 bA	83 cA	67 dA
CT	37 aA	42 aB	52 bB	62 cA
Storage pores				
NCT	123 abA	137 bA	117 aA	111 aA
CT	123 aA	133 aA	124 aB	125 aB
Residual pores				
NCT	32 aA	26 aA	28 aA	31 aA
CT	27 aA	27 aA	28 aA	28 aA

† For each parameter values on the same row followed by the same lowercase letter are not significantly different; values on the same column followed by the same capital letter are not significantly different.

recent addition of the compost organic matter, whilst in February, May, and July there were at least two reasons. One is that the organic matter of the quality compost was stable and remained undecomposed for longer [3]. The other is that most of the organic C of the compost was protected by the soil structure at a level similar to that of the preexisting organic C, as recently demonstrated by Sparvoli et al. [14]. The greater organic C content might have also stimulated a greater biological activity both in plants and microorganisms, unbalanced toward anabolic processes, which resulted in increased production of organic matter.

The influence of the newly incorporated organic C on the soil structure was investigated from two different points of view. (i) Water stability of aggregates, to evaluate if the added organic matter could prevent dispersion of soil particles, due to rainfall events or runoff or water action. (ii) Bulk density of the whole soil, plus porosity and pore size distribution of undisturbed aggregates, are used to evaluate the modifications of soil architecture and spatial arrangements of particles and aggregates.

Water stability is described in Table 2 by an index (WSI) that expresses the proportion of water stable aggregates greater than 250 μm. The WSI has been found to be very sensitive at measuring the effects of various organic C contents on soil structure of agricultural soils [15]. Moreover, the WSI changes during the growing season [16]. It usually reaches a maximum in late spring/early summer and then decreases to a minimum in winter. In NCT, the WSI of the soil ranged between 2.4 in February and 6.3 in July, always under the value of 10, which indicates a very bad structural stability. The seasonal variations were smaller but consistent with those observed in agricultural soils [17]. This suggests a similar, though less effective, mechanism in this urban soil, where the strong interactions between the finer mineral constituents and the organic matter were limited by the sandy texture and the low-organic C content. The WSI of CT soil was similar to NCT in December and February, but sharply increased in May, almost trebling its previous values, and especially in July, indicating a little but significant

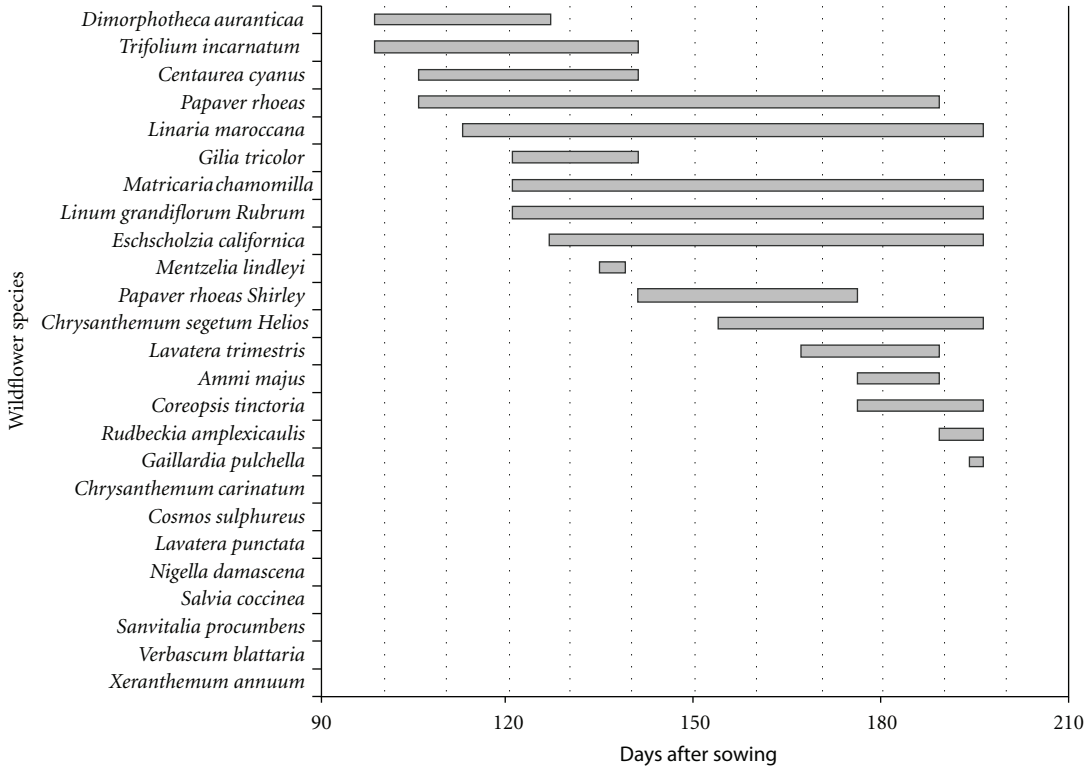

Figure 3: Flowering periods in no compost-treated soil.

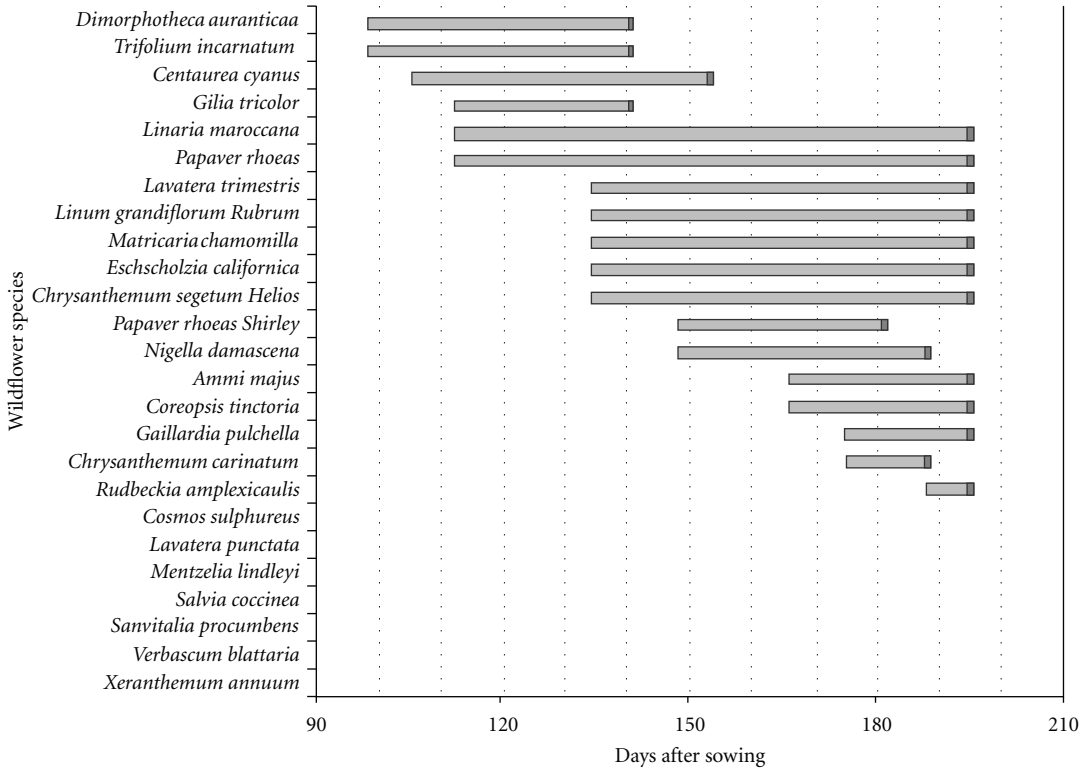

Figure 4: Flowering periods in compost-treated soil.

increase in the formation of stable aggregates, which were resistant to the impact of water.

Bulk density of NCT showed similar values at all the sampling dates (Table 2). The minimum value was in December, probably due to the recent tillage, which produced a more open and unstable structure. The trend of CT bulk density was quite different. The value in February was the same as December and significantly higher than May and July. In this Compost-treated soil, there were no evidence of a collapse in the structural arrangement induced by the tillage. On the contrary, the proportion of the void space was maintained and gradually increased. Bulk density is a simple and useful parameter for evaluating soil structure, but it is still raw. A more effective description is given by pore size distribution, especially when pore classification reflects their function in the soil. In Figures 1 and 2, we used en expanded classification, where increasing values of pore diameter correspond to decreasing values of water tension (i.e., 1 μm = pF 3.5, 3 μm = pF 3; 10 μm = pF 2.5), which is preferable because it relates to the dominant water processes. Pore size classes were also grouped according to the Greenland's [18] terminology (Table 3), which describes transmission pores (<50 μm), storage pores (0.5–50 μm), and residual pores (<0.5 μm) stressing their functional relevance. Transmission pores make air and water movement into the soil easier. Storage and residual pores are responsible for the storage of water and mineral nutrients, respectively.

Looking at the patterns in the two Figures and values in Table 3, two main considerations can be drawn. Firstly, NCT soil showed a larger variability among values of different sampling dates in every size class. There were clear maximums for each distribution curve and they were in different classes. On the other hand, in CT soil, the variability among the three sampling dates was much more narrow, higher values were distributed in more classes, and the maximum peak is in the same class (3–10 μm). Secondly, storage pores remained quite constant and transmission pores increased constantly with time in CT, whilst in NCT the sharp increase of transmission pores was coupled with declining storage pores.

From a general examination of the overall pore size distribution data, it seems that compost-treated soil has acquired a more stable architecture of the void spaces, with a more balanced distribution in the different size classes, with a tendency to increase pores that retain water or let it flow in the bulk soil.

3.2. Plant Growth. The wildflower mixture used in this work has been chosen for practical and aesthetical reasons. It should ensure an early and lasting cover of the seeded area, grow to a height that does not disturb drivers, and offer a pleasant array of flowers and colours. Thus, the duration and the quantity of flowering are the main parameters discussed here. The duration of flowering of the single species until June is shown in Figures 3 and 4. Although the quality of the floristic composition was the same in the two areas, they differed in terms of flowering in three of the species: *Mendzelia lindley* was present only in NCT, *Nigella damascena,* and *Chrisanthemum carinatum* flowered only in CT. As a total number, there were 18 flowered species in CT, while there were 17 in NCT. The mean duration of flowering of the single species was 39 days in NCT and 45 days in CT. Only five species had a longer blooming in the NCT soil. Among these, *Papaver rhoeas* and *Matricaria Camomilla* often behave like ruderals, dominating the planting and so decreasing the growth of the others, which reduces the ornamental effect. In this case, in fact, the quantitative data of each species (not shown) confirmed that these two species had a high blooming peak. Moreover, when they decreased, the other species were not able to develop satisfactorily. It can be argued that the beneficial effects of the compost treatment contrasted and reduced the ruderal behaviour of some species. In Figure 5, the overall quantity of flowering is presented. Data are expressed as a percentage of the maximum flowering, which had been recorded in NCT soil on April 20. The duration of the flowering was the same in the two areas, but NCT showed a very high peak and then a rapid decrease, whilst CT showed a higher quantity of flowers for a longer period. To conclude, the planting in the CT soil was richer in terms of individuals and longer in time, compared with the NCT soil.

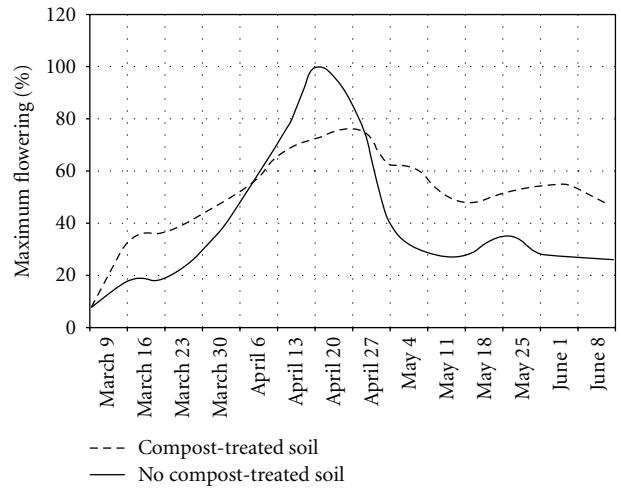

Figure 5: Flowering intensity expressed as percent of maximum flowering, recorded in NCT soil on April 20.

4. Conclusions

Seven month after the treatment, the compost-treated soil showed better soil physical parameters, in terms of less compaction and presence of pores that retain water or let it flow in the bulk soil. These characteristics were more constant over time, due to the increased structural stability of the aggregates. The enhanced physical quality of the soil, when treated with compost, reflected better conditions for growth and for development of the wildflowers, which produced a higher quantity of flowers for a longer time. The combined use of compost and wildflowers can be considered a good practice for the management of poor quality derelict urban soils.

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Research Article

Impact of Waste Materials and Organic Amendments on Soil Properties and Vegetative Performance

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Waste materials, and materials derived from wastes, possess many characteristics that can improve soil fertility and enhance crop performance. These materials can be particularly useful as amendments to severely degraded soils associated with mining activities. This study evaluated biosolids, composts, log yard wastes, and two organic soil treatments for improved soil fertility and vegetative performance using side-by-side comparisons. Each plot was seeded with a standardized seed mix and evaluated for a series of soil chemical and physical parameters, total vegetation response, species diversity, ecological plant response, and invasion indices. All treatments were successful at improving soil fertility and promoting a self-sustaining vegetative cover. The level of available nitrogen had a strong impact on vegetative coverage, species distribution, and extent of unseeded vegetation. For example, high nitrogen treatments promoted a grass-dominated (low forb) plant community with a low content of unseeded vegetation. In contrast, low nitrogen treatments promoted a more balanced plant community with a mixture of grass and forb species and greater susceptibility to unseeded vegetation establishment.

1. Introduction

The use of waste materials as soil amendments has received increased attention in recent years for agronomic applications as well as soil reclamation projects. Adding these materials to soils can be viewed as serving a dual purpose: (1) for disposal of solid waste from municipalities and agricultural operations and (2) as a means to improve chemical and physical soil properties which in turn promotes improved crop performance.

A variety of materials have been investigated for their suitability as soil amendments. For example, applications of composted municipal solid waste and composted crop residues were shown to increase soil fertility and improve structural stability in agricultural soils [1, 2]. Similarly, municipal biosolids have been used to improve soil chemical and physical properties in numerous studies [3–5]. Log yard fines (LYF) can increase water holding capacity and porosity [6] but have also been shown to reduce crop performance due to nitrogen immobilization [7]. For this reason, biosolids are often mixed with LYF and other wood byproducts as a means to reduce C : N and maintain nitrogen availability [8, 9]. Others have utilized agricultural limestone or wood ash in biosolids mixtures to reduce metal bioavailability [3, 10].

The chemical properties of the waste material can also have significant impacts on crop performance. For example, a high nitrogen content favors fast-growing grass species which is often desirable for reclamation and revegetation projects [11]. However, the dominance of grasses can lead to competitive exclusion of forb species [12] and can initiate a strong decline in plant species richness [10, 13]. Additional soil factors including pH, electrical conductivity, water holding capacity, and available phosphorus have also been shown to impact plant community composition [14].

Several microbial processes including substrate-induced respiration, potential ammonium oxidation, nitrogen mineralization, and enzyme activity increased in response to compost application [15, 16]. Multiple studies using a variety of substrates found no negative impacts on microbial diversity [16–18]. The potential does exist for deleterious impacts of

organic waste additions, due primarily to the presence of heavy metals [19–21] and nutrient runoff [22, 23]. Many of these concerns are addressed by careful characterization of the waste material and through adjustment of the application rate [24, 25].

The above discussion indicates that a given waste material must be evaluated for both its beneficial components and negative impacts within the context of a specific land use and crop performance objective. Given the variety of waste material types available, it was desirable to conduct a side-by-side comparison in order to evaluate each material's impact on crop performance under the same environmental and soil conditions. Furthermore, although multiple investigations have documented the impact of organic amendments in agricultural systems, far fewer studies have investigated these impacts in soil-plant systems associated with mine site reclamation. In many cases, mine spoils represent a completely destroyed soil-plant ecosystem. Restoration of these sites provides a unique opportunity to study the development of newly placed soils and associated succession of plant communities as modified by organic amendments. In the current study, seven waste-based and organic soil amendments were evaluated for their impacts on soil fertility and crop performance. The "crop" in this case was a grass-forb seed mix being evaluated for revegetation of the severely degraded site. Specific research objectives were to document (1) changes in soil properties, (2) overall vegetation success, and (3) plant community diversity as a function of organic amendment.

2. Methods

2.1. Site Description. The Silver Dollar Mine site is located west of Osburn, Idaho, USA (47°30.22′N; 115°59.39′W). The site is dominated by a waste rock pile produced during mine development and sorted from the ore during the mining process. The experimental plots were situated on a steep north-facing slope (1 : 2 slope, H : V) at an elevation of about 760 m. Average total monthly precipitation ranged from 3.8 cm in July to 11.4 cm in November, with a total annual precipitation of 96 cm. Average monthly temperatures were 0.5/−6°C (max/min) in January and 25.8/8.4°C in August.

2.2. Site Preparation/Plot Installation. The site was regraded using a Cat D5 Dozer, and nine plots (6 m × 30.5 m) were installed with a berm (1 m × 0.6 m) separating each plot. The western- and eastern-most plots were reserved for controls; the remaining plots were assigned on a random basis. Amendment types, application rates, and application methods were specified by the collaborators participating in the study (Table 1). Project collaborators included the local wastewater treatment facility (biosolids + woodash), a local lumber mill (log yard fines (LYF) + urea fertilizer), and two commercial composts (biosolids- and wood products-based). In addition, two commercial organic amendments were investigated. Kiwi Power is designed to reestablish mycorrhizal fungi, soil bacteria, and other beneficial soil organisms necessary to restore severely degraded soils. Biosol is an organic fertilizer material designed to increase microbial biomass and build humus. Installation of the plots began September 25, 2002 and concluded October 23, 2002. Each plot was seeded, either by hand or by hydroseeding, using a standardized seed mix (Table 2). No additional work, modification, or maintenance was conducted on the plots for the remainder of the five-year study with one exception—the LYF + urea plot was reseeded in August 2003. This was necessary due to a complete failure of seed germination in year 1.

Table 1: Amendment type, application rates, and application methods.

Amendment type	Rate and application method
Control (topsoil)	30 m^3 topsoil (surface applied, not incorporated, amendment depth approx. 15 cm)
Biosolids + woodash	20 m^3 class B biosolids mixed with wood ash (0.75 : 1) (surface applied, not incorporated, amendment depth approx. 10 cm)
Log yard fines (LYF) + urea fertilizer	37 m^3 log yard fines (<3/4″) mixed with urea fertilizer (10% v/v) (surface applied, not incorporated, amendment depth approx. 15 cm)
Kiwi Power	Fertile Fibers Plus, Kiwi Power, Strong Hold + Tacker and Atlas Soil Lock (materials were mixed and surface applied by hydroseeder)
Biosolids-based compost	15 m^3 of compost (surface applied, not incorporated, amendment depth approx. 10 cm)
Wood products-based compost	15 m^3 of compost (surface applied, not incorporated, amendment depth approx. 10 cm)
Biosol	38 kg Biosol Mix (7-2-3) plus 2.3 kg Wood Fiber Mulch (materials were mixed and surface applied by hydroseeder)
Log yard waste (LYW)	15 m^3 of log yard waste (surface applied, not incorporated, amendment depth approx. 10 cm)
Control (fertilizer)	23 kg of fertilizer (16-16-16) plus tackifier (materials were mixed and surface applied by hydroseeder)

2.3. Soil and Vegetation Assessment. The plots were inspected on a monthly basis during each field season beginning in April 2003 and concluding in August 2007. Quantitative determination of revegetation success was conducted each July using Bureau of Land Management standard methods [26]. Percent coverage was measured using a cover-point optical projection scope. One hundred points were recorded at 1 m intervals along a randomly located transect within each plot. Each point identified an individual plant, rock, bare soil, or litter.

Duplicate soil samples were collected from the control plots following regrading but prior to addition of plot

Table 2: Composition and application rates of standardized seed mix.

Common name	Species/variety	Amount/acre	Pct by wt.	Min. pct.
Slender wheatgrass	*Elymus trachycaulus* ssp. *Trachycaulus* var. Revenue	6.4 kg	22.3	21.9
Idaho fescue	*Festuca idahoensis* var. Joseph	3.9 kg	13.4	13.2
Sheep fescue	*Festuca ovina* var. Covar	3.2 kg	11.1	10.9
Mountain brome	*Bromus marginatus* var. Bromar	3.5 kg	12.2	12.0
Meadow brome	*Bromus biebersteinii* var. Paddock	3.9 kg	13.4	13.2
White yarrow	*Achillea millefolium*	0.34 kg	1.1	1.1
Blue flax	*Linum lewisii* var. Appar	1.9 kg	6.7	6.6
Rocky Mountain penstemon	*Penstemon strictus*	0.68 kg	2.2	2.2
White dutch clover	*Trifolium repens* L.	0.23 kg	0.8	0.8
Canada bluegrass	*Poa compressa*	0.34 kg	1.1	1.1
Big bluegrass	*Poa ampla* var. Sherman	0.68 kg	2.3	2.3
Canby bluegrass	*Poa canbyi* var. Canbar	0.68 kg	2.2	2.2
Cicer milkvetch	*Astragalus cicer*	3.2 kg.	11.1	10.9
Fireweed	*Epilobium angustifolia*	0.28 kg	0.1	0.1
Weed seed	—	—	—	0.5 (Max)
Inert and other crop	—	—	—	1.5 (Max)

amendments; these results are reported as the control (unamended) in the following figures and tables. Soil profile characteristics were evaluated in the field at the end of year 5. During years 1, 3, and 5, a composite (3x) soil sample was collected from the 0–10 cm depth of each plot. Soil fertility parameters (ammonium-N, nitrate-N, available P and K, pH, and EC) and physical properties (percent sand, silt, clay, coarse fragments, and textural class) were determined using standard methods [27]. Available N was calculated as the sum of ammonium- and nitrate-N. Organic matter content was determined by colorimetry [28]. Total recoverable metals were determined using EPA Method 3050B/6010 [29]. All laboratory work was conducted at the University of Idaho Analytical Sciences Laboratory. Standard quality assurance/quality control protocols were followed for all analytical work [30].

2.4. Data Analysis. Diversity-related indices, namely Shannon-Wineer index (H′) and evenness (E), were calculated by year for each treatment. The invasion index (I) was also calculated for each treatment where I is the number of introduced species/total number of species. Diversity indices and plant cover data were subjected to analysis of variance using the generalized linear model [31]. Single degree of freedom contrasts were used to compare mean responses. A 95% significance level ($P < 0.05$) was used for all statistical comparisons.

3. Results and Discussion

3.1. Soil and Amendment Properties. Following site preparation but prior to amendment application, the surface soil material was a mixture of waste rock and fine material. The unamended control (Table 3) exhibited properties endemic to the Silver Dollar site, namely an alkaline (pH 8.3) sandy loam with 58% coarse fragments. The electrical conductivity (EC) of the unamended control was 0.35 dS/m and heavy metal concentrations (As, Ba, Cd, Cr, Pb) were below hazard limits for regulated metals [32]. This is not surprising since milling and smelting activities took place off-site. The macronutrient content of the unamended control was quite low with available N and P concentrations of 1.5 ug/g and 1.0 ug/g, respectively (Figure 1). Organic matter content was also low at 0.9% and available K was moderate at 79 ug/g. Thus, the chemical and physical data indicate that low soil fertility was the primary factor limiting sustainable plant growth in the unamended soil. A secondary issue was the low water holding capacity.

Each treatment decreased soil pH relative to the unamended control (Table 3). The pH of the amended plots ranged from 6.3 to 7.8 with the biosolid + woodash and control (fertilizer) treatments exhibiting the highest pH. Overall, the pH remained relatively consistent among the amended plots throughout the five-year study period (data not shown). The highest EC values were observed in the biosolids + woodash and LYF + urea treatments (Table 3). Although these higher EC values are near critical values for agronomic crops (2–4 dS/m), none of the vegetation exhibited symptoms of a salinity problem.

During the first year (2003), the organic matter content varied from ∼1% in the controls and liquid-based amendments (Kiwi Power and Biosol) to 15–34% in the solid-based amendments (biosolid, composts, LYF, and LYW) (Figure 1(a)). The organic matter content in several of the treatments (biosolids, LYF + urea, and biosolids-compost) increased between 2003 and 2005 (Figure 1(a)). Each of these treatments exhibited substantial vegetative growth resulting in high crop residues and organic carbon recycling. Each treatment associated with the high organic matter amendment (biosolid, composts, LYF, and LYW) exhibited a marked decrease in organic matter between 2005 and 2007.

Each treatment increased the available P and K content with the extent of increase being strongly dependent on

Table 3: Metal content, electrical conductivity (EC), particle size distribution, and soil texture for unamended and treated plots.

	As (ug/g)	Ba (ug/g)	Cd (ug/g)	Co (ug/g)	Cr (ug/g)	Cu (ug/g)	Fe (ug/g)	Mn (ug/g)	Mo (ug/g)	Ni (ug/g)	Pb (ug/g)	Zn (ug/g)	pH	EC dS/m	Sand %	Clay %	Silt %	>2mm %	Texture
Unamended control	59	220	0.08	15	13	81	20000	1400	<3.8	16	100	170	8.3	0.35	66	16	18	58	Sandy loam
Control (topsoil)	16	240	<0.75	13	54	25	27000	830	<3.8	32	19	91	6.3	0.24	46.4	9.6	44.0	59	loam
Biosolids + woodash	34	850	2.8	11	58	130	16000	2200	<3.8	45	34	470	7.5	3.8	72.4	7.6	20.0	35	Sandy loam
LYF + urea	12	310	<0.75	11	71	33	22000	930	<3.8	43	22	110	7.2	3.4	52.4	7.6	40.0	38	Sandy loam
Kiwi Power	46	230	<0.75	18	35	140	29000	2400	4.6	32	26	74	7.8	0.78	70.4	11.6	18.0	57	Sandy loam
Biosolids compost	22	260	0.75	13	63	110	22000	1400	4.6	41	37	190	7.4	1.3	56.4	7.6	36.0	34	Sandy loam
Wood compost	33	400	<0.75	8.8	74	130	17000	1500	<3.8	41	29	110	6.5	0.31	66.4	9.6	24.0	51	Sandy loam
Biosol	47	480	<0.75	12	66	96	23000	2000	<3.8	39	80	150	7.8	1.6	60.4	15.6	24.0	57	Sandy loam
LYW	23	240	<0.75	7.3	62	35	16000	1100	<3.8	36	16	78	6.8	0.54	64.4	9.6	26.0	33	Sandy loam
Control (fertilizer)	62	350	3.7	11	46	86	22000	1300	<3.8	31	99	920	7.8	0.80	66.4	13.6	20.0	68	Sandy loam

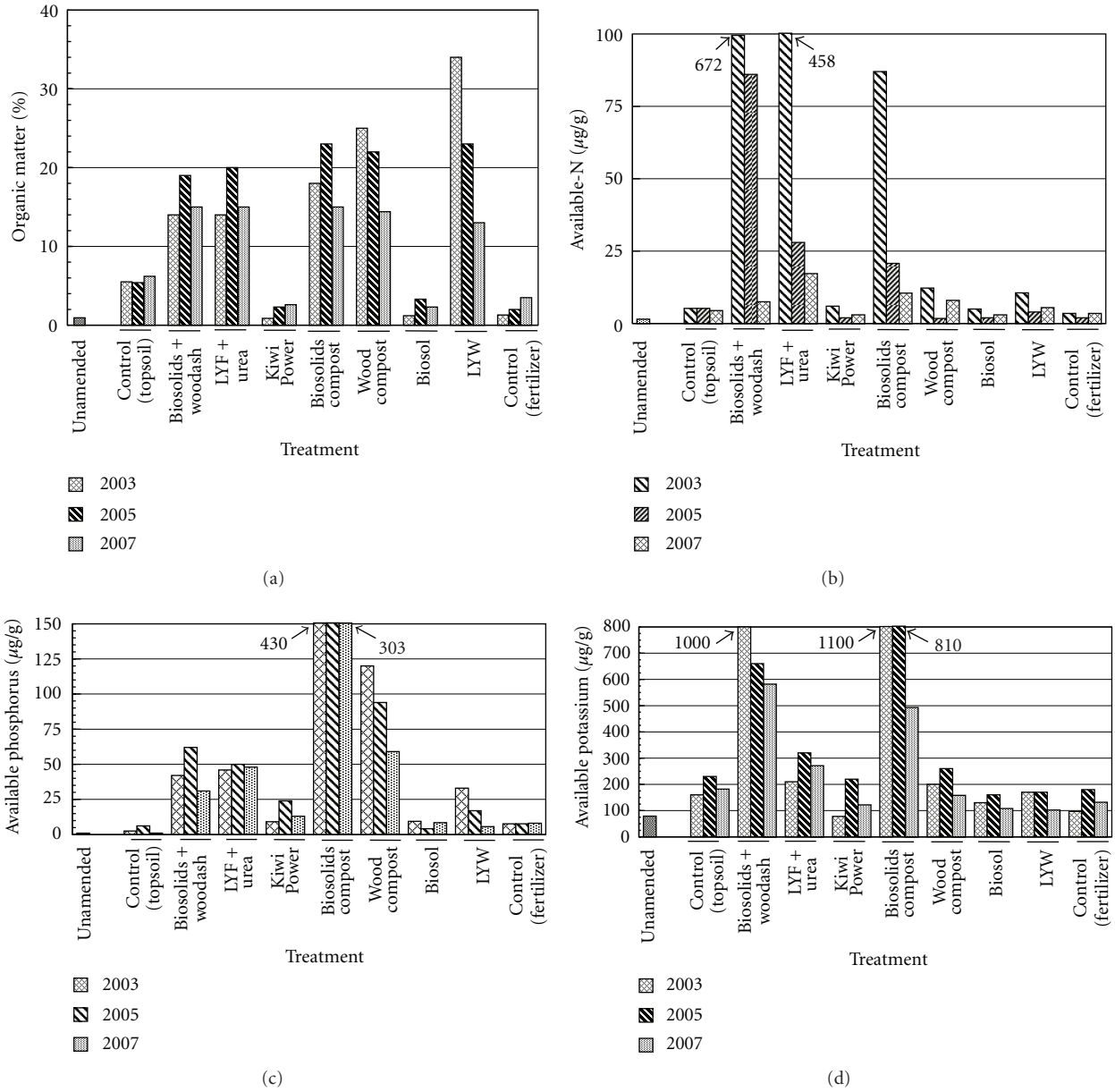

Figure 1: Trends in (a) organic matter content, (b) available N, (c) available phosphorus, and (d) available potassium in the unamended and treated plots for years 2003, 2005, and 2007.

the nature of the amendment (Figures 1(c) and 1(d)). Available P values ranged from <2 to >600 ug/g while available K ranged from 80 to 1000 ug/g. To put these numbers into perspective, available P and K levels in excess of 8 and 100 ug/g, respectively, are considered sufficient for nonirrigated legume-grass pastures in northern Idaho [33].

Available N also varied significantly among the treatments, ranging from ~5 ug/g in the Kiwi Power and Biosol to >450 ug/g in the biosolids and LYF + urea treatments (Figure 1(b)). Each treatment with high available N (biosolid, biosolid-compost, and LYF + urea) exhibited a substantial decrease between 2003 and 2005, and again between 2005 and 2007. Potential fates of available N include leaching, plant uptake, and volatilization. A significant fraction of available N was lost via surface runoff as previously described by McGeehan [34] and this was most significant in the biosolids + woodash, LYF + urea, and biosolids-compost treatments. The high available N associated with these plots also supported very heavy vegetative growth, primarily of perennial grasses which exhibit high uptake rates and N sequestration [35, 36]. However, given the magnitude of declines observed, it is likely that ammonia volatilization played the most significant role in decreased available N. Large and rapid loss of N is commonly observed in surface-applied biosolids with volatilization rates exceeding 50% of total N [37–39].

This mechanism was further enhanced by the high pH levels.

3.2. Profile Properties. The addition of the various amendments had differing impacts on the soil profile of each plot. It should be noted that a true pedogenic soil profile takes hundreds to thousands of years to form. Thus, it is somewhat of a stretch to describe the profile of each plot using standard soils terminology. Nonetheless, the amendments did alter the surface properties of each plot in ways that will have lasting impacts on the sustainability of the plant cover. For example, the addition of roughly 20 m^3 of biosolids, compost, or log yard waste resulted in an overburden depth of 10–15 cm. This overburden tended to be dark in color with a very friable (easily crumbled) texture. Such characteristics are associated with highly productive and fertile topsoil and, hence, these plots supported very good plant growth resulting in the presence of profuse fine roots in the overburden. Since the organic materials were surface applied but not incorporated, there was an abrupt boundary between the overburden and underlying waste rock with very few roots penetrating this boundary. This abrupt boundary remained very distinct throughout the study. Also, the physical condition of the overburden improved over the course of the 5-year study. For example, the biosolids were very sticky and tended to smear during application. However, as this material dried and weathered for several years, the result was a very light material with physical properties that are ideal for plant growth. Likewise, the log yard waste and composts underwent both physical and biological weathering, resulting in a very friable material with excellent tilth and other desirable physical properties.

In contrast, the Kiwi Power, Biosol, and Fertilizer Control did not receive large quantities of organic amendments. Consequently, these plots exhibited a thin organic surface layer developed from decaying plant debris. Despite the lack of a thick, organic overburden, these plots still supported good plant growth as evidenced by the moderate root presence. It is likely that these plots will continue to build organic matter content over time and slowly improve soil physical properties.

3.3. Vegetation Assessment

3.3.1. Plant Cover. Each treatment was successful in promoting a self-sustaining plant cover during 2003 and maintaining plant growth throughout the five-year study (Figure 2). During the first year, the extent of coverage varied considerably, ranging from 19% to 77% in the LYW and biosolid-compost treatments, respectively. The wood-compost treatment exhibited low coverage in year 1, most likely as a result of a high C : N. Plant coverage increased in all treatments between years 1 and 2. These changes were associated with increased growth of grass species in the biosolids + woodash, biosolids compost, and Biosol plots and increased forb growth in the Kiwi Power, wood compost, and LYW plots. Slender wheatgrass and brome species were the most extensive grasses observed during years 1 and 2 while yarrow and white dutch clover were the most frequently observed forbs. The majority of plots maintained plant coverage in the 75–90% range from year 3 to year 5 (Figure 2).

3.3.2. Unseeded Vegetation. Unseeded vegetation accounted for a significant portion of coverage in several treatments during the study period (Figure 2). For example, the topsoil-control exhibited a high incidence of weeds beginning in year 1 with >50% of the plant cover due to the establishment and growth of hare's foot clover. In contrast, the Kiwi Power, wood compost, and LYW plots did not exhibit substantial increases in weeds until years 3 and 4 (Figure 2). These large increases were primarily the result of black medic and sweet clover. It should be noted that several plots (i.e., biosolids + woodash, LYF + urea, biosolids compost, and Biosol) exhibited very little weed invasion. The majority of the unseeded species can be classified as common weeds of the Northwestern US [40] that are easily disseminated by wind, animals, and other vectors. However, given the disproportionately high percentage of unseeded vegetation present in the topsoil-control in year 1, it is likely that many weed seeds were transported to the site in the topsoil amendment. The role of topsoil as a seed bank is well established, and imported soil has been reported to introduce both desirable and undesirable invasive species [41, 42].

3.3.3. Species Distribution and Diversity. Grass species dominated the plant communities in the high N treatments. As an example, the species composition by year for the biosolids + woodash treatment is plotted in Figure 3(a). Similar results were obtained for the LYF + urea and biosolid-compost treatment. Although wheatgrass was the dominant grass in year 1, the 2004–2007 data (years 2–5) show a trend of declining wheatgrass with concurrent increases in bromes and fescues in of the grass-dominated plots. It is unclear whether the gradual decline in wheatgrass is a natural successional characteristic or a response of this species to a decrease in available N. The lower N treatments exhibited a more even distribution between grass and forb species throughout the study period. As an example, species composition for the wood-compost is plotted in Figure 3(b). Similar results were obtained for the Kiwi Power and LYW treatments. Yarrow, fescue, and bromes tended to increase steadily in years 1, 2, and 3. White clover was common on several of the lower N treatments in year 1 but was rarely encountered during years 2–5. In contrast, cicer milkvetch was not observed during years 1 and 2 but increased significantly during the final three years of the study.

The relationship between vegetation and N availability was further evaluated by pooling the plant response data into high versus low treatments for statistical analysis. As shown in Figure 4(a), significantly higher Shannon diversity indices (H') were obtained for the high N treatments in three out of the five study years. This reflects the greater number of grass species associated with these treatments, particularly in the final two years. Species evenness (E) was very similar between the high and low N treatments (Figure 4(b))

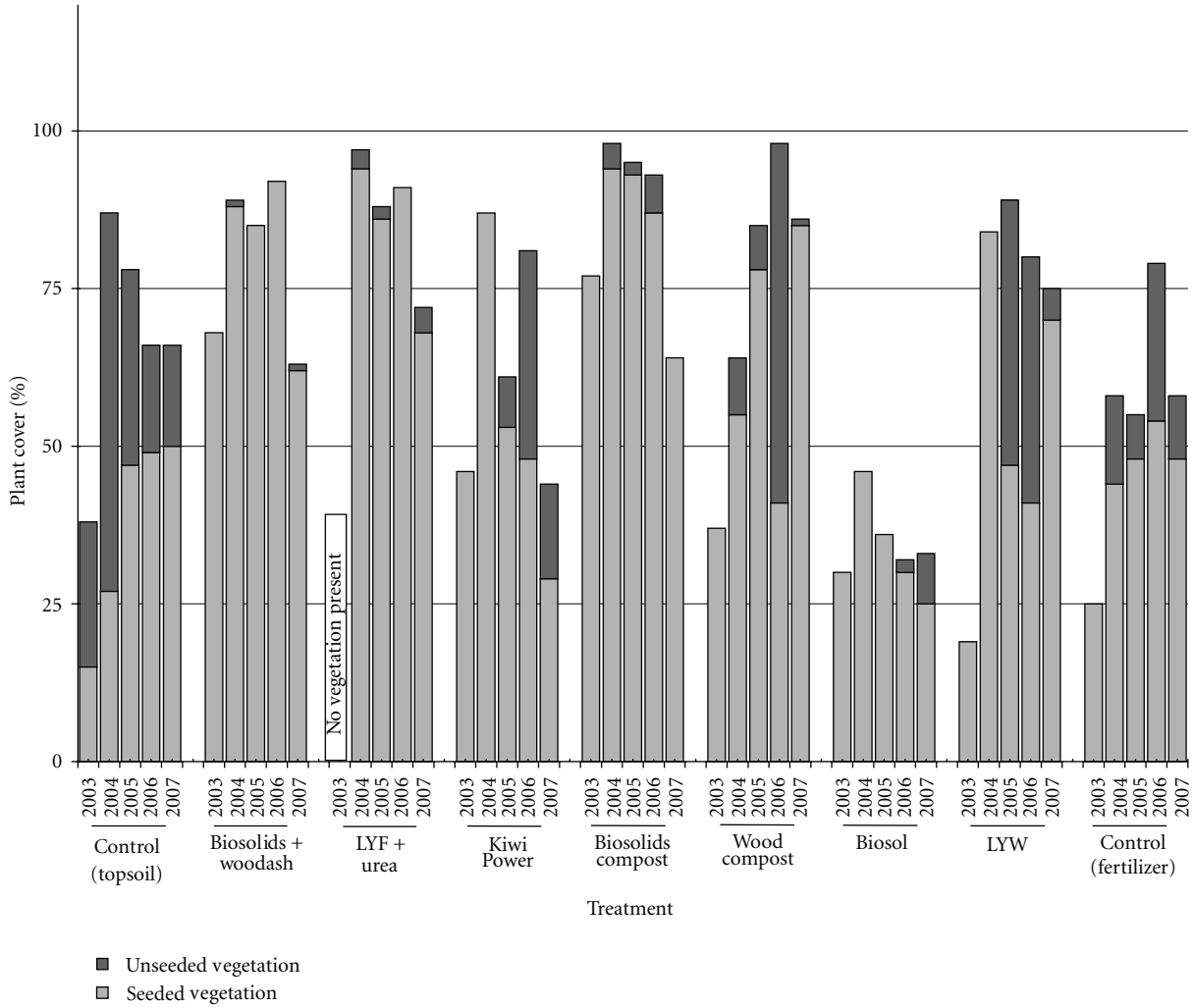

Figure 2: Comparison of total plant cover across all treatments for years 2003, 2004, 2005, 2006, and 2007.

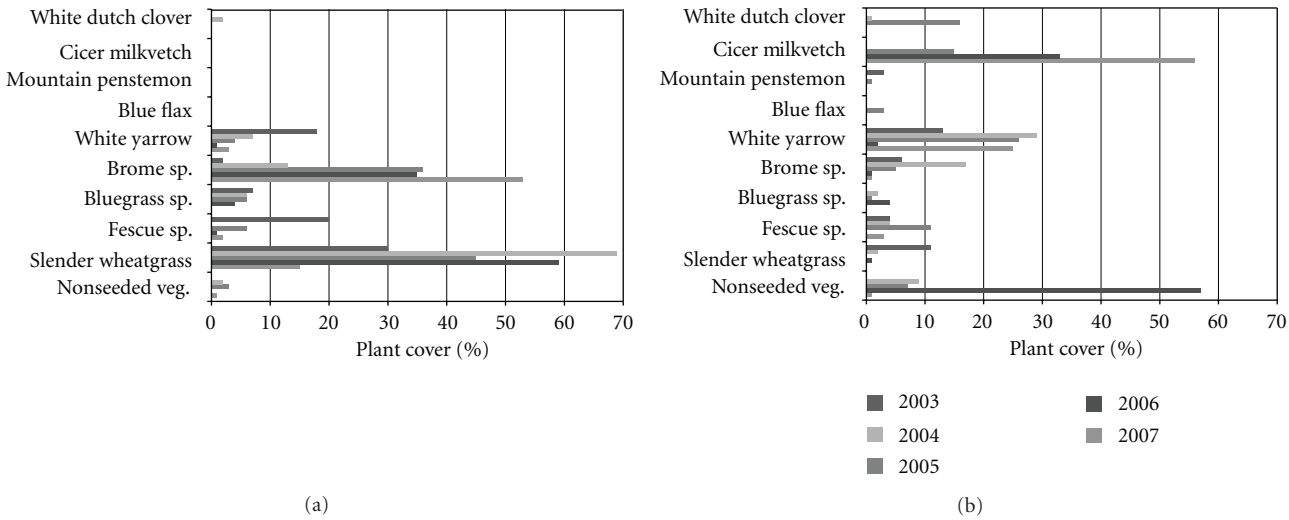

Figure 3: Species composition on (a) high nitrogen treatment (biosolids + woodash) and (b) low nitrogen treatment (wood compost) for years 2003, 2004, 2005, 2006, and 2007.

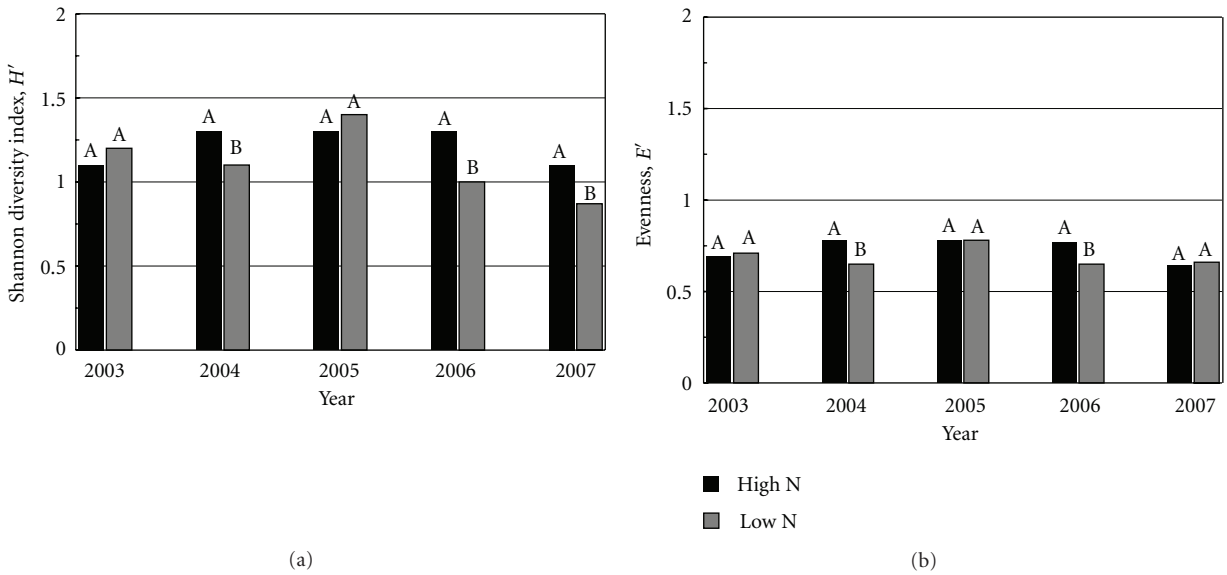

Figure 4: Mean responses for (a) Shannon diversity index and (b) species evenness in pooled high versus low nitrogen treatments by year. Responses associated with different letters are significantly different ($P < 0.05$).

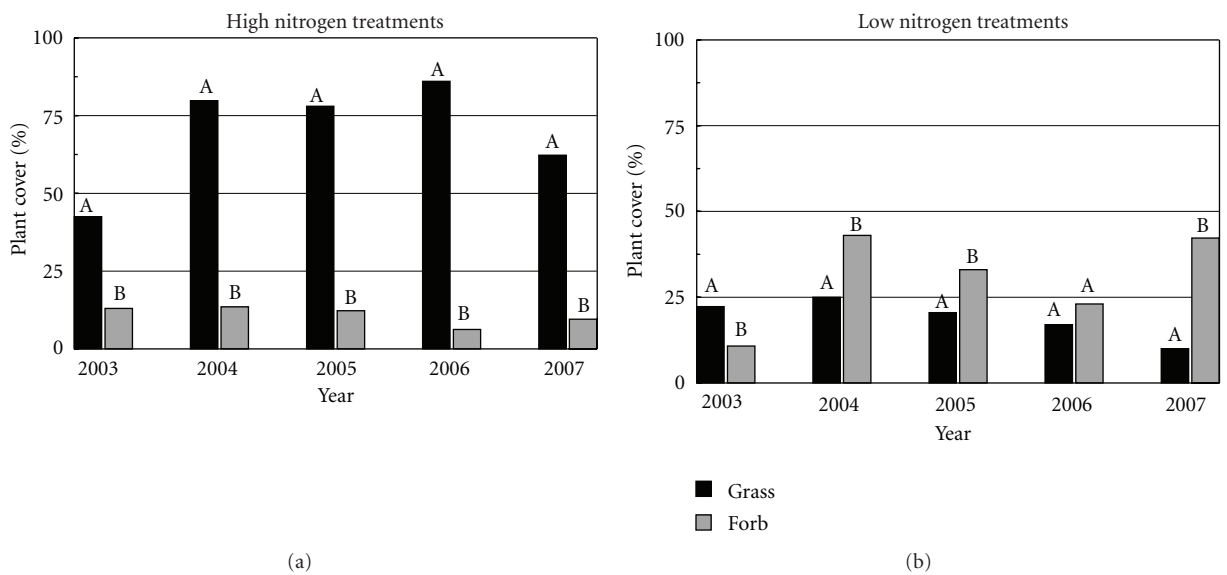

Figure 5: Mean vegetation response in (a) pooled high nitrogen treatments and (b) pooled low nitrogen treatments by year. Responses associated with different letters are significantly different ($P < 0.05$).

indicating no significant differences in the relative abundance of species between these groups, with the exception of 2006.

A comparison of vegetative response based on ecological vegetative groupings (grass versus forb) is shown in Figure 5. In each of the five years, the percent cover due to grasses was significantly higher than forb cover in the pooled high N treatments (Figure 5(a)). In contrast, forbs were significantly higher in 3 of 5 years in the low N treatments. Furthermore, the invasion index (I) was markedly impacted by N treatment. Counts of weed species were very low in the first two years of the study but increased substantially in the low N treatments in Years 3, 4, and 5 (Figure 6).

Several studies report increased weed growth in high nitrogen environments [43, 44]. The opposite trend was observed in our study where, as Figure 6 clearly shows, an inverse relationship exists between available nitrogen and the invasion index. That is, a low invasion index is associated with the high N treatments and vice versa. A likely mechanism explaining this result is a competition for

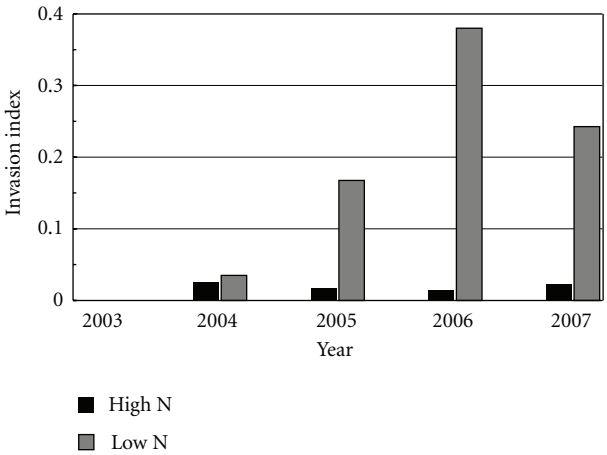

Figure 6: Mean invasion indices in pooled high- versus low-pooled nitrogen treatments by year. Note: the number of zero values precluded statistical analysis.

nutrients and light which can favor rapidly growing grasses [45, 46]. The growth habit of the weed species may be an additional factor with low, radially spreading species being less able to compete for light and, hence, be favored by the more open cover of the low nitrogen treatments [47].

4. Conclusions

The overall goal of this study was to evaluate waste-based and organic amendments for their ability to improve soil fertility and crop performance. Each amendment resulted in significant improvements in soil fertility parameters and all were successful in establishing plant cover during the first growing season and sustaining this cover throughout the five-year study.

Organic matter content and available N, P, and K were significantly increased by the various amendments although the extent of increase was determined by the composition of the amendment. All solid amendment types (composts, biosolids, and LYF/LYW) greatly increased the organic matter content. However, only the biosolids, biosolid-based compost, and LYF mixed with urea fertilizer contributed large amounts of available N. The wood products-based compost and LYF contributed lower amounts of available N. Each treatment associated with high inputs of organic matter and available N exhibited significant declines in these properties over the course of the study.

The fertility status of each amendment had a strong impact on crop performance. More specifically, available nitrogen was a critical factor in determining total plant cover, species distribution, and the incidence of unseeded vegetation. For example, high nitrogen amendments promoted a grass-dominated cover with low numbers of forbs. Wheatgrass was the dominant species in these plots during years 1 and 2 but a more equal distribution of wheatgrass, bromes, fescues, and bluegrass was observed in years 3–5. Throughout the study, these plots had the highest plant coverage and maintained very robust and thick grass growth. These characteristics were successful in preventing the establishment and spread of invasive weed species. In contrast, amendments with lower available nitrogen promoted a more diverse grass-forb mixture. No single grass species was dominant; instead a variety of grasses were intermixed with white yarrow, white clover, and cicer milkvetch. These plots had lower plant coverage and more patchy plant growth. Consequently, a higher incidence of invasive weed species was observed.

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Research Article

Productivity and Nutritive Quality of Johnsongrass (*Sorghum halepense*) as Influenced by Commercial Fertilizer, Broiler Litter, and Interseeded White Clover (*Trifolium repens*)

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In the southeastern USA, there is an abundance of broiler litter from commercial poultry production that is available for use as fertilizer, but cropland and pastureland amended with broiler litter often exhibit greatly increased soil-test P. We evaluated productivity and nutritive quality of Johnsongrass (*Sorghum halepense*) that was interseeded with or without white clover (*Trifolium repens*) and which commercial fertilizer (ammonium nitrate and diammonium phosphate) or broiler litter was applied on the basis of soil-test P; broiler litter was supplemented with ammonium nitrate to be isonitrogenous with commercial fertilizer. Forage dry matter yield and foliar concentrations of crude protein, cell wall constituents, P, K, and Cu were not different among fertilizer treatments, and concentration of Zn was only slightly greater for forage amended with broiler litter than commercial fertilizer. Results indicate that broiler litter can be a cost-effective alternative to commercial fertilizer for warm-season forage production when applied on the basis of soil-test P.

1. Introduction

Poultry production is a major agricultural industry in the southeastern USA, and significant quantities of broiler litter are generated and available for use as fertilizer for pasture and row crops. Within the state of Alabama alone, approximately 1.36 million metric tons of broiler litter are produced annually [1], over 90% of which is disposed through application to cropland and pastureland [2]. In areas of concentrated poultry production, soils often become concentrated with nutrients as a result of repeated land application of broiler litter over extended periods of time [3]. Studies have shown that repeated land application of animal manures to agricultural fields can potentially cause environmental problems [4]. Phosphorus runoff and resulting eutrophication is one of the most common environmental problems associated with use of organic fertilizers [2, 5].

High-producing warm-season forages have significant capacity for assimilating nutrients from land-applied broiler litter [6, 7]. In the southeastern USA, application of broiler litter to Bermudagrass (*Cynodon dactylon*) pasture has been used successfully for producing high biomass yields, and, in doing so, reducing adverse effects of broiler litter application on soil quality [8]. Studies have shown that Johnsongrass (*Sorghum halepense*) can produce as much or more biomass than common Bermudagrass, making it an attractive candidate for nutrient management.

Broiler litter is commonly land-applied on the basis of crop requirement for N; however, this practice has resulted in elevated concentrations of soil P [9]. Low nutrient concentration and bulk density compared with commercially available synthetic fertilizer make long-distance transportation of broiler litter cost-prohibitive. However, pressure compaction of broiler litter increases its bulk density without adversely

affecting its nutrient concentration [10], conceivably making its transportation from areas of intensive poultry production to limited-resource agricultural areas more economically feasible [11]. Also, application of broiler litter on the basis of soil-test P may prevent accumulation of P in soils and thus minimize environmental hazards associated with land application of animal manures.

The objective of the research reported herein was to evaluate productivity and nutritive quality of Johnsongrass that was interseeded with or without white clover (*Trifolium repens*) and which isonitrogenous treatments of commercial fertilizer (diammonium phosphate), compacted broiler litter, or noncompacted broiler litter were applied on the basis of soil-test P.

2. Materials and Methods

2.1. Research Site. The experiment was conducted in the summers of 2007 and 2008 at the Black Belt Research and Extension Center in Marion Junction, AL (32°28′50.29″N latitude, 87°15′26.61″W longitude, 57 m above MSL). The Black Belt physiographic region is of special interest in the context of the current research because it is characterized by a resource-poor agricultural landscape with historically limited access to broiler litter from areas of concentrated poultry production. Twenty-four field plots (3 × 6 m each) consisting of Vaiden and Houston clay soils were demarcated and treated on June 8, 2007 with glyphosate (N-(phosphonomethyl) glycine) to kill existing vegetation. Plots were tilled on June 15 and seeded on June 18, 2007. Plots were organized into four blocks (replicates), each of which comprised six plots representing six experimental treatments. Soil nutrient ratings and values were determined, and fertilization recommendations were made on the basis of soil tests conducted by the Auburn University Soil Testing Laboratory.

2.2. Compaction of Broiler Litter. Broiler litter was collected from a poultry operation in Talladega County, AL, and transported to Auburn University. Initial moisture concentration in litter was determined using a model IR-200 moisture analyzer (Denver Instruments, Arvada, CO). Water was then added to and mixed with a portion of the litter in a concrete mixer to achieve a moisture concentration of approximately 40%. Immediately after mixing, moistened litter was subjected to 192 MPa of pressure for 1 min until 4 layers of litter were compacted into cubes that measured approximately 30.5 × 30.5 × 20 cm. Cubes were stored for 5 days before they were chipped using a commercial mulch chipper, and then were transported with a load of noncompacted broiler litter to Marion Junction, AL, and applied to plots.

2.3. Forage Establishment, Management, and Harvesting. Johnsongrass (*Sorghum halepense*) was seeded into all plots at a recommended rate of 28 kg/ha, and ladino clover (*Trifolium repens* cv. "Regal Graze") was seeded into half of the plots in each block at a rate of 5.6 kg/ha to achieve a 1 : 4 ratio of clover to Johnsongrass. Plots were fertilized on June 18, 2007 with compacted broiler litter (BL), noncompacted BL (BL-N), or commercial fertilizer (CF) such that each clover-status × fertilizer-source treatment was represented once in each block. The CF was a mixture of ammonium nitrate (34-0-0) and diammonium phosphate (18-46-0) that was formulated to provide the equivalent of 56.0 kg P_2O_5 and 67.3 kg N/ha. Broiler litter application rate was determined on the basis of soil-test P, and litter-amended plots were supplemented with additional N from ammonium nitrate in order to meet the recommended rate of N application (67.3 kg N/ha) and be isonitrogenous with CF. All fertilizer was applied by hand and soil-incorporated prior to initial planting. In May, 2008, fertilizer was hand-applied onto the soil surface but not incorporated so as to not damage plant tissues.

Primary-growth forage was harvested in each year of the experiment (August 9, 2007 and August 1, 2008) when Johnsongrass had reached a late vegetative (boot) stage of maturity, followed by a second harvest of vegetative-regrowth forage (October 2, 2007 and September 22, 2008). Forage was cut with a flail-chopping mower to leave an aboveground stubble height of approximately 10 cm. Fresh-cut forage was weighed on a portable scale, and a sample from each plot was then placed into a teared paper bag and weighed. Samples were oven-dried at 60°C for 72 hours, and dry matter (DM) yield was calculated for each plot based on dry-weight data.

2.4. Laboratory Analyses. Dried, air-equilibrated samples were ground in a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) to pass a 1-mm screen, and final DM concentration was determined by oven drying at 100°C according to procedures of AOAC [12]. Forage N concentration was determined by the Kjeldahl procedure [12], from which crude protein (CP) was calculated as N × 6.25. Concentrations of neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were analyzed by procedures of Van Soest et al. [13]. Forage samples were prepared for mineral analyses by dry-ashing, wet-digestion with 1 N HNO_3, and solubilization in 1 N HCl [14], and concentrations of P, K, Cu, and Zn were then determined by inductively coupled argon plasma (ICAP) spectroscopy (Spectro Ciros CCD, Germany).

2.5. Statistical Analysis. Data were analyzed using the PROC MIXED procedure (SAS Institute Inc., Cary, NC) for a complete block design with a 2 × 3 factorial arrangement of treatments (4 replicates per treatment). Independent variables included block (replicates), clover status, fertilizer source, and the clover-status × fertilizer-source interaction. Vegetative regrowth harvests were treated as repeated measures of primary harvests, and year was considered as a random effect in the statistical model. Treatment means were separated by the LSMEANS procedure (SAS Inst. Inc., Cary, NC) when protected by F-tests significant at α of 0.10 and are reported as least squares means ± SE.

3. Results

3.1. Temperature and Precipitation. During the study period, monthly mean air temperatures were slightly higher than 30-year averages for Marion Junction, AL (Table 1). For the months of June, July, and August 2007, monthly precipitation was 11, 46, and 25% lower, respectively, than the 30-year average (Table 1). In 2008, June and July monthly precipitation was 13 and 53% lower, respectively, than the 30-year average. Precipitation in August 2008 was 171% higher than the 30-year average; however, total precipitation in September 2008 was 96% lower than the 30-year average for Marion Junction, AL. Total precipitation for the months during the experimental period was 61 and 17% below the 30-year average in 2007 and 2008, respectively.

3.2. Dry Matter Yield. No differences ($P = 0.204$) were observed between Johnsongrass and Johnsongrass-clover forage or among fertilizer-source treatments ($P = 0.838$) for DM yield (Table 2).

3.3. Crude Protein. Crude protein concentration (Table 2) was greater ($P = 0.074$) in Johnsongrass-clover than Johnsongrass forage but was not different ($P = 0.602$) among the three fertilizer-source treatments.

3.4. Cell Wall Constituents. Neutral detergent fiber concentration (Table 2) was not different ($P = 0.130$) between Johnsongrass and Johnsongrass-clover forage or among fertilizer-source treatments ($P = 0.221$). Similarly, concentration of ADF (Table 2) was not different ($P = 0.968$) between Johnsongrass and Johnsongrass-clover forage or among fertilizer-source treatments ($P = 0.834$). However, a forage × fertilizer source interaction ($P = 0.098$) was observed such that Johnsongrass fertilized with BL-N had lower ($P = 0.081$) ADF concentration than CF-amended Johnsongrass. Also, Johnsongrass amended with CF had greater ($P = 0.075$) concentration of ADF than CF-amended Johnsongrass-clover. Interseeding with clover had no effect ($P = 0.737$) on forage concentration of ADL (Table 2); also, fertilizer source did not affect ($P = 0.342$) ADL concentration. However, a forage × fertilizer source interaction ($P = 0.051$) was observed such that ADL concentration was greater ($P = 0.013$) in CF- than BL-N-amended Johnsongrass and within CF forages was greater ($P = 0.041$) for Johnsongrass than Johnsongrass-clover.

3.5. Minerals. Foliar concentration of P (Table 2) was not different ($P = 0.306$) between forages or among fertilizer-source treatments ($P = 0.504$). The Johnsongrass-clover mixture had greater ($P = 0.002$) foliar concentration of K (Table 2) than Johnsongrass, and BL-amended forages tended to have greater ($P = 0.122$) foliar concentration of K than CF-amended forage. No difference ($P = 0.870$) was observed between Johnsongrass and Johnsongrass-clover in foliar Zn concentration (Table 2). Forages amended with CF had a lower foliar Zn concentration than both BL-N ($P = 0.022$) and BL-C ($P = 0.064$) treatments, but there was no difference ($P = 0.656$) in foliar Zn concentration between the BL-N and BL-C treatments. However, foliar concentration of Cu (Table 2) was not different between forages ($P = 0.261$) or among fertilizer-source treatments ($P = 0.459$).

Table 1: Monthly mean air temperatures and precipitation for May–October 2007 and 2008 and 30-year averages for Marion Junction, AL.

Month	Mean, °C			Precipitation, mm		
	2007	2008	30-yr avg.	2007	2008	30-yr avg.
May	22	22	22	3	78	104
June	26	27	26	101	98	113
July	29	27	27	70	61	129
August	29	26	27	64	230	85
September	24	24	24	67	4	100
October	18	17	18	66	33	75
Total				371	504	606

4. Discussion

Broiler litter used for fertilization of forages in 2007 contained 62% DM, 3.75% N, 1.4% P, and 3.6% K on an ambient air-equilibrated basis. In 2008, broiler litter contained 80% DM and 3.4% N, 1.4% P, and 3.7% K on an ambient air-equilibrated basis. Application rates of broiler litter based on soil-test P were equivalent to 1.358 and 1.752 kg/ha in 2007 and 2008, respectively. This method of application required supplementation with ammonium nitrate to meet crop N requirements because experimental plots were deficient by 16.3 and 19.0 kg N/ha in 2007 and 2008, respectively. To meet Alabama Cooperative Extension System recommendations for N, plots were supplemented with ammonium nitrate to achieve a total of 75 kg N/ha in both years.

Ball et al. [6] have reported that Johnsongrass can routinely produce between 4.500 and 11.200 kg of hay per ha over an entire growing season. In this experiment, DM yield averaged 6.856 kg/ha for each harvest across years, forages, and fertilizer-source treatments. In the first year (2007), cumulative yield of primary growth and vegetative regrowth harvests averaged 10.078 kg/ha across forages and fertilizer-source treatments. In the second year (2008), the corresponding value for seasonal productivity was 17.344 kg/ha across forages and fertilizer-source treatments. In 2007, total annual precipitation was 61% lower than the 30-yr average for Marion Junction, AL; however, forage production was still within the range of typical seasonal yields reported by Ball et al. [6], which illustrates the ability of Johnsongrass to withstand significant drought [15]. In 2008, total annual rainfall was only 17% below the 30-yr average, which provided more optimal conditions for growth. Total seasonal productivity of forages in 2008 illustrates the exceptionally high productivity potential of this warm-season grass.

Crude protein (N × 6.25) is an important determinant of nutritive quality of forages. Johnsongrass interseeded with clover contained 6% more CP than Johnsongrass alone,

Table 2: Yield of dry matter (DM) and foliar concentrations (DM basis) of crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and select minerals from Johnsongrass interseeded with (+C) or without (−C) white clover and amended with commercial fertilizer (CF), noncompacted broiler litter (BL-N), or compacted broiler litter (BL-C).

	Fertilizer treatment			
	CF	BL-N	BL-C	Mean
DM Yield (kg/ha)				
−C	7.590	6.656	7.314	7.187
+C	6.555	6.810	6.208	6.524
Mean	7.073	6.733	6.761	
CP (%)				
−C	10.2	10.0	9.7	9.9[a]
+C	10.6	10.5	10.4	10.5[b]
Mean	10.4	10.3	10.0	
NDF (%)				
−C	63.2	63.8	65.4	64.1
+C	64.2	66.8	67.2	66.0
Mean	63.7	65.3	66.3	
ADF (%)[c]				
−C	36.3	34.8	35.2	35.4
+C	34.8	35.6	35.8	35.4
Mean	35.5	35.2	35.5	
ADL (%)[d]				
−C	3.4	3.0	3.2	3.2
+C	3.1	3.2	3.2	3.2
Mean	3.2	3.1	3.2	
P (mg/kg)				
−C	0.17	0.18	0.18	0.18
+C	0.17	0.17	0.18	0.17
Mean	0.17	0.18	0.18	
K (mg/kg)				
−C	0.85	0.99	0.93	0.92
+C	1.01	1.09	1.09	1.06
Mean	0.93	1.04	1.01	
Zn (mg/kg)				
−C	37.6	40.9	40.2	39.5
+C	37.2	40.7	40.1	39.3
Mean	7.4[e]	40.8[f]	40.1[f]	
Cu (mg/kg)				
−C	4.5	5.4	4.9	5.0
+C	5.1	5.9	5.7	5.6
Mean	4.8	5.6	5.3	

[a,b]Clover treatment means without a common superscript differ ($P < 0.10$); SE = 1.43, $n = 48$.
[c]Clover treatment × fertilizer treatment interaction ($P < 0.10$); SE = 0.89, $n = 16$.
[d]Clover treatment × fertilizer treatment interaction ($P < 0.05$); SE = 0.58, $n = 16$.
[e,f]Fertilizer treatment means without a common superscript differ ($P < 0.10$); SE = 2.05, $n = 32$.

which was expected because legumes normally have a higher concentration of protein than grasses. Alfalfa, for example, routinely contains approximately 20% or more CP, which is much higher than that in most forage grasses [16]. In the present study, mean foliar concentration of CP was 10.2% across forages and fertilizer-source treatments, which is more than adequate to support maintenance of a mature, nonlactating beef cow or modest daily liveweight gain in a growing beef steer [17]. Fertilizer source did not have an effect on CP concentration. Wood et al. [8] observed no difference in CP concentration in Bermudagrass that had been fertilized with ammonium nitrate or broiler litter, although CP concentration differed among forages receiving different rates of N application. In the current study, N-application rates were uniform among all fertilizer-source treatments. Across years, forages, and fertilizer-source treatments, regrowth harvests contained 9% less CP than primary-growth harvests. Similarly, Johnson et al. [18] reported that CP concentration declined over a growing season in Bermudagrass that had been harvested multiple times.

Neutral detergent fiber consists of partially and nonuniformly digestible fractions of total cell-wall constituents that are inversely related to voluntary DM intake, whereas ADF includes the least digestible and indigestible cell-wall constituents that are inversely related to DM digestibility [16]. Foliar concentration of NDF across forages and fertilizer-source treatments was 65.1% in the present study. Similarly, Adeli et al. [19] reported a range of 63.9 to 66.7% NDF in Johnsongrass that had been fertilized with swine effluent. Neutral detergent fiber concentrations were not different between Johnsongrass and Johnsongrass-clover forages or among fertilizer-source treatments. Previous studies have shown a significant decrease in NDF concentration in Bermudagrass grown in mixture with a legume [20, 21], consistent with the agronomic generalization that concentration of NDF in legumes is typically lower than that of grasses when compared at comparable stages of physiological maturity [16].

Foliar concentration of ADF was 35.4% across forages and fertilizer-source treatments in the present study. Similarly, Adeli et al. [19] observed a mean concentration of 39.2% ADF in Johnsongrass across multiple fertilizer-application rates, harvests, and years. No fertilizer-source or forage treatment effects were observed for ADF concentration in the present study, in agreement with Adeli et al. [19] who reported that fertilizer source had no effect on ADF concentration in Johnsongrass. Within the Johnsongrass treatment, forage amended with commercial fertilizer had 5% greater ADF concentration than that amended with noncompacted broiler litter. However, this difference, while statistically significant, would not be expected to have a material effect on *in vivo* digestibility by the ruminant animal. Similarly, Johnsongrass fertilized with commercial fertilizer had 5% greater ADF concentration than Johnsongrass-clover forage, but this difference is probably too small to predict a material effect on digestibility in the live animal.

Lignin is an indigestible polyphenolic compound that is covalently bound via ester and ethereal linkages with structural carbohydrates in the secondary cell wall. It is a major protractor of forage DM digestibility *in vivo* because of the negative effect of lignification on digestibility of NDF and ADF, of which ADL is a structural and analytical subset [16]. Foliar concentration of ADL was 3.2% across all harvests, years, forage, and fertilizer-source treatments in the present study and was not different between forage or among fertilizer-source treatments.

Relative feed value (RFV) is calculated by reference to a digestible DM intake that has been adopted to standardize a mature legume forage (e.g., full-bloom alfalfa) containing 53% NDF and 41% ADF to an RFV of 100 [22]. As such, it integrates intake and digestibility predicted from concentrations of NDF and ADF, respectively, into a single index that is used widely for describing forage nutritive quality and determining market value of grass and legume hays in the USA and Canada [23]. Mean RFV was 87.9 and was not different between Johnsongrass and Johnsongrass-clover forages or among fertilizer-source treatments. As such, nutritive quality of forage in the present study is estimated to be approximately 88% of that of a medium-quality alfalfa hay. By comparison, Franzluebbers et al. [24] reported that Coastal Bermudagrass ranged in RFV from 85 to 100 in their study.

Foliar concentration of P was not different between Johnsongrass and Johnsongrass-clover forages or among fertilizer-source treatments. Previous reports have indicated that P concentrations are generally lower in legumes than grasses [25]. Additionally, Wood et al. [8] reported no difference in concentrations of P between Bermudagrass that had been fertilized with broiler litter or ammonium nitrate.

Foliar K concentration was not different between Johnsongrass and Johnsongrass-clover forages or among fertilizer-source treatments concentrations but tended to be greater in forages amended with broiler litter than commercial fertilizer. Wood et al. [8] reported 38% greater K concentration in Bermudagrass receiving broiler litter than ammonium nitrate. Also, Johnsongrass-clover forage contained 12% more foliar K than Johnsongrass, in agreement with Whitehead et al. [25] who reported greater concentrations of foliar K in white clover than in common grasses. This finding, in conjunction with the observed increase in concentration of CP in the Johnsongrass-clover treatment, suggests that there may have been a sufficient amount of clover to alter at least some of the elemental compositional characteristics of the mixed-species treatment, even though the clover component may not have achieved its full growth potential.

One of the advantages of organic fertilizers over commercial fertilizer is their content of microelements that benefit plant productivity and nutrition of the grazing animal. However, it is important to monitor these for possible accumulation in soil and potential toxicity to livestock and humans [26]. Kingery et al. [4] reported that plant tissue samples collected from tall fescue (*Lolium arundinacea*) pastures receiving annual applications of broiler litter for 15 to 28 years had greater concentrations of Cu and Zn than unlittered pastures. Broiler litter can contain elevated concentration of Cu because it is used as an additive in poultry feed. However, foliar concentration of Cu did not differ between forages or fertilizer-source treatments in the present study, in agreement with previous studies on Bermudagrass fertilized with broiler litter and ammonium nitrate [8]. Zinc is a component of several key metalloenzymes that is routinely added to poultry feed and may be excreted at relatively high concentrations in fecal material [27]. Forages receiving commercial fertilizer had approximately 8% lower concentration of Zn than those receiving the broiler litter treatments, in contrast to findings by Wood et al. [8] who reported no difference in foliar concentrations of Zn in Bermudagrass fertilized with either broiler litter or ammonium nitrate. Foliar concentrations of Cu and Zn in the present study are within the range of requirements and well below the maximum tolerable limits for these minerals in cattle, sheep, and horses [28].

5. Conclusions

Results of this study indicate that pressure-compacted broiler litter supported productivity and nutritive quality of Johnsongrass that were comparable to those from noncompacted broiler litter. Also, broiler litter applied on the basis of soil-test P and supplemented with ammonium nitrate to meet crop N requirement supported productivity and nutritive quality of Johnsongrass comparable to that from commercial fertilizer. Pressure compaction may enable economical transportation of broiler litter from areas of intensive poultry production to resource-poor agricultural areas, providing limited-resource farmers with a cost-effective alternative to commercial fertilizer while at the same reducing P loading onto soils in areas of intensive poultry production.

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Research Article

Organic Amendments and Earthworm Addition Improve Properties of Nonacidic Mine Tailings

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In many mined areas, lack of topsoil limits conversion of disturbed landscapes to former or other productive uses. We examined the use of biosolids (10 or 20% by dry mass), with or without sawdust, pulp sludge, and the contribution of an earthworm species (*Dendrobaena veneta*) to improve the properties of nonacidic mine tailings. Pulp sludge more rapidly immobilized excessive NH_4^+ concentrations from biosolids early in the study; however, total mineral N concentrations were similar in pulp sludge and sawdust treatments by week 29. Although NO_3^--N concentrations were generally greater in treatments with earthworms, these trends were not statistically significant ($P > 0.05$). In general, Bray P concentrations were greater in the presence of earthworms. Soil thin sections showed that earthworms mixed organic residues into elongated spherical units within mine tailings. Organic residues in combination with earthworm addition may improve the chemical and microstructural properties of non-acidic mine tailings, producing a substrate conducive for plant establishment.

1. Introduction.

Mining operations around the world produce large quantities of residuals such as tailings, overburden, and waste rock. Past mineral extractions in the Sierra de Cartagena (SE Spain) for >2,500 years left behind large volumes (i.e., usually 700,000–900,000 m^3 per deposit) of wastes composed of overburden rocks and tailings [1]. A copper and gold mine in northern British Columbia (Canada) generated nearly 25 million tonnes of milled waste rock in 2007 [2]. In Canada and other jurisdictions, industry is required to convert disturbed landscapes to former or other productive uses following mine extraction and processing activities. High-quality topsoil is often limiting at mine sites, so mines often need to utilize on-site substrates (such as mine tailings) to create mine soils for reclamation activities.

Establishment of vegetation is key to reclamation of mine tailings and other residuals [2–5]. Plant cover minimizes the dispersion of particulate matter and contaminants through wind and water erosion, and improves the aesthetic value of unvegetated landscapes [6, 7]. However, mine soils require suitable physical and chemical properties in order to support the growth of plants and associated soil organisms. Tailings are typically fine sands low in organic matter and plant available macronutrients such as N and P.

Various industrial, environmental, and municipal waste treatment facilities generate organic materials that can be added to mine residuals to increase soil organic matter (SOM) and/or nutrient contents [8–12]. Biosolids, also called sewage sludges, are particularly well suited for this purpose due to their relatively high N and P contents [10, 13]. The addition of organic residues to mine soils accelerates soil forming processes, and this practice is also a means of promoting carbon sequestration through the conversion of waste substrates into SOM [12, 14]. Plant-derived organic inputs also contribute to SOM and play an important role in initiating soil development [15, 16].

In natural soils, pedogenic mixing of inorganic and organic materials is best achieved by soil organisms, particularly soil invertebrate ecosystem engineers [17]. Earthworms

intimately mix organic and inorganic materials into soil aggregates through ingestion and excretion [18, 19]. Earthworms have also been reported to stimulate soil microbial activity and enhance the availability of soil nutrients such as nitrogen and phosphorus [17, 20, 21]. Earthworms have the potential to enhance the reclamation mine residuals, but even nonacidic mine residuals provide a hostile environment to these sensitive animals [22]. The addition of organic materials may help overcome these limitations by providing an energy source, enhancing nutrient levels, stimulating microbial activity, promoting soil structural development, and increasing water-holding capacity [22, 23].

Addition of biosolids to mine tailings (or to other inorganic substrates) can lead to high concentrations of NH_4^+/NH_3, which is toxic to earthworms at relatively low concentrations [24]. The coapplication of high C/N ratio organic substrates with biosolids may provide the benefit of net NH_4^+-N immobilization into the microbial biomass; this organic N can then undergo slow net N mineralization over a period of time. Addition of these supplementary substrates may have additional benefits of enhancing soil physical and chemical properties relative to tailings only receiving biosolids.

The primary objective of this 29-week laboratory study was to determine the influence of pulp sludge or softwood sawdust coapplied with biosolids on selected chemical and physical properties of nonacidic mine tailings. A secondary objective was to test the role of an earthworm species (*Dendobaena veneta*) for altering the selected physical and chemical properties of these amended mine tailings. Although *Dendobaena veneta* is considered to be an epigeic earthworm, we selected this species because it is hardy and has been shown to be active in mineral soils, including those containing organic or inorganic contaminants [25]. It is also widely used in vermicomposting and is readily available. To the best of our knowledge no studies have reported the use of this earthworm in the treatment of mine tailings.

2. Materials and Methods

2.1. Collection and Characterization of Substrate Materials. Inorganic and organic materials were obtained from industrial and municipal sources in north central BC (Canada). Desulphurized mine tailings were obtained from the Mount Polley copper mine near Likely, BC; anaerobically digested sewage sludge (BC Class B biosolids) was obtained from the Prince George municipal wastewater treatment facility; softwood sawdust (mainly from pine) was obtained from a local sawmill; pulp mill sludge was obtained from the Canfor Intercon Kraft pulp mill, Prince George, BC. Materials were allowed to air-dry for several weeks on plastic sheets prior to use in this study.

Substrates were well homogenized prior to characterization. Mine tailings were subjected to the following chemical analyses: total C, N, and S (dry combustion), total P, and total elemental analyses using a combination of lithium metaborate fusion followed by ICP-AES (major elements), lithium borate fusion followed by ICP-MS (trace elements) and 1 : 1 concentrated HNO_3 : HCl digestion followed by ICP-MS (Hg, Se). Organic substrates were homogenized and subjected to the following chemical analyses: total C, N, and S (dry combustion), total P (microwave digestion followed by ICP OES), extractable NH_4^+-N and NO_3^--N via KCl extraction followed by AutoAnalyzer [26]. Elements were determined by digestion in 1 : 1 concentrated HNO_3 : HCl followed by ICP-OES (metals, metalloids) or cold-vapor atomic fluorescence spectrophotometry (Hg). X-ray diffraction (XRD) and elemental concentrations were used to estimate (±5%) the mineralogy of the tailings. Particle size distribution of the mine tailings was determined by dry sieving [27].

As expected, concentrations of total N (4.95 g 100 g^{-1}) and total P (4.94 g 100 g^{-1}) in biosolids were orders of magnitude greater than those in sawdust, pulp mill sludge, and mine tailings (Table 1). Ammonium–N, NO_3^--N and Bray-P in biosolids were 2490, 2.7, and 3042 mg kg^{-1}, respectively, immediately prior to use in this study; concentrations in pulp sludge and sawdust were negligible (data not shown). Potassium (4.77 g 100 g^{-1}) and Cu (656 mg Cu kg^{-1}) contents in mine tailings were much higher than organic substrates (Table 1). Semiquantitative estimates of the mineral composition of the tailings (via XRD and elemental composition) were 8.5% kaolinite, 2.3% goethite, 0.6% clinochlore, 1% quartz, 9.5% calcite, 0.4% muscovite, 77% feldspars, and 0.6% ilmenite. Particle size analysis of mine tailings showed the following proportions of size classes by mass (mean; standard deviation in brackets) <63 μm – 12 (3.8)%, 63–125 μm – 58 (5.4)%, 125–250 μm – 26 (3.7)%, 250–500 μm – 3 (0.9)%, 500–1000 μm – 0.8 (0.4)%, and >1000 μm – 0%.

2.2. Experimental Setup and Treatments. The experimental design consisted of 2 levels of earthworm addition (with and without) and 8 combinations of organic materials added to mine tailings (Table 2). There were three replications for each treatment (i.e., a total of 48 experimental units). Each mixture was contained within a 4-L white plastic bucket (19 cm ø × 15 cm height) that had ∼ forty 2 mm holes drilled on its wall to maintain sufficient aeration. Mine tailings were added at a rate of 1500 g (equivalent oven-dry basis) to each container with the appropriate amounts of organic substrates. Moisture content was adjusted to 75% of water-holding capacity (WHC; see below) with tap water and the experimental units were incubated at 20°C for 7 weeks prior to earthworm addition to appropriate experimental units (Table 2). A plastic lid (with ∼ thirty 2 mm holes) covered each bucket during incubation to minimize water evaporation. The 7-week incubation was conducted to stimulate microbial activity and to encourage net immobilization of NH_4^+-N that originated from the biosolids prior to the addition of earthworms. As mentioned above, high concentrations of NH_4^+/NH_3 are known to be toxic to earthworms [24].

2.3. Earthworm Addition, Experimental Conditions, and Sample Collection. At week 7, subsamples from all experimental units were removed for chemical analysis, and fifteen earthworms (*Dendrobaena veneta*) were added to one-half of

Table 1: Elemental composition of mine tailings, biosolids, pulp sludge, and sawdust used in this experiment.

Element	Units	Mine tailings	Biosolids	Pulp sludge	Sawdust
Si	g 100 g^{-1}	24.2	ND	ND	ND
Al	g 100 g^{-1}	8.92	1.7	0.06	0.004
Fe	g 100 g^{-1}	6.24	1.4	0.09	0.005
Ca	g 100 g^{-1}	3.47	3.1	0.91	0.07
Mg	g 100 g^{-1}	1.24	0.47	0.15	0.01
K	g 100 g^{-1}	4.77	0.19	0.01	0.02
Na	g 100 g^{-1}	2.97	ND	ND	ND
Mn	g 100 g^{-1}	0.09	0.06	0.01	0.01
C	g 100 g^{-1}	0.80	36.8	42.9	51.3
N	g 100 g^{-1}	0.007	4.95	0.027	0.050
S	g 100 g^{-1}	0.89	1.36	0.01	<0.01
P	g 100 g^{-1}	0.17	2.31	0.01	<0.01
As	mg kg^{-1}	20.9	5.2	<0.5	<0.5
Cd	mg kg^{-1}	0.53	2.8	<0.5	<0.5
Co	mg kg^{-1}	18.5	4.0	<2.0	<2.0
Cr	mg kg^{-1}	10	35	4	<2
Cu	mg kg^{-1}	660	2.3	3.8	3.0
Pb	mg kg^{-1}	7	62	<30	<30
Hg	mg kg^{-1}	0.157	6.73	0.011	0.079
Mo	mg kg^{-1}	8	15	<4	<4
Ni	mg kg^{-1}	7	25	<5	<5
Se	mg kg^{-1}	3	10	<2	<2
Zn	mg kg^{-1}	8	790	8	8

Concentrations are presented on an oven-dry equivalent (mass) basis.
ND: not determined.
Hg concentrations in biosolids from this facility are usually less than 1 mg kg^{-1}.

Table 2: Experimental treatments used in this study. Proportions (g amendment 100 g^{-1} mine tailings) of organic amendments are presented on an equivalent oven-dry basis relative to initial mine tailings.

			Treatment code	
Biosolids	Sawdust	Pulp sludge	Without earthworms	With earthworms
10	10	0	T1-WO	T1-W
10	0	10	T2-WO	T2-W
10	5	5	T3-WO	T3-W
10	0	0	T4-WO	T4-W
20	10	0	T5-WO	T5-W
20	0	10	T6-WO	T6-W
20	5	5	T7-WO	T7-W
20	0	0	T8-WO	T8-W

Appropriate treatments received earthworms 7 weeks after the substrates were mixed and the incubation was started.

the treatments (Table 2). The incubation was conducted in a lighted room to discourage the escape of earthworms from the experimental units. Average (±SD) live weight (with full guts) of the earthworms was 1.15 ± 0.11 g per individual. Earthworms tunneled into the organic amended tailings immediately after addition except for treatments T4-W and T8-W where earthworms remained on the surface and died within a day of addition; treatments T4-W and T8-W were discontinued due to the immediate death of earthworms.

At week 13, the amended tailings from all experimental units were removed, placed into trays, and examined for earthworm survival. Subsamples of tailings were also removed for chemical analysis. Earthworms in treatment T5-W did not survive to week 13. Instead of discontinuing T5-W, fifteen new earthworms were added to the experimental units of this treatment at week 16.

Incubations were continued at 20°C for the duration of the experiment (week 29). Moisture loss was minimal and

small water additions were only required every 2-3 weeks. Earthworm populations were counted and tailings samples were collected for chemical analyses and WHC measurements at the conclusion of the experiment. Samples were briefly stored at 4°C prior to chemical analyses.

2.4. Laboratory Analyses. Amended tailings were analyzed for selected physical and chemical properties. Water-holding capacity was determined by placing 300 g of tailings into a 500 mL plastic cylinder (bound with cheese cloth on the bottom) and tapping firmly three times in order to standardize packing. The tailings were then water-saturated from the bottom of the container. After 24 h of saturation, the tailings were allowed to drain freely for 10 h under conditions designed to minimize evaporation; free drainage had ceased at this time and subsamples were then removed for moisture content analysis. Gravimetric moisture content (g H_2O 100 g^{-1} oven-dry (OD) tailings) was determined by drying tailings overnight at 105°C. The pH was measured in 1 : 4 solids to deionized H_2O (g : mL) suspension [28]. Electrical conductivity of tailings was determined in saturated paste extracts using a conductivity meter [29]. Total C and N were determined on <100-mesh samples (air-dried, then ground in a ball mill) by dry combustion using an elemental analyzer [30]. Available N (NH_4^+-N and NO_3^--N) was determined by extraction with 2 M KCl, followed by colorimetric N determination [26]. Potentially mineralizable N (NH_4^+-N plus NO_3^--N) was determined by 7-day anaerobic incubation at the end of the study [31]. Cation exchange capacity (CEC) and exchangeable K, Ca, Mg, and Na were determined at pH 7.0 using the NH_4OAc method [32]. Available P was determined on samples at the end of the incubation by the Bray 1 procedure [33].

2.5. Microstructural Analysis. After the laboratory analyses, we thought that additional spatial information about the soil structure would be useful to complement our observations. We made thin sections from two selected treatments (T2-WO and T2-W) to qualitatively describe the changes in soil microstructure due to organic and earthworm amendments. Thin sections were prepared by impregnating the sample with epoxy resin and subsequent grinding and polishing using corundum to a 30 μm soil section. We described the sections under a Nikon Eclipse E600 polarizing microscope at the University of Northern British Columbia (Prince George, Canada) for micromorphological characteristics following the concepts and terminology in [34].

2.6. Statistical Analysis. The observed parameters were analyzed using a 1-way ANOVA in Statistica, v 6 [35]. Differences between treatments were determined by Student Newman Keuls (SNK) test. Earthworm numbers were transformed (log10) prior to statistical analysis.

3. Results

3.1. Chemical Properties of Amended Mine Tailings at Beginning of Incubation. Subsamples obtained 24 h following the mixing of substrates showed the relatively neutral pH and moderate electrical conductivity in the experimental treatments (Table 3). Nitrate-N concentrations were low, but NH_4^+-N concentrations were very high with treatment averages ranging from 254 to 654 mg N kg^{-1}. Treatments receiving 20% biosolids had significantly greater NH_4^+-N and total N than treatments receiving 10% biosolids.

3.2. Dynamics of Nitrogen in Response to Amendment Additions. Within each of the two biosolid addition rates, the concentration of NH_4^+-N at week 7 was lowest in treatments T2-WO (Figure 1(a)) and T6-WO (Figure 1(b)), the two treatments that also received 10% pulp sludge. In contrast, treatments T4-WO (Figure 1(a)) and T7-WO (Figure 1(b)), the two biosolids-only treatments, had very high NH_4^+-N concentrations at week 7. By week 13, all treatments had very low NH_4^+-N concentrations, except for the two biosolids-only treatments.

Concentrations of NO_3^--N increased over the experiment in most treatments, but did not show any significant earthworm effects (Figures 2(a) and 2(b)). By week 13, NO_3^--N concentrations were generally much greater in treatments receiving 20% biosolids than those treatments receiving 10% biosolids.

For each of the two biosolid addition rates, the decline in C/N ratio for amended tailings was more extensive and more rapid for treatments also receiving 10% pulp sludge (Figures 3(a) and 3(b)). The presence of earthworms did not have a significant effect on C/N ratios. Treatments receiving 20% biosolids generally had lower C/N ratios than treatments receiving 10% biosolids.

3.3. Selected Properties of Amended Mine Tailings at End of Study. Comparison of treatment effects on nitrogen parameters showed that the presence of earthworms did not significantly influence C/N ratio, NH_4^+-N, or potentially mineralizable N at the end of the study (Table 4). Although NO_3^--N concentrations tended to be greater for an amendment treatment when earthworms were present, these differences were usually not statistically significant (Table 4). Potentially mineralizable N, total N, and NO_3^--N concentrations were generally greater in treatments receiving 20% biosolids as compared to treatments receiving 10% biosolids.

Earthworms did not have significant effects on pH, WHC, or CEC (Table 5). Although average values for EC tended to be greater for amendment treatments when earthworms were present, these differences were not statistically significant. The two biosolids-only treatments (T4-WO and T8-WO) had the greatest EC values measured in the study. Values of CEC were generally greater in treatments receiving 20% biosolids as compared to those receiving 10% biosolids. There was a positive correlation between NO_3^--N concentrations and EC at the end of the study ($r = 0.773$, $P < 0.0001$), as well as throughout the experiment ($r = 0.833$, $P < 0.0001$).

The presence of earthworms was associated with higher Bray P concentrations within amendment treatments by the end of the study. Average Bray P was significantly greater

Table 3: Selected chemical properties of amended mine tailings sampled 24 hours after experimental setup. Means ± standard deviation ($n = 3$). Values designated by the same letter (within a column) are not significantly different from each other ($P < 0.05$).

Treatment	pH (water)	EC (dS m^{-1})	NH4$^+$-N (mg kg^{-1})	NO$_3^-$-N (mg kg^{-1})	Total N (g 100 g^{-1})
			Treatments with 10% biosolids		
T1-WO	7.37 ± 0.03c	3.19 ± 0.05d	345 ± 20c	0.3 ± 0.1a	0.34 ± 0.03b
T2-WO	7.72 ± 0.06a	2.34 ± 0.04e	254 ± 9d	0.3 ± 0.1a	0.30 ± 0.04b
T3-WO	7.35 ± 0.01c	3.16 ± 0.06d	261 ± 70d	1.9 ± 0.2a	0.30 ± 0.09b
T4-WO	7.43 ± 0.02bc	5.29 ± 0.12b	387 ± 19c	0.1 ± 0.1a	0.35 ± 0.01b
			Treatments with 20% biosolids		
T5-WO	7.24 ± 0.06d	4.69 ± 0.09c	615 ± 10a	0.2 ± 0.03a	0.60 ± 0.02a
T6-WO	7.45 ± 0.03b	3.50 ± 0.06d	491 ± 25b	1.3 ± 0.1a	0.57 ± 0.04a
T7-WO	7.26 ± 0.02d	4.44 ± 0.27c	524 ± 31b	0.9 ± 0.3a	0.64 ± 0.06a
T8-WO	7.27 ± 0.05d	6.34 ± 0.06a	654 ± 26a	0.3 ± 0.3a	0.60 ± 0.07a

Concentrations are expressed on an oven-dry equivalent basis.
For reference, pH and EC of pure mine tailings were 8.31 ± 0.05 and 1.80 ± 0.04 dS m^{-1}, respectively ($n = 3$).

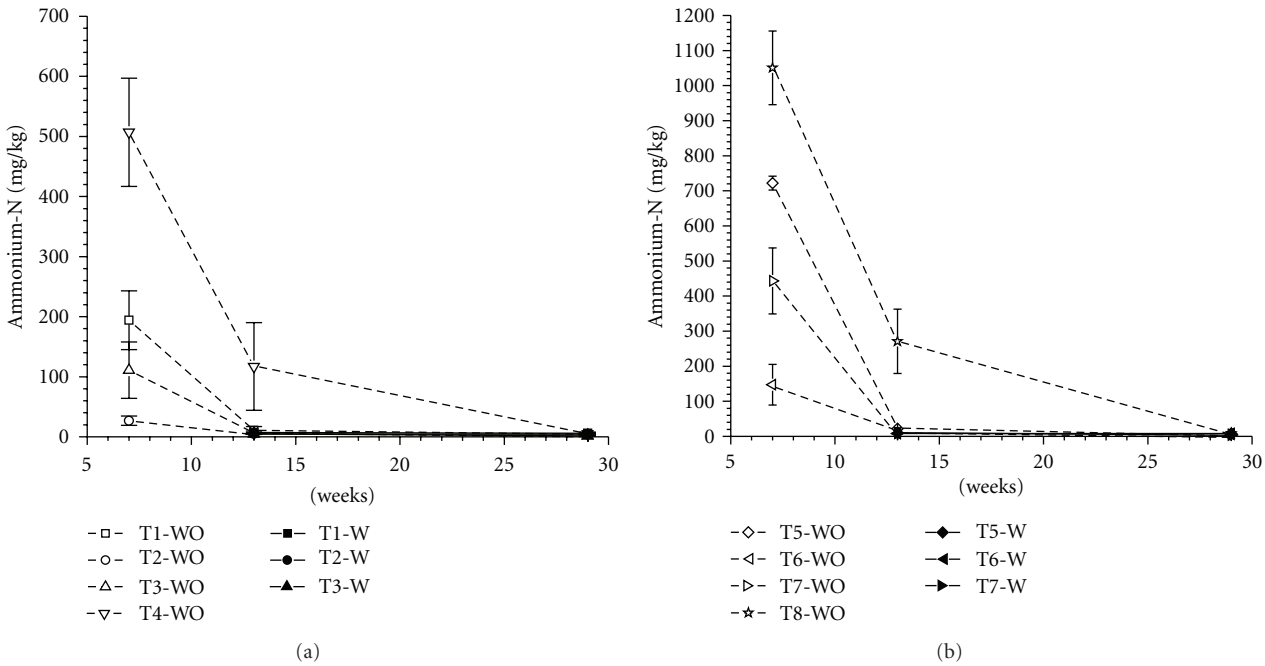

Figure 1: Mean NH$_4^+$-N concentrations in amended mine tailings; (a) treatments that included 10% biosolids addition; (b) treatments that included 20% biosolids addition. Error bars indicate (±1 SD; $n = 3$). See Table 2 for explanation of treatment codes.

in several amendment mixtures when earthworms where present as compared to when they were absent (Table 5).

3.4. Earthworm Populations and Other Observations. Earthworms in treatments that received pulp sludge (T2-W, T3-W, T6-W, and T7-W) were large and very active throughout most of the study. Earthworm activity in these treatments was very apparent by week 13, with young earthworms being present in experimental units with larger earthworms. Abundant fungal growth was clearly visible at week 7, the time of earthworm addition, in treatments that received pulp sludge. In general, by the end of the study earthworms were smaller and less active than those observed at week 13. Earthworm numbers at the end of the study tended to be greater in treatments that included pulp sludge (T2-W, T3-W, T6-W, and T7-W) and least in treatments that only received biosolids and sawdust (T1-W and T7-W; Table 6).

Treatments with earthworms often exhibited small tunnels within the tailings at the bottom of the experimental units, and mineral surfaces exhibited shinier surfaces relative to treatments without earthworms; this was more apparent in pulp sludge-amended treatments. Although not measured in this study, the addition of pulp sludge appeared to aggregate the tailings more than those receiving sawdust. The "aggregates" observed in this study included true aggregates

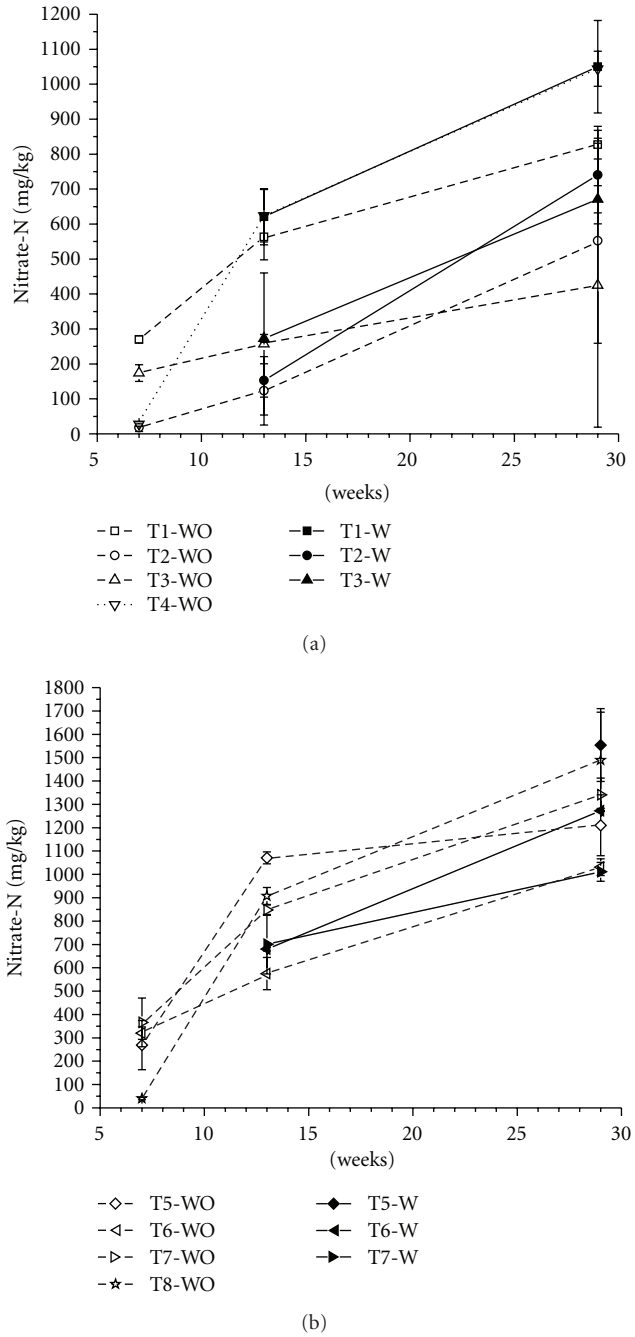

Figure 2: Mean NO_3^--N concentrations in amended mine tailings; (a) treatments that included 10% biosolids addition; (b) treatments that included 20% biosolids addition. Error bars indicate (±1 SD; $n = 3$). See Table 2 for explanation of treatment codes.

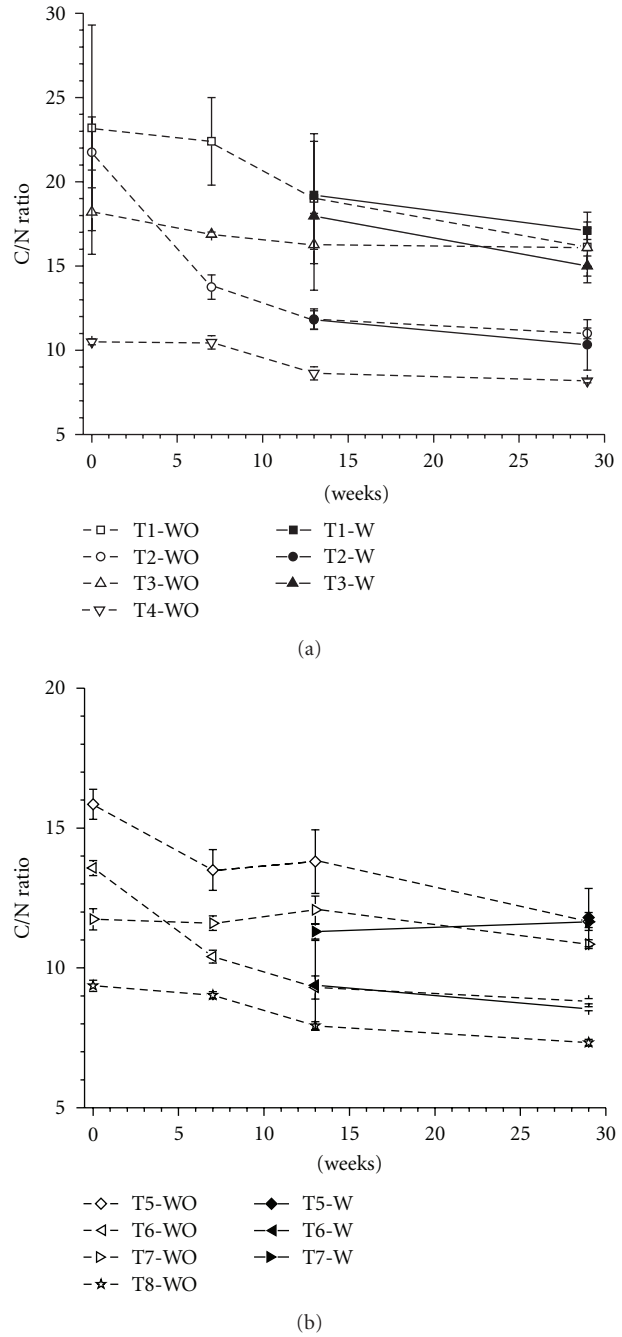

Figure 3: Mean C/N ratio of amended mine tailings; (a) treatments that included 10% biosolids addition; (b) treatments that included 20% biosolids addition. Error bars indicate (±1 SD; $n = 3$). See Table 2 for explanation of treatment codes.

(i.e., primary particles aggregated weakly into secondary aggregates) and also residual biosolid particles and other residual organic debris (e.g., wood sawdust) from the organic amendments.

3.5. Soil Microstructure. By week 29, organic residues added to the mine tailings were still recognizable in soil thin sections (Figure 4). These residues were intimately mixed with the inorganic materials in T2-W (with earthworms) (Figures 4(a) and 4(b)) while discrete residues were present in isolated patches in T2-WO (without earthworms; Figures 4(c) and 4(d)). The contiguous discrete rounded microfabric units in T2-W formed an outline of elongated (~ 8 mm × 4 mm) spherical units composed of organic and mineral materials (Figure 4(a)). The discrete units of residues in T2-WO exhibited a cracked pattern (Figure 4(d)) not observed in

Table 4: Mean (±standard deviation) C/N ratio, ammonium-N, nitrate-N, potentially mineralizable N (7-day anaerobic incubation), and total nitrogen at the end of the study (week 29). Values designated by same letter (within a column) are not significantly different from each other by SNK ($P < 0.05$; $n = 3$).

Treatment	C/N ratio	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	Mineralizable-N (mg kg^{-1})	Total N (g 100 g^{-1})
			Treatments with 10% biosolids		
T1-WO	16.1 ± 1.1ab	4.3 ± 0.7d	827 ± 41cde	71.2 ± 16.8cde	0.39 ± 0.04c
T1-W	17.1 ± 0.5a	6.0 ± 1.9bcd	1050 ± 132bcd	37.3 ± 11.4e	0.35 ± 0.02cd
T2-WO	11.0 ± 0.3c	4.1 ± 0.1d	553 ± 293ef	73.9 ± 5.6cde	0.36 ± 0.03cd
T2-W	10.3 ± 0.4c	4.3 ± 1.5d	740 ± 139de	51.0 ± 14.6de	0.34 ± 0.01cd
T3-WO	16.1 ± 2.1ab	1.9 ± 0.2e	425 ± 406f	65.4 ± 14.1cde	0.31 ± 0.02cd
T3-W	15.0 ± 0.6b	1.7 ± 0.2e	672 ± 39de	30.4 ± 7.9e	0.30 ± 0.01d
T4-WO	8.2 ± 0.1d	4.9 ± 0.6d	1043 ± 50bcd	102.9 ± 10.3bcd	0.39 ± 0.02c
			Treatments with 20% biosolids		
T5-WO	11.7 ± 0.3c	6.9 ± 0.8abc	1210±131abc	153.6 ± 48.6ab	0.64 ± 0.09ab
T5-W	11.8 ± 1.0c	7.9 ± 0.8ab	1554±156a	184.3 ± 23.3a	0.69 ± 0.05a
T6-WO	8.8 ± 0.1d	5.3 ± 0.9cd	1031±36bcd	141.7 ± 41.8ab	0.59 ± 0.03b
T6-W	8.5 ± 0.1d	8.0 ± 0.5a	1272±40ab	199.6 ± 33.9a	0.69 ± 0.05a
T7-WO	10.8 ± 0.2c	4.5 ± 0.2d	1341±72ab	144.7 ± 25.7ab	0.61 ± 0.004ab
T7-W	11.7 ± 0.2c	3.7 ± 0.6d	1011±40bcd	112.4 ± 9.8bc	0.58 ± 0.02b
T8-WO	7.3 ± 0.1d	8.4 ± 1.1a	1490±206a	157.5 ± 22.0ab	0.63 ± 0.01ab

Concentrations are expressed on an oven-dry (mass) equivalent basis.
See Table 2 for explanation of treatments.

Table 5: Mean (± standard deviation) pH, electrical conductivity, Bray extractable phosphorus, cation exchange capacity, and water holding-capacity at the end of the study (week 29). Values designated by same letter (within a column) are not significantly different from each other ($P < 0.05$; $n = 3$).

Treatment	pH	EC (dS m^{-1})	Bray P (mg P kg^{-1})	CEC (cmol$_c$ kg^{-1})	WHC (g H_2O 100 g^{-1})
			Treatments with 10% biosolids		
T1-WO	6.77 ± 0.01bcd	9.00 ± 0.38de	237 ± 20cd	5.81 ± 0.48ef	68.8 ± 3.6abc
T1-W	6.64 ± 0.07 de	10.04 ± 0.63 de	324 ± 123bc	7.32 ± 0.60cdef	66.3 ± 3.1bcd
T2-WO	6.91 ± 0.03bc	7.58 ± 0.58ef	195 ± 57cd	5.32 ± 0.66f	54.1 ± 7.8d
T2-W	6.66 ± 0.05 cde	10.01 ± 1.30de	487 ± 63a	7.26 ± 0.38cdef	58.6 ± 1.4cd
T3-WO	7.27 ± 0.31a	5.65 ± 2.70f	224 ± 16cd	7.68 ± 0.44cdef	64.1 ± 5.6bcd
T3-W	6.83 ± 0.04bcd	7.76 ± 0.40ef	482 ± 78a	6.19 ± 0.51def	68.7 ± 6.3abc
T4-WO	6.97 ± 0.01b	16.0 ± 0.25a	117 ± 24d	4.87 ± 1.26f	40.0 ± 1.6e
			Treatments with 20% biosolids		
T5- WO	6.72 ± 0.12cd	11.37 ± 0.83cde	270 ± 22bc	9.77 ± 0.48abc	81.9 ± 3.4a
T5-W	6.57 ± 0.02def	12.51 ± 1.45 cd	349 ± 23abc	10.36 ± 0.53abc	77.7 ± 10.2ab
T6-WO	6.46 ± 0.02ef	11.24 ± 3.65cde	296 ± 22bc	8.46 ± 0.68bcde	64.7 ± 3.0bcd
T6-W	6.36 ± 0.06f	13.89 ± 0.60bc	393 ± 12ab	8.74 ± 2.16bcd	74.3 ± 2.7ab
T7-WO	6.63 ± 0.04cde	12.50 ± 0.91cd	257 ± 12 bc	11.63 ± 0.97a	70.0 ± 6.6abc
T7-W	6.90 ± 0.10bc	9.88 ± 0.37de	463 ± 118a	11.21 ± 1.35ab	73.9 ± 4.9ab
T8-WO	6.63 ± 0.03cde	20.1 ± 0.94a	271 ± 11bc	10.6 ± 2.22ab	55.6 ± 4.9d

Bray P, CEC, and WHC are expressed relative to oven-dry (mass) tailings.

rounded microfabric units (Figure 4(b)). Accumulations of near pure organic matter normally exhibit a cracked pattern upon desiccation [36]. In a typical microfabric in T2-WO, a large section (i.e., upper 75% in Figure 4(a)) is composed of mixed organic matter and mine tailings. This contrasts with T2-WO where the microfabric is composed mainly of mine tailings, with isolated patches of discrete residues (Figure 4(c)).

4. Discussion

The mine tailings in this study consisted mainly of feldspar mineral particles of a very fine sand texture. Concentrations of trace elements were generally low, with the exception of copper (660 mg kg^{-1}). For comparison, the Canadian Soil Quality Guidelines for copper in agricultural and industrial soils are 63 and 91 mg kg^{-1}, respectively [37]. The biosolids

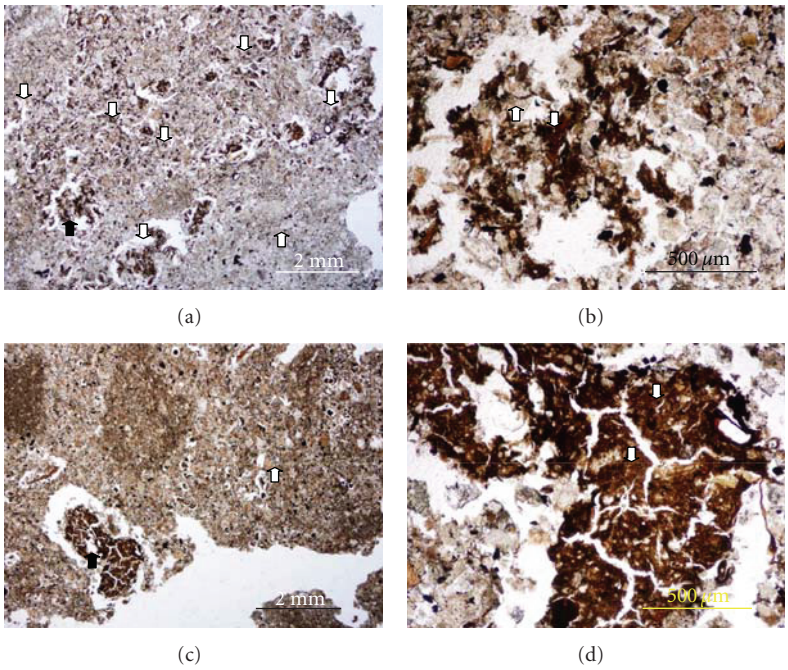

Figure 4: Microfabric of mine tailings amended with organic residues with or without earthworms. After 29 weeks, the organic residues (white down arrow) were intimately mixed in (a) T2-W (10% biosolids, 10% pulp sludge, with earthworms), compared to (c) discrete unit showing cracked pattern in T2-WO (10% biosolids, 10% pulp sludge, without earthworms). Close-up images of sections marked by (black up arrow) in (a) and (c) show the (b) intimate mixing of organic residues (white down arrow) with inorganic materials (white up arrow) and (d) the cracked pattern of organic residues (white down arrow) not mixed with inorganic materials.

Table 6: Mean earthworm numbers (± standard deviation) in each of the experimental units at the end of the study ($n = 3$).

Treatment	Earthworm numbers
T1-W	7.0 ± 3.6ab
T2-W	19.7 ± 17.6a
T3-W	12.7 ± 0.6ab
T5-W	2.0 ± 2.8b
T6-W	20.3 ± 10.4a
T7-W	11.7 ± 3.2 ab

Treatments T4-W and T8-W were discontinued at week 7 when earthworms died immediately after addition.

amendments are increasingly being used in the reclamation of mine soils mainly to improve plant nutrient levels and to improve soil chemical and physical properties [10]. In this study, we show that coapplication of other organic substrates can enhance soil properties compared to the addition of biosolids alone. The epigeic earthworm *Dendobaena veneta* only survived in biosolids-amended mine tailings when other organic substrates were coapplied. Pulp sludge addition appeared to be more beneficial to earthworms than softwood sawdust.

4.1. Effect of Immobilizer Substrates on Soil Properties. Very low concentrations of N and P (e.g., <0.01% and <0.20%, resp., in this study) are often the most limiting characteristics to plant growth in nonacidic mine tailings. The current study is consistent with other research that shows addition of biosolids improves total N content of mine residuals such as tailings [38]. Although total N concentrations after 29 weeks of organic residue addition were <0.7%, these concentrations are typical of temperate cultivated soils [30] and are also comparable to soil organic N accumulation in pioneer species in acidic mine tailings in southeast Spain [15]. This increase in total N coupled with more available P in organic and earthworm amended tailings may be sufficient to initiate soil formation and promote plant growth in nonacidic mine tailings. In the Czech Republic, Frouz and Nováková [39] argued that soil formation and organic matter accumulation is crucial to the restoration of ecosystem functions in post-mining landscape.

In addition to further improving soil physical properties, the use of "immobilizer" substrates can be used to manipulate nitrogen originating from N-rich substrates. Chaves et al. [40] define immobilizer substrates as those which have net N immobilization potential. Biosolids used in this study initially had a low C/N ratio (7.4) and very high NH_4^+-N concentrations (2490 mg NH_4^+-N kg^{-1}). High NH_4^+-N concentrations may lead to excessive N losses in field soils through volatilization (NH_3) or as NO_3^--N leaching following nitrification [41]. High NH_4^+/NH_3 concentrations are also toxic to some soil invertebrates such as earthworms [24] and young plants. Immobilizer substrates can temporarily immobilize mineral N (NH_4^+-N or NO_3^--N) into organic matter (e.g., microbial biomass) which then gradually remineralize the nitrogen over time, ideally in synchrony with plant demand [40].

In this study, pulp sludge was a more effective immobilizer substrate than wood shavings. At 7 weeks, the mineral N (NH_4^+-N+ NO_3^--N) concentration in the 10% biosolids : 10% pulp sludge treatment (T2-WO) was only 45 mg N kg^{-1} soil, compared to 464 mg N kg^{-1} in the 10% biosolids : 10% wood shavings (T1-WO). For comparison, the 10% biosolids-only treatment (T4-WO) had a mineral N concentration of 535 mg N kg^{-1}. The more rapid and extensive drop in C/N ratio in T2-WO compared to T1-WO over the first 7 weeks is consistent with greater microbial activity (and loss of C as respired CO_2-C) in the pulp sludge amended tailings compared to the sawdust-amended tailings (Figure 3(a)). Total carbon concentration decreased by 28% in the T2-WO treatment over the first 7 weeks, while the change in the T1-WO treatment was negligible (data not shown). Results for mineral N and C/N for the 20% biosolids treatments at 7 weeks followed the same trends but pulp sludge concentrations were not great enough to reduce mineral N concentrations to the same extent as in the 10% biosolids treatments (Figure 3(b)). The very high concentrations of NH_4^+ -N in the 10% biosolids-only treatment (T4-WO; 507 mg N kg^{-1}), 20% biosolids-only treatment (T8-WO; 1051 mg N kg^{-1}), and the 20% biosolids: 10% sawdust treatment (T5-WO; 721 mg N kg^{-1}) at 7 weeks likely contributed to the death of the earthworms that were added at that time [24]. Similar NH_4^+ -N and NO_3^- -N concentrations, and potentially mineralizable N, at the end of the study (within a biosolids application rate) suggest that all organic matter amendments had similar effects on available N over the longer term.

The decrease in pH observed in this study supports earlier literature that soils receiving organic substrates, due largely to formation of carbonic and other acids during decomposition processes and increased nitrification, increases the acidity of the artificial soils. The slight decrease in pH might render P more available to plants [42].

The greater CEC of tailings when amended with high amounts of biosolids (20%) is expected because organic materials are major sources of CEC in soils. Total acidities in isolated humus fractions separated from well-decomposed organic residues range from 300 to 1400 $cmol_c$ kg^{-1} humus [43].

4.2. Influence of Amendments and Earthworms on Mine Tailings. Earthworms can be classified into three ecological types: epigeic, endogeic, and anecic, based on their burrowing habits and habitat [44]. *Dendrobaena veneta* has been described as an epigeic earthworm (i.e., prefers organic materials such as organic litter layers or in compost). To the best of our knowledge, this is the first report of successful inoculation of *Dendrobaena veneta* addition to nonacidic sandy mine tailings. Earthworms generally do not do well in sandy substrates [22]; however, the tailings in this study were composed largely of feldspars, which are likely less abrasive than silica-based sands.

The survival of earthworms in amended nonacidic mine tailings (very fine sand) is quite significant because macrofaunal colonization of mine wastes is critical to the development of soils necessary to support plant establishment in postmine sites [15, 45]. Earthworms are "ecosystem engineers" because they transform massive soil to favorable habitats for other organisms [46]. They intimately mix organic and inorganic materials into soil aggregates through ingestion and excretion [18]; they are the main producers of granular structure in many soils. We argue that the elongated spherical units observed in the microstructure of biosolid-pulp sludge amended soils with earthworms (i.e., T2-W) represent the initial stages in the formation of soil aggregates. Similar microstructural units observed in acid mine tailings amended with organic residues are the precursor to the formation of granular structure [47]. Granular structure is ideal for plant growth because it promotes proper aeration, porosity and drainage. Soil aggregation is one of the best indicators of fertile soils because it bridges the physics and biochemistry of soil systems [48]. Shaw and Pawluk [49] reported that the formation of favourable soil structure is associated with the high concentrations of clay-bound sugars in fecal materials of earthworms.

We believe that the survival and activity of *Dendobaena veneta* in the mine tailings of this study were related to the proper combination of organic residues. The addition of immobilizer substrates was critical to the survival of this earthworm species. In addition, pulp sludge was a better substrate than sawdust to stimulate earthworm numbers and activities; this may have been due to more rapid and extensive decomposition (larger loss of C over the study period; also, greater reduction in C/N ratio) and stimulation of microbial activity. These trends are consistent with the observation of greater earthworm vigour and size in an environment of low ammonium and likely large microbial populations (e.g., fungal biomass) to feed on. It is notable that the earthworms in this study were tolerant of the very high electrical conductivity (EC > 10 dS m^{-1}) in several treatments by the end of the study. The significant positive correlation between soil EC and NO_3^--N concentrations lead us to believe that high EC at the end of the study was largely due to the high NO_3^--N concentrations. Although tolerant of these high EC values, the earthworms were lethargic, likely due to salt stress. The presence of plants in a field reclamation program would provide a sink for NO_3^--N and should result in a reduction in EC, thereby providing a more favorable habitat for earthworms.

We conclude that proper amounts and types of organic residues in combination with earthworm addition may improve the chemical and microstructural properties of nonacidic mine tailings, producing a substrate conducive for plant establishment. This manufactured soil meets the definition of a Technosol [50]. Addition of earthworms with organic residues can be an option to safely manage residuals from numerous stockpiles and tailings ponds at many mined sites around the world. We recommend that future studies also consider the inoculation of earthworm species native to the mine location where tailings are obtained.

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Research Article

Effects of Paper-Mill Sludge as a Mulch versus Topsoil Incorporation on Potassium Uptake and the Grain Yield of Rain-Fed Wheat in a High Specific Surface Loess Soil with Illite Dominance in Clay Fraction

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A field experiment with rain-fed winter wheat investigated the nutritional aspects of paper-mill sludge as a mulch and incorporated into the topsoil. Treatments with chemical fertilizers were also used as controls. Paper-mill sludge used as mulch with high rate (100 MG ha^{-1}) and also the combined N and K mineral fertilizer treatments increased yield when a low potassium otherwise caused potassium deficiency in wheat with high specific surface soil. High soil Ca : K molar ratio by incorporation lowered potassium uptake and yield, with visual symptoms of potassium deficiency. A very high Gapon selectivity coefficient (K_G) for K exchange against Ca + Mg (16.58 $(L/mole)^{0.5}$) produced a nonlinear normalized exchange isotherm in favor of potassium with these soils containing high illite. Ca and K which are released by sludge decomposition are diverged in soil when mobilized by rain infiltration, lowering Ca : K molar ratio. Low soil Ca : K molar ratio may be expected by surface sludge application relative to incorporation, due to greater rain infiltration through upper soil layers and their effluent pore volumes per unit depth. Ca from triple superphosphate by the P, N, and K mineral fertilizers combined also reduced potassium uptake and yield relative to N and K combined.

1. Introduction

The application of organic residues at or near to the surface as mulches or incorporated in the topsoil influences the behavior of soil water and temperature regime and supplies additional nutrients for crop growth. Soil physical and nutritional limitations are enhanced by marginal rain and temperatures due to more limited root growth [1] or transfer of ions from soil to roots [2]. Limitations of phosphate supply and un-sustainability of fossil-fuel-based fertilizers and their transport cost add to the significance of organic residues for agricultural use [3]. They also contain a large quantity of potassium [4]. A combined sludge from Mazandaran Mill (Iran) was used in this research.

Three different methods are used commercially to break down wood fiber (comprised principally of cellulosic compounds) mechanically or chemically to create wood pulp. Mechanical mills use stone discs (with or without heat) to grind the fibers. Kraft mills use sodium hydroxide and sodium sulphide to break down the fibers, while sulphite mills use calcium sulfite, magnesium sulfite, or ammonium sulfite [5]. Many mills use chlorine to bleach the paper, while other mills use hydrogen peroxide [6].

Solid wastes arising from above processes, commonly referred to as sludges, are available in three different classes. Primary or clarifier sludges are composed principally of wood fibers that have physically settled out of the wood/water slurry, or waste water, during initial virgin water treatment and are unsuitable for further processing. Secondary sludges arise from microbial decomposition used to remove suspended solids that were not removed during primary clarification of waste water. Secondary solids contain higher

concentrations of nitrogen and phosphorous, added to the slurry mixtures to aid microbial activity, increase flocculation, and expedite the subsequent removal of suspended particles in the waste water. An additional class of paper mill sludges are the tertiary or deinking sludges, which are similar to primary sludges in fiber composition but also contain wastes filtered from the fiber stream during screening and deinking processes resulting from newsprint, magazine, and used-paper recycling [7].

Combination of charge distribution and stereochemical factors gives K-vermiculite surface complexes great stability and is the molecular basis for the term potassium fixation [8]. Desiccation during growing season by dry spells may cause that K rapidly disappears into the edge-situated interlattice sites. Formation of inner sphere Cs surface complexes is enhanced by drying sample at 65°C for 48 hours [8]. Interlattice K ions are held extremely preferentially with respect to other cations with illite and are also, therefore, largely nonexchangeable (in dilute electrolyte solutions). Along the edges; however, some K ions may be exchanged against, for example, H or Ca, if the clay is brought in contact with solution of very low concentration (Bolt et al. [9]). Upon the intensification of agricultural production, the K supply becomes insufficient. Application of K fertilizer, however, gives only a meager response, because the increased concentration of K in the solution leads to entry of K in the vacated interlattice positions [9].

One may make use to some extent of the strong competition of NH_4 ions for the interlattice sites. Application of the NH_3 or NH_4 salts (urea fertilizer), thus, gives (temporarily) NH_4 fixation, suppressing the K fixation to some degree. The fixed NH_4 is then gradually liberated throughout growing season and oxidized to nitrate [9]. NH4 in feeding solution increased outflow K concentration in an open system (flow through reactor) with soils of the site of experiment [10].

A balanced supply of nutrients is essential for optimal plant growth. Deficiency or excess in supply in one nutrient not only affects the uptake and utilization of that nutrient but also of others [11]. Plant nutrient ratios can be used to asses crop nutrient balance. For example, K : Ca, K : Mg and K : Ca + Mg and other ratios are commonly used. When a nutrient ratio is optimal, optimum yield occurs unless some other limiting factor limits yield [2]. Because ions are taken up by plants in equivalents, it is important to be expressed as equivalent weights. If ions are expressed as percentages, over three times as much potassium as magnesium is required on a weight basis to obtain chemical equivalency. Therefore, meq/100 g of dry matter will be used as the primary means of expressing data in relation between monovalent potassium and divalent calcium and magnesium [12].

Both Ca and Mg compete with K for uptake; thus, soils high in one or both may require K fertilization for optimum K nutrition. K uptake would be reduced as Ca and Mg are increased; conversely, Ca and Mg would be reduced as K is increased. Thus, the K availability is somewhat more dependent on its concentration relative to Ca and Mg than on the total quantity of K present [2].

Mechanisms for the penetration of trace elements such as heavy metals in the soil are fairly simple as these small amounts will hardly influence the overall composition of the soil exchange complex. Obviously one then takes the major ion of the same valence as the minor one, and the distribution ratio for the minor cation is also constant during leaching. The transport of trace elements, thus, follows a linear exchange isotherm and moves in the soil with a step front, if it is introduced in that manner [9]. Major elements like K, Na, and Ca follow normally non-linear exchange isotherms with relative favor in exchange and leaching. Metal chelation (by organic amendments) is also important in soils because it increases the solubility of metal ions and affects many important physical, chemical, and biological processes including plant root uptake [13]. Water-soluble concentrations of many minerals such as iron are not sufficient to support plant growth in normal soil pH. Various organic compounds offer various mole fractions of mineral species and availability to plants.

The objective of this research was to diagnose nutrients that affect wheat production in a high specific surface soil with illite as the dominant mineral in clay fraction and effects of sludge as mulch and incorporation on yield production.

2. Materials and Methods

2.1. Soil's Physical, Chemical, and Mineralogical Composition. A composite soil sample was obtained from the site of the experiment for physical, chemical, and mineralogical determinations. A standard digest technique for soil analysis [14] for carbon content percentage and total N applied (Table 1). Carbon content percentage was determined using a potassium dichromate method, P using a colorimetric method, Ca and Mg using an EDTA titration method, N by kjeltec, and K by flame photometry. Based on a general calibration for the NH_4OAc-K soil test [2, Chapter 9], the values for extracted potassium with these soils were highly greater than their suggested sufficiency levels (>160 ppm). Ca, Mg, and K were also determined in both soil paste and ammonium nitrate extracts to obtain normalized exchange isotherm using Gapon's selectivity coefficient for K – (Ca + Mg) exchange. Phosphorous with Olsen's soil test was very high (>20). Loess soils generally contain high quantities of P throughout their profile [2, Chapter 10]. $NO3^-$ with 2 molar KCl, was high (18 ppm). Electrical conductivity for soil-saturated extract was less than 4, and pH was about neutral (Table 1). Soil textural class was silty clay loam by hydrometer method [15]. Other determinations were organic carbon content, satura-percentage, total neutralizing value (%TNV). Bulk density was obtained through core sampling (Table 1).

Mica (illite) was the dominant soil mineral with X-ray diffraction technique [16] using a D8-ADVANCE model. Other minerals were smectites, kaolinites, and chlorites in a descending order [17].

2.2. Chemical Composition of Paper-Mill Sludge. Chemical determinations of sludge material were undertaken for C,

Table 1: Chemical and physical analysis of the soil and combined sludge.

Factors	Soil	Combined sludge
Sand (%)	10	—
Silt (%)	62	—
Clay (%)	28	—
Specific surface (m^2/g)	130	—
Saturation moisture (%)	49	273
Dry bulk density ($g\ cm^{-3}$)	1.7	0.25
EC ($dS\ m^{-1}$)	0.8	3.1
pH	7.7	7.38
Neutral material (%)	5.5	—
Organic C (%)	1.43	22.4
$NO_{3-}N$ ($mg\ kg^{-1}$)	18	600
Total N (%)	0.14	1.2
$NH_{4-}N$ ($mg\ kg^{-1}$)	0.12	0.97
Available P ($mg\ kg^{-1}$)	7	22
Total P (%)	—	0.25
Extractable K ($mg\ kg^{-1}$)	380	540
Total K (%)	—	0.128
Extractable Ca ($meq\ l^{-1}$)	6.6	—
Extractable Na ($meq\ l^{-1}$)	1.3	12.6
Available Ca ($mg\ kg^{-1}$)	—	1600
Total Ca (%)	—	0.412
Extractable Mg ($meq\ l^{-1}$)	5.8	—
Available Mg ($mg\ kg^{-1}$)	—	440
Total Mg (%)	—	0.145
CEC ($cmol\ kg^{-1}$)	20.3	82.6
Total Fe ($mg\ kg^{-1}$)	—	35
Total Mn ($mg\ kg^{-1}$)	—	22.8
Total Cu ($mg\ kg^{-1}$)	—	10.4
Total Zn ($mg\ kg^{-1}$)	—	15.7
Total Ni ($mg\ kg^{-1}$)	—	0.73
Total Cd ($mg\ kg^{-1}$)	—	0.42
Total Pb ($mg\ kg^{-1}$)	—	4.7
Total Cr ($mg\ kg^{-1}$)	—	0.12
Total As ($mg\ kg^{-1}$)	—	4.5
Total Se ($mg\ kg^{-1}$)	—	2.5
Total Hg ($mg\ kg^{-1}$)	—	2.7
Total Mo ($mg\ kg^{-1}$)	—	6

N, P, K, Ca, Mg, Fe, Mn, Cu, Zn, Ni, Ca, Pb, Cr, As, Se, Hg, and Mo (Table 1). The method used a standard digest technique for plant analysis [18] for N, P, K, Ca, Mg, and micronutrients and heavy metals. Micronutrients and heavy metals determined by atomic absorption. Total N, P, K, Ca, Mg, Fe, Mn, Cu, Zn, Ni, Cd, Pb, Cr, As, Se, Hg, and Mo for 50 t/ha paper-mill sludge application were, respectively, 600, 125, 64, 206, 72.5, 1.75, 1.14, 0.52, 0.785, 0.0365, 0.021, 0.235, 0.006, 0.225, 0.125, 0.135, and 0.3 Kg/ha and for 100 t/ha were, respectively, twice as much. C/N ratio was 18.6 with negligible or no potential harm for plants through N immobilization. In terms of potentially toxic elements [19], application rates were well below maximum permissible limits for Cu, Zn, Ni, Cd, Pb, Cr, As, Se, Hg and Mo. pH, EC, SP, and dry bulk density are also presented in Table 1.

The sludges obtained from Mazandaran wood and paper mill (Iran). In this mill, mechanical and chemical methods (both Kraft and sulfite processes) are used for pulping or wood fiber breakdown. The paper-mill sludge used for this research is a mixture of primary and secondary sludges (combined sludge).

2.3. Site and Treatments. The field trials were located in Rahmat Abad soil series on the estate of Gorgan university of Agricultural Sciences and Natural Resources (Pardis), Gorgan, Iran (approx. 37°45′ N, 54°30′ E. altitude 13 m O.D.). The average annual rainfall (1991–2004) was 545.8 mm, and mean air temperatures (1991–2004) were 8.5°C for January, 8°C for February.

Treatments were 50 t/ha (I_{50}) and 100 t/ha (I_{100}) paper-mill sludge incorporated with top soil (I treatments), 50 t/ha (M_{50}), and 100 t/ha (M_{100}) paper-mill sludge applied as mulch (M treatments). Applications were based on sludge dry weights. Other treatments were $N_0P_0K_0$, $N_{92}P_0K_0$, $N_0P_{50}K_0$, $N_0P_0K_{83}$, $N_{92}P_{50}K_0$, $N_{92}P_0K_{83}$, $N_0P_{50}K_{83}$, and $N_{92}P_{50}K_{83}$. Subscripts are Kg/ha N, P, and K. Fertilizer sources for N, P, and K were urea, triple superphosphate, and potassium sulfate. Triple superphosphate, potassium sulfate, and also one-third urea were incorporated to soils to a depth of 0.20 m in respective treatments immediately before drilling with a spade. One-third of urea was incorporated at tillering and the remaining one-third at heading using a manual furrower.

The land had been ploughed and disked before any treatments were applied. For the mulch treatments, the paper-mill sludge was applied uniformly over the land immediately after sowing manually. Where the sludge was to be incorporated (treatments I_{50} and I_{100}), it was initially applied uniformly as a mulch and then incorporated to a depth of 0.2 m manually using a spade before drilling.

Seeds were treated with 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxamide fungicide powder (Vitavax, Uniroyal Chemical Co., USA) before drilling. Fenoxaprop-p-ethyl 5 g/L herbicide (Puma Super, Bayer CropScience Inc., Canada) was applied at early March for curbing oat (*Avena sativa*). Other weeds such as turnip (*Brassica napus*), milk thistle (*Silybum marianum*) and bastard cabbage (*Rapistrum rugosum*) were removed later manually.

2.4. Experimental Design. A completely randomized block design was used with plot size 3 m × 2 m. Each treatment was replicated three times. Soil was a Typic Haploxerepts (Rahmat Abad soil series) of silty clay loam texture from a loess origin. Paper-mill sludge with a dry bulk density of 250 $Kg\ m^{-3}$ and approximately 70% water content was applied on December 30, 2004 and laid on M treatments immediately after drilling or incorporated in the I treatments immediately before drilling at the same day both. Wheat

(Triticum aestivum Var. Tajan) was drilled on 30 December, 2004 with seeds 0.02 m apart and 0.15 m between the rows, equivalent to 3300000 seeds per hectare.

2.5. Measurements. At harvest 1 m^2 from the centre of each plot was harvested manually for determining fresh yield. A subsample was used for determining dry matter for grain and straw, harvest index, and yield components including stem length, head length, heads number per square meter, grains weight per head, and 1000 grain weight.

Chemical determinations of plant shoot material were undertaken for N, P, K, Ca, and Mg with a different subsample at harvest. The method used a standard digest technique [18], N was determined using Kjeltec, P using a colorimetric method, K by flame photometry, and Ca and Mg by an EDTA titration method.

The following chemical determinations were also made at harvest [14]: soil pH, organic carbon content (using a potassium dichromate method), available P (from a sodium bicarbonate extract), K (using a 1 N NH_4OAc extractant), Ca and Mg (in soil-saturated paste extract). Soil available mineral–N (NH_4–N + NO_3–N) was determined in a potassium chloride extract. The soil samples were taken randomly somewhere in plots from central location between two adjacent random rows from 0–0.20 m depth on June 5, 1995, three replicates per plot, and mixed. Data analysis used an ANOVA method with the SAS statistical package and regressions with Excel. Raw data are in Amini [20].

3. Results

3.1. Grain Yield and the Yield Parameters. Differences between treatments for dry grain yield, grains per head, grain weight per head, heads per square meter, and head length were significant by analysis of variance ($P < 0.01$) but were not significant for 1000 grain weight, stem length, and harvest indices ($P > 0.05$).

Dry grain yield for $N_{92}P_0K_{83}$ (4729.8 kg/ha) and high rate of mulch (4825.3 kg/ha) were greater than other treatments (Table 2). Their differences with $N_0P_0K_0$ (3207.7 kg/ha) were significant. Grain yield with incorporation treatments (2264.3 and 2158 kg/ha for I_{50} and I_{100}, resp.) were less than other treatments, but differences with $N_0P_0K_0$ were not significant. All treatments increased significantly heads per square meter relative to $N_0P_0K_0$. It seems all additional sources of nutrients (notably N, P, and K) increased head density. Nutrient applications (such as starters) may be important for enhancing head density and early growth, because crop response to mobile and immobile nutrients may occur even in high-testing soils in cool and moist conditions [2, chapter 10] such as sub optimal topsoil temperatures in winter. Head densities per square meter were greatest with both incorporation treatments (410 and 398.3 for I_{50} and I_{100}, resp.). A greater early growth and head density are not always suggesting a greater final yield.

Relative to other treatments, incorporations (I_{50} and I_{100}) returned minimum grains per head (19.9 and 19.26, resp.), grain weight per head (0.55 and 0.54 g, resp.), head length (4.83 and 5.16 cm resp.), and 1000 grains weight (28 and 28.76 g). For $N_0P_0K_0$ grains per head, grain weight per head, head length, and 1000 grains weight were 33.66, 1.26 g, 7.66 cm, and 37.71 g, respectively, and their differences with incorporations (I_{50} and I_{100}) were statistically significant. A low dry grain yield relative to $N_0P_0K_0$ with incorporation treatments is due to a reduced grain number, weight per head, head length, and 1000 grain weight despite greater heads per square meter. Maximum grains per head obtained by $N_{92}P_0K_{83}$ (37.46) and high rate of mulch (37.33) with no significant difference with $N_0P_0K_0$ (33.66) and other treatments. Maximum head length was with high rate of mulch (9.6 cm) with a significant difference with $N_0P_0K_0$ (7.66 cm). A high dry grain yield relative to $N_0P_0K_0$ with $N_{92}P_0K_{83}$ and high rate of mulch treatments is mainly due to greater heads per square meter. Heads per square meter were least with $N_0P_0K_0$. No effect on grain yield by low rate of mulch is possibly due to a low grain weight per head.

Stem lengths were greatest with $N_{92}P_0K_{83}$ (67.6 cm) and M_{100} (68.3 cm) with no significant difference with other treatments but incorporations. Total dry weights (grain + straw) were also greatest by $N_{92}P_0K_{83}$ and high rate of mulch applications but with $N_{92}P_{50}K_{83}$ was less than $N_{92}P_0K_{83}$.

The least harvest indices were with both rates of incorporations. Harvest indices for $N_{92}P_0K_{83}$ and mulch treatments were not significantly different relative to other remaining treatments due to concurrent straw and grain yield increase. Irrespective of statistics, harvest indices were greatest with $N_0P_{50}K_0$, $N_{92}P_{50}K_0$, $N_0P_{50}K_{83}$, and $N_{92}P_{50}K_{83}$ treatments.

3.2. Shoot Tissue Nutrient Concentration and Uptake, Soil Organic Carbon, Soil Nutrient Concentration, and (Ca + Mg) : K Ratios for Plant and Soil at Harvest. N, K, Ca, and Mg concentrations in shoot tissue at harvest were significantly different between treatments by analysis of variance ($P < 0.01$). P concentration was not significantly different ($P < 0.05$). N, P, K, Ca, and Mg plant shoot uptakes at harvest were significantly different between treatments by analysis of variance ($P < 0.01$).

Soil NO_3^-, NH_4^+, organic C, total N, available P, extractable K, and C/N ratio were significantly different between treatments at harvest by analysis of variance ($P < 0.01$). Soil extractable Ca and Mg, EC, and pH were not significantly different between treatments ($P > 0.05$). (Ca + Mg) : K ratios were significantly different among treatments by analysis of variance ($P < 0.01$) for plant tissue but not significantly different for soil ($P > 0.05$).

3.3. Shoot Tissue N Concentrations, Uptake, and Soil N Concentration. Percentage mean treatment tissue N concentrations with low (0.451%) and high rates of sludge incorporation (0.47%) and also $N_0P_{50}K_0$ (0.549%) were significantly less than other treatments (Table 3). Dry grain yield and total dry matter at harvest (Table 2) and the N uptake (Table 4) were less than other treatments with low and high rates of incorporations (35.53 and 36.60 Kg/ha N uptake, resp.), but they were greater than other treatments with the high rate of mulch (90.46 Kg/ha for N uptake). Despite high soil nitrate concentration with $N_{92}P_0K_0$, yield and tissue N concentrations were not significantly different with

Table 2: Harvest index, grain components (stem length, head length, heads per square meter, grains weight per head, 1000 grains weight), grain yield, and the total shoot dry matter.

Shoot dry treatment	Harvest index	Stem length (cm)	Head length (cm)	Grain heads per square meter	Grain weight per head (g)	Grains per head	1000-grain weight (g)	Dry grain yield (Kg/ha)	Total shoot dry matter (Kg/ha)
$N_{92}P_0K_0$	38.46^{a}	61.6^{abcd}	8.16^{ab}	313.3^{d}	1.1^{ab}	32.33^{ab}	34.58^{ab}	3465.3^{bcde}	9033^{cd}
$N_0P_{50}K_0$	42.83^{a}	62.3^{abcd}	7.5^{b}	360^{bc}	1.14^{ab}	32.2^{ab}	35.66^{a}	4105.3^{abcd}	9783^{cd}
$N_0P_0K_{83}$	39.4^{a}	63.3^{abcd}	7.66^{ab}	339.6^{cd}	1.06^{ab}	27.26^{bc}	39.16^{a}	3630.7^{abcd}	9233^{cd}
$N_{92}P_{50}K_0$	40.41^{a}	65.5^{ab}	8.66^{ab}	360.3^{bc}	1.18^{ab}	35.46^{ab}	33.33^{abc}	4262.8^{abcd}	10500^{c}
$N_{92}P_0K_{83}$	35.16^{abc}	67.6^{ab}	8.66^{ab}	359.6^{bc}	1.31^{a}	37.46^{a}	35.17^{ab}	4729.8^{ab}	13450^{a}
$N_0P_{50}K_{83}$	41.4^{a}	64^{abc}	8.16^{ab}	353.67^{cd}	1.1^{ab}	32.1^{ab}	34.56^{ab}	3957.3^{abcd}	9600^{cd}
$N_{92}P_{50}K_{83}$	40.67^{a}	65.5^{ab}	8.5^{ab}	355^{c}	1.24^{a}	36.26^{ab}	34.13^{abc}	4401.6^{abc}	10800^{bc}
I_{50}	28.58^{c}	56.3^{d}	4.83^{c}	410^{a}	0.55^{c}	19.9^{c}	28^{c}	2264.3^{ef}	7883^{d}
M_{50}	36.56^{abc}	60.3^{bcd}	7.8^{ab}	333.3^{cd}	0.92^{b}	27.23^{bc}	34^{abc}	3076.1^{def}	8517^{cd}
I_{100}	27.75^{c}	56.6^{cd}	5.16^{c}	398.3^{ab}	0.54^{c}	19.26^{c}	28.76^{bc}	2158^{f}	7783^{d}
M_{100}	36.49^{abc}	68.3^{a}	9.6^{a}	373^{abc}	1.29^{a}	37.33^{a}	34.66^{ab}	4825.3^{a}	13233^{ab}
$N_0P_0K_0$	37.92^{ab}	61.3^{abcd}	7.66^{ab}	254^{e}	1.26^{a}	33.66^{ab}	37.71^{a}	3207.7^{cdef}	8450^{cd}

Means within the same column followed by the same letter are not statistically different at $P = 0.05$ (Fisher's LSD).

$N_0P_0K_0$. Urea fertilizer application lowered percentage of tissue N concentration with $N_{92}P_0K_{83}$ treatment (0.518) relative to all other urea containing treatments through dilution effect by increasing yield. In K-fixing soils, urea may increase fertilizer potassium availability for plants through ammonium release and competition for K fixation. This may lead to increased tissue potassium concentration and uptake. Soil nitrate concentration (Table 5) may not be closely correlated with tissue nitrate concentration due to dilution effect; increased plant uptake may also diminish soil concentration. Soil nitrate concentrations, with both rates of incorporation and also mulch treatments, $N_0P_{50}K_0$ and $N_0P_0K_0$ were significantly less than $N_0P_0K_0$ and with $N_{92}P_0K_0$ and $N_{92}P_{50}K_{83}$ significantly greater than $N_0P_0K_0$. Greatest nitrate concentrations occurred with nitrogen-bearing treatments.

The ammonium from urea fertilizer application is converted into nitrite by *Nitrosomonas* autotrophic bacteria and then promptly to nitrate by autotrophic nitrobacteria activities under aerobic conditions. These bacteria obtain energy through nitrogen oxidation and carbon from the atmospheric carbon dioxide. The dominant soil N mineral form would be expected to be nitrate at harvest (soil sampling time), a long time after fertilization (Table 5).

3.4. Soil Organic Carbon. Soil organic C content with all rates of mulch and incorporation treatments was significantly greater than other treatments at harvest (Table 5), indicating that paper-mill sludge was not yet completely decomposed at harvest. Soil organic C with low and high rates of mulch were less than low and high rates of sludge incorporation, respectively, which shows a more rapid decomposition by mulch application. This increases total N release and other elements in soil including K, Ca, and Mg with mulch. Soil organic C with fertilizer treatments was not significantly different with $N_0P_0K_0$. EC_s (ds/m) were less than 0.7 by all treatments.

3.5. Shoot Tissue Potassium Concentration, Uptake, and Soil Extractable Potassium Concentration. Incorporation treatments with least tissue potassium concentrations are placed in f statistical group in Table 3 and are significantly different with $N_0P_0K_0$. $N_{92}P_0K_{83}$ and $N_0P_{50}K_{83}$ treatments with the greatest tissue potassium concentrations are placed in statistical group a in Table 3.

Potassium plant tissue concentration for high rate of mulch application is placed in b statistical group and is significantly greater than $N_0P_0K_0$. Potassium uptake by this treatment is in statistical group a, due to high yield production. K uptake with $N_{92}P_0K_{83}$ was also significantly greater than other treatments (Table 4). Potassium uptake by $N_{92}P_{50}K_{83}$ was significantly less than $N_{92}P_0K_{83}$. Least K uptakes occurred with both rates of sludge incorporation.

1 N NH_4OAc soil potassium concentrations by both incorporation treatments were significantly less than other treatments. The greatest soil extractable potassium concentrations were with $N_{92}P_0K_{83}$ and high rate of mulch treatments. Their differences with $N_0P_0K_0$ treatment were not statistically significant however (Table 5). Plant tissue potassium concentrations and uptakes were less with $N_0P_0K_0$ however. Lack of correlation between soil potassium concentration and plant potassium uptake suggests 1 N NH_4OAc is not suitable for extracting plant-available potassium from soils characterized by a great specific surface and illite dominance in clay fraction. This was elaborated upon in Amini [20].

3.6. Normalized Exchange Isotherm. Normalized exchange isotherm was constructed (Figure 1) using Gapon's selectivity coefficient for K – (Ca + Mg) exchange [9]. K_G was 16.58 $(L/mole)^{0.5}$ for soils of the experimental site with illite

Table 3: Tissue nutrient concentrations for shoot at harvest (%).

Treatment	N	P	K	Ca	Mg
$N_{92}P_0K_0$	0.56^{c}	0.204^{d}	0.391^{d}	0.12^{d}	0.14^{a}
$N_0P_{50}K_0$	0.453^{e}	0.253^{a}	0.343^{e}	0.136^{bc}	0.11^{bc}
$N_0P_0K_{83}$	0.552^{c}	0.223^{bcd}	0.458^{c}	0.125^{cd}	0.105^{bc}
$N_{92}P_{50}K_0$	0.593^{b}	0.219^{bcd}	0.351^{e}	0.125^{cd}	0.115^{bc}
$N_{92}P_0K_{83}$	0.518^{d}	0.245^{ab}	0.707^{a}	0.141^{b}	0.155^{a}
$N_0P_{50}K_{83}$	0.602^{b}	0.232^{abc}	0.72^{a}	0.161^{a}	0.155^{a}
$N_{92}P_{50}K_{83}$	0.552^{c}	0.229^{abcd}	0.366^{de}	0.131^{bcd}	0.12^{b}
I_{50}	0.451^{e}	0.233^{abc}	0.281^{f}	0.135^{bc}	0.105^{bc}
M_{50}	0.519^{d}	0.222^{bcd}	0.365^{de}	0.135^{bc}	0.105^{bc}
I_{100}	0.47^{e}	0.236^{abc}	0.292^{f}	0.135^{bc}	0.12^{b}
M_{100}	0.68^{a}	0.24^{ab}	0.628^{b}	0.14^{b}	0.12^{b}
$N_0P_0K_0$	0.549^{c}	0.212^{cd}	0.38^{de}	0.12^{d}	0.12^{b}

Means within the same column followed by the same letter are not statistically different at $P = 0.05$ (Fisher's LSD).

Table 4: Nutrient uptakes for shoot at harvest (Kg/ha).

Treatment	N	P	K	Ca	Mg
$N_{92}P_0K_0$	50.59^{cde}	18.46^{c}	35.37^{cd}	10.75^{cd}	12.64^{bcde}
$N_0P_{50}K_0$	44.27^{ef}	24.8^{b}	33.68^{cde}	13.34^{bcd}	10.76^{defg}
$N_0P_0K_{83}$	50.96^{cde}	20.56^{bc}	42.3^{c}	11.57^{cd}	9.63^{defg}
$N_{92}P_{50}K_0$	62.26^{bc}	23.06^{bc}	36.82^{c}	13.17^{bcd}	12.09^{cdef}
$N_{92}P_0K_{83}$	69.67^{b}	32.94^{a}	95.03^{a}	19.03^{a}	20.86^{a}
$N_0P_{50}K_{83}$	57.72^{bcd}	22.27^{bc}	70.31^{b}	15.54^{ab}	14.9^{bc}
$N_{92}P_{50}K_{83}$	59.58^{bc}	24.7^{b}	39.55^{c}	14.24^{bc}	12.94^{bcd}
I_{50}	35.53^{f}	18.4^{c}	22.13^{e}	10.64^{cd}	8.11^{g}
M_{50}	44.13^{ef}	19^{bc}	31.01^{cde}	11.54^{cd}	9.07^{fg}
I_{100}	36.6^{f}	18.38^{c}	22.74^{de}	10.5^{cd}	9.34^{efg}
M_{100}	90.46^{a}	31.73^{a}	83.41^{a}	18.49^{a}	15.81^{b}
$N_0P_0K_0$	46.4^{def}	17.91^{c}	31.35^{cde}	10.34^{d}	10.14^{defg}

Means within the same column followed by the same letter are not statistically different at $P = 0.05$ (Fisher's LSD).

Table 5: Soil nutrient concentrations at harvest.

Treatment	NO_3–N (mg kg^{-1})	NH_4–N (mg kg^{-1})	Organic C (%)	Total N (%)	Avai. P (mg kg^{-1})	Avai. K (mg kg^{-1})	Ext. Ca (meq l^{-1})	Ext. Mg (meq l^{-1})	EC (dS m^{-1})	pH	C/N
$N_{92}P_0K_0$	38.8^{a}	0.1^{d}	1.25^{e}	0.12^{e}	18.06^{d}	380^{b}	5.1^{a}	4.1^{a}	0.46^{b}	7.55^{abcd}	10.41^{ab}
$N_0P_{50}K_0$	7.66^{d}	0.12^{d}	1.23^{e}	0.12^{e}	24^{c}	385.6^{ab}	5.23^{a}	4.3^{a}	0.58^{ab}	7.54^{abcd}	10.26^{ab}
$N_0P_0K_{83}$	18^{c}	0.15^{c}	1.31^{de}	0.13^{e}	15.7^{d}	392^{ab}	4.8^{a}	3.8^{a}	0.58^{ab}	7.45^{cd}	10.07^{abc}
$N_{92}P_{50}K_0$	30.6^{b}	0.1^{d}	1.17^{e}	0.12^{e}	27.3^{bc}	380^{b}	5.2^{a}	4.2^{a}	0.67^{a}	7.55^{abcd}	9.77^{bcde}
$N_{92}P_0K_{83}$	22^{c}	0.12^{d}	1.19^{e}	0.13^{e}	14.9^{d}	407.6^{a}	5.1^{a}	4.2^{a}	0.59^{ab}	7.75^{a}	9.15^{e}
$N_0P_{50}K_{83}$	18^{c}	0.15^{c}	1.37^{de}	0.14^{e}	35.6^{a}	387.3^{ab}	5.33^{a}	4.3^{a}	0.51^{ab}	7.62^{abc}	9.8^{bcde}
$N_{92}P_{50}K_{83}$	36^{a}	0.15^{c}	1.3^{de}	0.14^{e}	27^{bc}	394^{ab}	5^{a}	4^{a}	0.47^{b}	7.69^{ab}	9.27^{de}
I_{50}	7.33^{d}	0.22^{b}	2.39^{b}	0.23^{b}	17.36^{d}	340.3^{c}	5.66^{a}	4.96^{a}	0.51^{ab}	7.48^{bcd}	10.41^{ab}
M_{50}	9.3^{d}	0.12^{d}	1.48^{d}	0.16^{d}	17.23^{d}	383.6^{ab}	5.5^{a}	4.73^{a}	0.45^{b}	7.57^{abc}	9.26^{de}
I_{100}	9.3^{d}	0.27^{a}	2.86^{a}	0.27^{a}	17.1^{d}	346.6c	6.16^{a}	5.2^{a}	0.59^{ab}	7.52^{abcd}	10.6^{a}
M_{100}	10^{d}	0.2^{b}	1.81^{c}	0.19^{c}	30.1^{ab}	405.3^{ab}	6^{a}	4.8^{a}	0.66^{a}	7.5^{bcd}	9.52^{cde}
$N_0P_0K_0$	10.33^{d}	0.1^{d}	1.2^{e}	0.12^{e}	16.9^{d}	393.3^{ab}	4.66^{a}	3.5^{a}	0.46^{b}	7.33^{d}	10^{abcd}

Means within the same column followed by the same letter are not statistically different at $P = 0.05$ (Fisher's LSD).

dominance in clay fraction. This ratio is generally less than unity for most minerals and soils [21]. Potassium is held apparently with greater preference relative to divalent cations in close vicinity of colloids in soils exhibiting high specific surface and truncated diffuse double layers. The K ion fits snugly into the hexagonal cavity that is built into layer silicate surfaces and becomes trapped or fixed in a nonexchangeable form. Exchange equations cannot adequately quantify this kind of behavior [22]. This may be less evident when N and K fertilizers are used combined or with sludge containing N and K because, according to Bolt et al. [9], NH_4 is fixed roughly to the same extent as K ions with illites, suppressing the K fixation to some degree.

3.7. Shoot Tissue Phosphorous Concentration, Uptake, and Soil Phosphorous Concentration. The greatest phosphorous tissue concentrations relative to other treatments occurred with $N_0P_{50}K_0$, $N_{92}P_0K_{83}$, and high rate of mulch (Table 2). The greatest phosphorous plant uptakes obtained with $N_{92}P_0K_{83}$, and high rate of mulch application (Table 4) and the greatest soil phosphorous concentrations with $N_0P_{50}K_{83}$ (Table 5).

3.8. Shoot Tissue Calcium Concentration, Uptake, and Soil-Soluble Calcium Concentration. Plant tissue Ca concentration with $N_0P_{50}K_{83}$ was significantly greater than other treatments (Table 3). No notable trend observed for Ca uptake (Table 4). With high rate of sludge incorporation, soil-soluble calcium concentrations were greater than other treatments and with $N_0P_0K_0$, less than others irrespective of statistics (Table 5).

3.9. Shoot Tissue Magnesium Concentration, Uptake, and Soil-Soluble Magnesium Concentration. With $N_{92}P_0K_0$, $N_{92}P_0K_{83}$, and $N_0P_{50}K_{83}$, plant tissue Mg concentrations (Table 3) were significantly greater than other treatments. Remaining treatments are not significantly different with $N_0P_0K_0$. No notable trend observed for Mg uptake (Table 4).

Similar to calcium concentrations, soil-soluble magnesium concentrations were not also significantly different between treatments (Table 5). The high quantity of available calcium and magnesium in paper-mill sludge (Table 1) would be expected to increase their quantity in soil solution with mulch and incorporation, relative to other treatments. A quick deposition in soils as carbonates may prevent high quantities in solution.

3.10. Equivalent Levels of Ca, Mg, and K (meq/100 g Plant Dry Weight) and also the Ratios of (Ca + Mg) : K in Plant Tissues and the Soil. With both incorporation treatments, (Ca + Mg) : K in plant tissues was significantly greater than $N_0P_0K_0$ and irrespective of statistics greater than other treatments (Table 6). Plant tissue K concentrations (meq/100 g) with incorporations were significantly less than mulch treatments, but their Ca and Mg (meq/100 g) were not statistically different. (Ca + Mg) : K ratios in plant tissues were significantly less than $N_0P_0K_0$ with $N_{92}P_0K_{83}$, M_{100}, $N_0P_0K_{83}$, and $N_0P_{50}K_{83}$. (Ca + Mg) : K in plant tissues were not significantly different from $N_0P_0K_0$with $N_0P_{50}K_0$, $N_{92}P_0K_0$, $N_{92}P_{50}K_0$, $N_{92}P_{50}K_{83}$ and low rate of mulch. With $N_{92}P_{50}K_{83}$, (Ca + Mg) : K in plant tissues was significantly greater than $N_{92}P_0K_{83}$, with $N_{92}P_{50}K_{83}$ potassium in plant tissues (meq/100 g plant dry weight) was significantly less than $N_{92}P_0K_{83}$, and with $N_{92}P_{50}K_0$ magnesium was less than $N_{92}P_0K_0$ (Table 6). K, Ca, and Mg concentrations in soil (meq/100 g soil) at harvest were not different by these treatments (Table 6). The greatest soil (Ca + Mg) : K ratio was with high rate of incorporation with a significant difference with $N_0P_0K_0$.

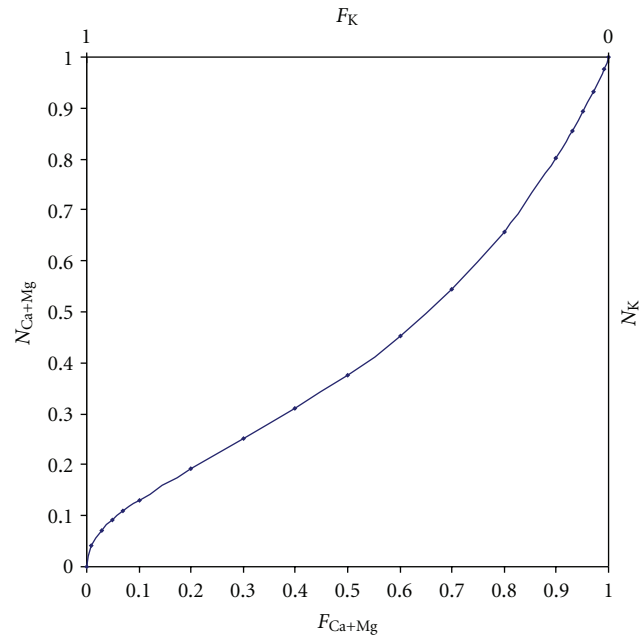

Figure 1: Normalized exchange isotherm following the Gapon equation for K – (Ca + Mg) exchange with K_G = 16.58 (l/mole)$^{0.5}$, corresponding to total electrolyte level of 0.01 normal. Ca exchange is slightly unfavorable when potassium is present except for very low concentrations.

4. Discussion

N, P, and K nutrient sources may be used as starter fertilizers for increasing tiller number per unit surface or the yield [2, chapter 10]. All treatments increased head number per square meter relative to control (Table 2). Therefore, all treatments might have worked as starters, but no significant effect on final grain and shoot yield were observed with individual N, P, or K applications. More nutrient availability in root zone with different fertilizers and sludge treatments might have enhanced root cell division and elongation after emergence in winter, leading to more absorption of nutrients and tiller production by plants. Unless the most limiting plant growth factor is met, greater tiller production does not mean a greater yield.

Short stems and heads, low heads density and weight, and the grain shrinkage were wheat potassium deficiency symptoms with both rates of sludge incorporation. Thin plants, leaf deformation with a faint color, and the chlorosis

Table 6: Means of meq/100 g K, Ca and Mg and (Ca + Mg) : K ratio in soil and plant.

Treatment	Soil K	Soil Ca	Soil Mg	Plant K	Plant Ca	Plant Mg	(Ca+Mg) : K (soil)	(Ca+Mg) : K (plant)
$N_{92}P_0K_0$	0.97^{a}	0.25^{a}	0.2^{ab}	10.04^{d}	6^{d}	11.66^{a}	0.47^{ab}	1.75^{b}
$N_0P_{50}K_0$	0.98^{a}	0.25a	0.21^{ab}	8.8^{e}	6.83^{b}	9.16^{b}	0.48^{ab}	1.81^{b}
$N_0P_0K_{83}$	1^{a}	0.233^{a}	0.19^{ab}	11.74^{c}	6.25^{cd}	8.74^{b}	0.42^{ab}	1.27^{c}
$N_{92}P_{50}K_0$	0.97^{a}	0.26^{a}	0.21^{ab}	8.99^{e}	6.25^{cd}	9.58^{b}	0.48^{ab}	1.75^{b}
$N_{92}P_0K_{83}$	1.04^{a}	0.25^{a}	0.21^{ab}	18.14^{a}	7.08^{b}	12.91^{a}	0.44^{ab}	1.1^{cd}
$N_0P_{50}K_{83}$	0.99^{a}	0.26^{a}	0.21^{ab}	18.66^{a}	8.08^{a}	12.91^{a}	0.47^{ab}	1.11^{cd}
$N_{92}P_{50}K_{83}$	1^{a}	0.24^{a}	0.173^{ab}	9.38^{de}	6.58^{bcd}	9.99^{b}	0.42^{b}	1.78^{b}
I_{50}	0.87^{b}	0.28^{a}	0.24^{ab}	7.21^{f}	6.75^{bc}	8.61^{b}	0.61^{ab}	2.12^{a}
M_{50}	0.98^{a}	0.27^{a}	0.23^{ab}	9.35^{de}	6.75^{bc}	8.74^{b}	0.51^{ab}	1.65^{b}
I_{100}	0.88^{b}	0.3^{a}	0.26^{a}	7.49^{f}	6.75^{bc}	10^{b}	0.65^{a}	2.24^{a}
M_{100}	1.03^{a}	0.29^{a}	0.24^{ab}	16.11^{b}	7^{b}	9.99^{b}	0.51^{ab}	1.05^{d}
$N_0P_0K_0$	1^{a}	0.23^{a}	0.17^{b}	9.51^{de}	6^{d}	10^{b}	0.39^{b}	1.68^{b}

Means within the same column followed by the same letter are not statistically different at $P = 0.05$ (Fisher's LSD).

of leaf margins were apparent since May 5, 2005 with these treatments. Potassium plant tissue concentration and uptake and also the shoot and grain yield were least with both incorporation treatments. Potassium uptake and concentration with highest yielding treatments ($N_{92}P_0K_{83}$ and high rate of sludge as mulch) were greatest, however, indicating potassium deficiency in soils. Potassium could be the target element for sludge application in these soils. Significant greater potassium uptake by $N_{92}P_0K_{83}$ and high rate of mulch compared to remaining treatments is due to greater plant growth and soil potassium availability. Accordingly low soil potassium availability to roots with both incorporation treatments could cause low potassium uptake.

The antagonist effect induced by calcium from the triple superphosphate may reduce plant potassium uptake and yield. Superphosphate with $N_{92}P_{50}K_{83}$ lowered potassium concentration in plant tissues (meq/100 g plant dry weight) relative to $N_{92}P_0K_{83}$ but with $N_{92}P_{50}K_0$, magnesium concentration relative to $N_{92}P_0K_0$, with no effect on plant tissue Ca concentration (Table 6). Ca, Mg, and K compete entering roots. Soil (Ca + Mg) : K ratio and exchangeable potassium affect K uptake by roots. Potassium and magnesium uptake by roots are adversely affected by the antagonistic effect of increased Ca concentration from superphosphate. These antagonistic effects on yield production were greater with treatments supplementing more available potassium such as $N_{92}P_0K_{83}$. K, Ca, and Mg concentrations (meq/100 g soil) in soil at harvest were not different by superphosphate. Soil supplies for these nutrients may exceed uptakes at the end of growing season with diminishing metabolic activities, restoring initial predrilling concentrations.

Soil K (meq/100 g) and similarly plant tissue K (meq/100 g) were significantly less than mulch with sludge incorporation, but no differences were found for soil and plant tissue Ca and Mg (meq/100 g). This suggests a correlation between plant tissue K and soil extractable K with NH_4OAc for mulch and incorporation treatments. (Ca + Mg) : K ratio in plant tissue with incorporation was greater than mulch. Potassium deficiency with incorporation treatments is due to limited potassium uptake as a result of antagonistic effects by Ca or Mg. As it was noted earlier, Ca from triple superphosphate also reduced potassium uptake and yield by $N_{92}P_{50}K_{83}$ relative to $N_{92}P_0K_{83}$. Normalized exchange isotherm (Figure 1) is a case of slightly unfavorable exchange (close to linear exchange) for Ca + Mg when K is present. Ca + Mg and K are all mobile with an almost linear exchange. Ca + Mg is more mobile than K, however, due to slightly unfavorable exchange isotherm. This suggests a greater K : Ca ratio and plant potassium uptake in layers close to surface with organic mulch as compared to incorporation. Number of pore volume rain infiltration and nutrient mobilization (also divergence between Ca and K) is greater in surface soil layers near mulch as compared to average 0.2 m incorporated plough layer. The greatest soil (Ca + Mg) : K ratio at harvest was with high rate of incorporation with a significant difference with $N_0P_0K_0$ (Table 6).

Irrespective of low shoot dry matter production and grain yield relative to $N_{92}P_0K_{83}$ and M_{100}, soil nitrate concentrations with $N_{92}P_0K_0$ and $N_{92}P5_0K_{83}$ were both greater than all treatments at harvest, suggesting N could not be the most limiting plant growth factor. Least N uptake and tissue concentration obtained by treatments with the least dry grain and shoot dry matter production, namely, low and high rate of sludge incorporation. Stunted growth and N uptake with incorporation treatments is likely due to limited soil N and K availability and the root growth. A significant greater soil organic carbon percentage and total N at harvest by incorporation treatments relative to mulch suggests lower microbial activity and decomposition rate due possibly to high EC (Table 1), salt index, or toxicity close to sludge-incorporated particles. Salts may be leached from mulch cover by rainfall, keeping salt concentration favorable for decomposing microbial activity.

Plant tissue N concentration with high rate of mulch application was greater than $N_0P_0K_0$ and with $N_{92}P_0K_{83}$ was

lower, suggesting again that soil N concentration is not possibly the most limiting plant growth factor. Shoot and grain yields with treatments containing only N ($N_{92}P_0K_0$) or K ($N_0P_0K_{83}$) were not different with $N_0P_0K_0$, suggesting ammonium ion from urea, neither potassium from potassium sulfate fertilizers affect soil potassium availability. The ammonium ion from urea fertilizer with $N_{92}P_0K_{83}$ may increase potassium residence time and concentration in diffuse double layer and soil solution in potassium-fixing soils (containing illite) and, hence, plant tissue concentration and uptake (Table 4). High soil potassium concentration with $N_{92}P_0K_{83}$ at harvest supports this conclusion. Rezaei and Movahedi Naeini [23] found that ammonium increased potassium desorption in batch experiments with these soils. Yield is expected to increase with high rate of mulch with a similar mechanism involving both ammonium and potassium ions.

5. Conclusions

Decomposition rate with sludge mulch was greater than incorporation. Optimal application rate of sludge application for nutrient supply is less for mulch than incorporation. 100 t/ha sludge with 22.4% C by mulch application adds 1% OC to top 0.2 m surface soil with many benefits to arid and semiarid soils.

Cations released by sludge decomposition are diverged along their traveling path in soil due to their nonlinear exchange isotherm and variable mobility. Due to greater rain infiltration, traveling distance and divergence is greater for surface applied cations by mulch relative to incorporation. Cation competition for entering roots and their antagonistic effects is minimized by mulch application.

Plant potassium uptake and yield were both increased by paper-mill sludge application as mulch but decreased as incorporation in this research. Great care must be exercised with conventional tillage where mulch is eventually ploughed under and incorporated. Since sludge mulch is eventually ploughed under, two consecutive trials are suggested for future research, with mulch first and then with incorporation. Paper-mill sludge could be used successfully for alleviating soil potassium deficiency if no detrimental effect is detected on yield with the second trial after incorporation.

Heavy metal additions by 50 and 100 t/ha dry weight sludge applications (Table 1) were not excessive and hazardous [24]. Mineralization rate with high rate of mulch application was greater than incorporation. A greater yield by mulch suggests no adverse effect by chlorotic organic compounds such as dioxins isomers, furans, and polychlorinated biphenyls. Likelihoods of excessive hazardous organic compounds and heavy metal load must be considered prior to sludge application. This likelihood is greater with frequent applications.

High (Ca + Mg) : K in soil solution creates a tense competition between Ca + Mg and K for entering roots in soils with high specific surface [20] and low potassium diffusion to soil solution. Therefore, even short-lived sources of increased Ca and Mg in soil solution are expected to disturb potassium uptake. In these soils containing illites, potassium uptake by roots is adversely affected by calcium-bearing superphosphate. Other potassium- or ammonium-based phosphates are recommended for future research and were already applied successfully by Talebizadeh and Movahedi Naeini [25].

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Research Article

Effects of Stubble Management on Soil Fertility and Crop Yield of Rainfed Area in Western Loess Plateau, China

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The combination of continuous cereal cropping, tillage and stubble removal reduces soil fertility and increases soil erosion on sloping land. The objective of the present study was to assessment soil fertility changes under stubble removal and stubble retention in the Loess Plateau where soil is prone to severe erosion. It was indicated that soil N increased a lot for and two stubble retention treatments had the higher N balance at the end of two rotations. Soil K balance performed that soil K was in deficient for all treatments and two stubble retention treatments had lower deficit K. The treatments with stubble retention produced higher grain yields than the stubble removal treatments. It was concluded that stubble retention should be conducted to increase crops productivity, improve soil fertility as well as agriculture sustainability in the Loess plateau, China.

1. Introduction

Crop stubble is a main agricultural waste material as well as a renewable resource, due to being rich in nitrogen (N), phosphorus (P), and potassium (K). China has a long tradition of efficient recycling of organic residues in agriculture, but this tradition is rapidly disappearing following the intensification of agricultural production, the increased use of mineral fertilizers, and the increasing urbanization and decoupling of crop production and animal production [1]. The intensification of agricultural production has greatly increased the agricultural production, but at the same time, it has contributed to a decrease in resource use efficiency, land degradation through increased wind and water erosion, and pollution of ground water and surface waters [2–4]. There are approximately 0.7 billion ton of organic residues produced each year in China, which contain 3, 0.70, and 7 million ton of N, P, and K, respectively, equivalent to 25% of the total chemical fertilizers used for farming system [5].

In the last few decades, there has been increased interest in the reuse of crop stubble for soil ecology [6, 7], crop system [8, 9], and atmospheric environment [10] worldwide. Retention of plant residues has been found to have many long-term benefits around the world. These crop stubble constitutes a mulch cover that protects the soil against run-off and erosion [11] and increases the percentage of organic matter in the surface soil layer [12, 13]. Nutrient loss due to runoff is also decreased [14]. The capacity of the soil surface to intercept rainfall is improved because of changes in soil roughness, soil surface porosity, and hydraulic conductivity of the topsoil. Mulching also reduces temperature extremes [15, 16] and direct evaporation [17, 18]. As a result, crop productivity is often improved. However, according to existing problems of rational and effective utilization of stubble resources under different soils and climatic conditions, the choice of the best suited utilization of stubble must be harmonious to particular agroecological environment.

On the western Loess Plateau in China, dryland cropping systems are dominated by wheat. The practice of 3 ploughs and 2 harrows is employed prior to sowing to prepare a seedbed, while all crop stubble and residues are normally removed from the field at harvest for animal feed or fuel

for heating or cooking [19]. The combination of continuous cereal cropping, tillage, and stubble removal reduced soil fertility and increased soil erosion on sloping land [20, 21]. However, little research on stubble retention had been undertaken in the semiarid areas on the western Loess Plateau where soil is prone to severe erosion. The objective of the present study was to assess soil fertility changes under stubble removal and stubble retention in the Loess Plateau.

2. Methods and Materials

2.1. Site Description. The field experiment was conducted from 2001 to 2009 at the Dingxi Experimental Station (35°28′N, 104°44′E, elevation 1971 m a.s.l.) of Gansu Agricultural University, Anding County, Gansu Province, northwest China. The site had a Huangmian soil [22], aligning with a Calcaric Cambisols in the FAO soil map of the world [23]. It is a sandy loam with low fertility. Soil organic carbon was below 7.63 g kg^{-1} (Table 1), representing the major cropping soil in the district [24], one of two dominant soils on the Loess Plateau. Long-term annual rainfall at Dingxi averages 391 mm, ranging from 246 mm in 1986 to 564 mm in 2003, with about 54% received between July and September. Daily maximum temperatures can reach up to 38°C in July, while minimum temperatures can drop to −22°C in January. Hence, summers are warm and moist, whereas winters are cold and dry. Annual accumulated temperature >10°C is 2239°C, and annual radiation is 5929 MJ m^{-2} with 2477 h of sunshine. The site had a long history of continuous cropping using conventional tillage. The crop prior to the experiment commencement in 2001 was flax (*Linum usitatissimum* L.).

2.2. Experimental Design and Treatment Description. The experiment had a fully phased 2 × 2 factorial design with 2 phases, replicated 4 times (blocks). Spring wheat (cv. Dingxi no. 35) and field pea (cv. Yannong) were sown in rotation in both phases represented in each year. Phase 1 (P/W) started with field pea followed by spring wheat, and phase 2 (W/P) started with spring wheat followed by field pea. Therefore, there were 32 plots in total. Plots were 4 m wide, 17 m long in block 1, 21 m long in blocks 2 and 3, and 20 m long in block 4. All treatments were described as follows (Table 2).

2.3. Sowing Rate, Fertilizers, and Field Management. All crops were sown by a small no-till seeder (5-6 rows in 1.2 m width) designed by the China Agricultural University. The no-till seeder, drawn by a 13.4 kW (18 HP) tractor, was designed to place fertilizers below the seeds using narrow points followed by concave rubber press wheels in one operation. Spring wheat was sown at 187.5 kg ha^{-1} in mid-March and harvested in late July to early August each year. Field pea was sown at 180 kg ha^{-1} in early April and harvested in early July each year. The row spacing was 20 cm for spring wheat and 24 cm for field pea using the no-till seeder.

Nitrogen and P were applied at 105 kg N ha^{-1} as urea (46% N) and at 45.9 kg P ha^{-1} as calcium superphosphate (6.1% P) for spring wheat, and 20 kg N ha^{-1} and 45.9 kg P ha^{-1} for field pea. No farm manure was used in this experiment. Field peas were not inoculated when sown as no appropriate rhizobia were available on the market. However, the site had history of field pea in the previous 3 years. Roundup (glyphosate, 10%) was used for weed control during fallow after harvesting as per the product guidelines. During the growing season, weeds were removed by hand. Pests and diseases were monitored and controlled as per conventional practice in the area.

2.4. Measurements

2.4.1. Soil Properties. Soil samples were collected from 0–30 cm depth for the determination of soil nutrient level after harvest (mid-August, 2007). Five cores were bulked into one sample for each plot using a standard 25 mm diameter soil corer. Soil sample were dried and sieved through 2 mm mesh. Soil organic carbon was determined by dichromate oxidation [25]. Total N in soil was determined by Semimicro-Kjeldahl method [26]. Nitrate nitrogen (NO_3^--N) and exchangeable ammonium nitrogen (NH_4^+-N) in soil was determined using $FeSO_4$/Zn reduction method described by Carter [27]. Total P in soil was determined using Sodium carbonate fusion described by Carter [27]. Available phosphorus (P) in soil was determined by extracting samples with 0.5 M $NaHCO_3$, and determining P colorimetrically using molybdate [28]. Total K in soil was determined using Flame photometry method [28]. Available K in soil was determined using 1 N ammonium acetate extraction-flame photometry method [28]. Nitrogen in grain and crop residues (straw and chaff) was determined using the method described by Lu [29]. Potassium in grain and crop residues (straw and chaff) was determined using the method described by Bao [28].

2.4.2. Nitrogen Fixation. Nitrogen fixation by field pea was estimated in 2005 using the method of ^{15}N natural abundance as described by Armstrong et al. [30]. At anthesis, 5 individual field pea plants were cut at ground level from each plot, bulked into one sample and dried at 60°C for 24 h. At the same time, 5 nonlegume plants (weeds) from the plot were also collected and oven-dried at 60°C as "reference plants". Both the legumes and reference plants were ground through 1 mm mesh, then subsampled and finely ground prior to analysis of ^{15}N natural abundance using continuous flow isotope ratio mass spectrometry (Europa Scientific ANCA System) [31].

2.4.3. Grain Yield. The whole plot was harvested manually using sickles at 5 cm above ground. The edges (0.5 m) of the plot were trimmed and discarded. Samples were then processed to obtain grain yield, straw and chaff. The grain yield from the harvesting area was recorded and converted to yield per hectare.

2.5. Calculations.

2.5.1. Nitrogen Balance. Nitrogen balance was calculated over 4 years with two complete rotation cycles. Nitrogen

Table 1: Soil chemical and physical properties at the start of experiment.

Depth (cm)	Bulk density ($g\,cm^{-3}$)	Organic matter ($g\,kg^{-1}$)	Total N ($g\,kg^{-1}$)	Total P ($g\,kg^{-1}$)	Olsen P ($mg\,kg^{-1}$)	Available K ($mg\,kg^{-1}$)	pH
0–5	1.29	13.15	0.85	0.83	5.81	290.09	8.30
5–10	1.23	12.86	0.87	0.84	5.02	274.00	8.40
10–30	1.32	11.95	0.78	0.79	2.14	202.47	8.30

Table 2: Details of treatments used in the long-term conservation tillage experiment.

Code	Treatments	Description
T	Conventional tillage with stubble removed	Fields were ploughed 3 times and harrowed twice after harvesting. The first ploughing was in August immediately after harvesting, the second and third ploughing were in late August and September, respectively. The plough depths were 20 cm, 10 cm, and 5 cm, respectively. The field was harrowed after the last cultivation in September and again in October before the ground was frozen. This is the typical conventional tillage practice in the Dingxi region.
NT	No-till with stubble removed	No-till throughout the life of the experiment. The straw was removed from the field and used as fuel or feed.
TS	Conventional tillage with stubble incorporated	Fields were ploughed and harrowed exactly as for the T treatment (3 passes of plough and 2 harrows), but with straw incorporated at the first ploughing. All the straw from the previous crop was returned to the original plot immediately after threshing and then incorporated into the ground.
NTS	No-till with stubble cover	No-till throughout the life of the experiment. The ground was covered with the straw of previous crop from August until the following March. All the straw from previous crop was returned to the original plot immediately after threshing.

inputs included N in fertilizers and N in seeds. The N in straw brought into the system (6.8 t/ha) in 2002 was also taken into account for TS and NTS treatments. Nitrogen output includes grain N and stubble N if stubble was removed (e.g., T and NT treatments). Nitrogen fixed by field pea in 2001–2004 was extrapolated using data in 2005 as no data were available in 2001–2004.

2.5.2. Nitrogen Fixation. Nitrogen fixation by field pea was calculated as follows:

$$\%\text{Ndfa} = \frac{\left(\delta^{15}\text{N}_{\text{weeds}} - \delta^{15}\text{N}_{\text{legume}}\right)}{\left(\delta^{15}\text{N}_{\text{weeds}} - \beta\right)}, \quad (1)$$

(see [30]), where %Ndfa is the percentage of plant total N derived from fixation, $\delta^{15}\text{N}_{\text{weeds}}$ is the natural abundance of ^{15}N in reference plant (weeds), $\delta^{15}\text{N}_{\text{legume}}$ is the natural abundance of ^{15}N in legume (field pea), and β represents a measure of the isotopic fraction associated with redistribution of N between roots and shoots.

2.5.3. Potassium Balance. Potassium balance was calculated after 5 years. Potassium inputs included K in straw and K in seeds. Potassium output includes grain K and stubble K if stubble was removed (e.g., T and NT).

2.6. Data Analysis. Analysis of variance was performed to determine the effects of different stubble management on soil fertility and grain yield. All statistical analyses of data were carried out through the SPSS package.

3. Results and Discussion

3.1. The Distribution of Soil Organic Matter and Total Nutrients under Different Stubble Management. Results showed that stubble retention increased soil organic matter at the 0–5 cm and 10–30 cm depth significantly ($P < 0.01$), while increased soil organic matter at the 5–10 cm significantly ($P < 0.001$) after 6 years in W/P rotation sequence (Table 3). In the top 5 cm depth, soil organic matter under NTS was the highest, while soil organic matter under TS was the highest at lower depths. The pattern for organic matter distribution in the W/P rotation sequence was similar to that in the P/W rotation sequence. Soil organic matter concentration was relatively uniformly distributed within the 0–30 cm depth under TS treatment. In contrast, NTS treatment resulted in a significant increase in soil organic matter at the soil surface.

Accumulation of soil organic matter at the soil surface was a result of surface placement of crop residues and a lack of soil disturbance that kept residues isolated from the rest of the soil profile. Decomposition of surface-placed residues is often slower than when incorporated in the soil profile [32, 33], primarily because of less optimal moisture conditions [34]. The apparent soil organic matter accumulation in stubble retention treatments noted by these results is consistent with the findings of Lao et al. [35] and

Table 3: Soil total nutrients as affected by stubble management in different rotation sequence ($g\,kg^{-1}$).

Depth (cm)	Treatment	Field pea-spring wheat (P/W)				Spring wheat-field pea (W/P)			
		Organic matter	Total N	Total P	Total K	Organic matter	Total N	Total P	Total K
0–5	T	13.90 ± 0.64	1.00 ± 0.05	0.85 ± 0.03	15.08 ± 0.64	13.30 ± 0.52	1.00 ± 0.04	0.90 ± 0.07	15.58 ± 0.76
	NT	14.01 ± 0.79	1.05 ± 0.02	0.90 ± 0.15	14.61 ± 0.34	13.78 ± 1.17	1.04 ± 0.03	0.96 ± 0.04	14.48 ± 0.34
	TS	15.26 ± 0.43	1.09 ± 0.03	1.06 ± 0.12	16.31 ± 0.92	14.56 ± 0.62	1.09 ± 0.04	1.07 ± 0.02	16.36 ± 0.33
	NTS	15.66 ± 1.12	1.13 ± 0.02	1.08 ± 0.04	16.77 ± 0.35	16.81 ± 1.50	1.21 ± 0.11	1.09 ± 0.03	16.92 ± 0.14
	Significant	**	*	*	*	***	*	**	**
5–10	T	13.83 ± 0.68	0.99 ± 0.04	0.80 ± 0.02	15.08 ± 0.60	13.14 ± 0.32	0.99 ± 0.03	0.85 ± 0.06	14.47 ± 0.71
	NT	14.00 ± 0.58	1.04 ± 0.04	0.82 ± 0.02	14.13 ± 0.22	13.70 ± 0.87	1.01 ± 0.02	0.95 ± 0.14	13.98 ± 0.61
	TS	15.11 ± 0.38	1.04 ± 0.04	1.00 ± 0.09	15.47 ± 1.73	14.24 ± 0.84	1.01 ± 0.01	0.99 ± 0.02	15.67 ± 0.26
	NTS	14.78 ± 0.17	1.07 ± 0.01	1.02 ± 0.13	16.17 ± 0.33	14.94 ± 0.62	1.12 ± 0.09	1.03 ± 0.08	16.31 ± 0.26
	Significant	***	ns	*	ns	***	*	ns	**
10–30	T	13.17 ± 0.79	0.99 ± 0.04	0.81 ± 0.06	14.53 ± 0.40	12.42 ± 1.09	0.96 ± 0.06	0.871 ± 0.03	14.54 ± 0.48
	NT	13.70 ± 0.82	0.99 ± 0.03	0.81 ± 0.05	13.86 ± 0.43	13.49 ± 0.48	1.01 ± 0.05	0.87 ± 0.12	14.21 ± 0.37
	TS	14.45 ± 0.59	1.00 ± 0.03	0.83 ± 0.04	15.10 ± 1.05	14.03 ± 0.91	1.01 ± 0.03	0.89 ± 0.02	15.27 ± 0.85
	NTS	14.15 ± 0.26	1.00 ± 0.05	0.84 ± 0.02	15.45 ± 0.66	13.80 ± 0.75	1.02 ± 0.04	0.86 ± 0.02	15.49 ± 0.04
	Significant	**	ns	ns	ns	*	ns	ns	ns

Significant level (ns: not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

Lin et al. [36]. Sun et al. [7] reported that soil organic matter increased 15.8% ∼18.1%, 6.6% ∼10.6%, and 1.3% ∼1.9% stubble retention compared with straw removal in the 0–10, 10–20, 20–40 cm depths in Wushan soil after 15 years.

Stubble-induced changes in soil total N are often directly related to changes in soil organic C. This similarity may be related to soil organic matter which could influence nutrient retention and supply [37]. Both stubble retention and no-tillage increased soil total N concentration significantly ($P < 0.05$) at the soil surface 0–5 cm (Table 3), but it was uniformly distributed with depths under T treatment. In the 5–10 cm, both stubble retention and no-tillage increased soil total N concentration significantly ($P < 0.05$) in W/P rotation sequence, but not in P/W rotation sequence. In the 10–30 cm depths, there was no difference in soil total N concentration ($P > 0.05$) among stubble management and tillage systems in either rotation sequences. Soil total N probably contains compounds that are more resistant to decomposition and which consequently can affect N dynamics in the soil. Reeves et al. [38] reported that under stubble management and no-tillage system, soil N losses were reduced, but short-term N availability was also reduced. The higher total N content associated with stubble retention treatments in this study can also be attributed to a reduction in soil erosion. The amount of soil lost due to soil erosion has been reported as high as 3720 $t\,km^{-2}\,year^{-1}$, rising to maxim of 3720 $t\,km^{-2}\,year^{-1}$, in this area during rainy season (July–September) [21]. The stubble retention treatments leaves crop residues on the soil surface and creates more large size soil aggregates, thus reducing soil erosion and contributing to the higher organic carbon and total N content in soil. In addition, because stubble yield was different for TS and NTS, the amount of crop stubble returned to the soil should be different as well. Thus, difference in total N was not only due to difference in disturbance by tillage operations, but also due to difference in the amount of crop residue returned to the soil under TS and NTS.

Unlike soil C and N, soil P does not readily undergo oxidation-reduction reactions in the common processes of organic matter decomposition. Total P concentration was significantly ($P < 0.05$) greater in the top 5 cm depth of stubble retention treatments in P/W rotation sequence, while significantly ($P < 0.01$) greater in the top 5 cm depth of stubble retention treatments in W/P rotation (Table 3). In the 5–10 cm depth, total P concentration was significantly ($P < 0.05$) greater in P/W rotation sequence, but not in W/P rotation sequence. In the 10–30 cm depths, there was no difference in soil total P concentration ($P > 0.05$) among residue management and tillage systems in either rotation sequences. NTS had the lowest total P concentration in W/P rotation sequence. Surface application of phosphate fertilizer, immobilization of phosphate fertilizer as well as stubble cover in surface soil may account for this result. It has been proposed that organic matter itself may be considered an important source for P recycling in the short and long term [39].

Total K was significantly ($P < 0.05$) greater in the top 5 cm depth of stubble retention treatments in P/W rotation sequence, while significantly ($P < 0.01$) greater in the top 5 cm depth of stubble retention treatments in W/P rotation (Table 3). In the second 5 cm, total K concentration was only significantly ($P < 0.01$) greater in W/P rotation sequence, but not in P/W rotation sequence. In the 10–30 cm depth, although NTS and TS showed higher total K concentration, there was no difference ($P > 0.05$) among stubble management and tillage systems in either rotation sequences. This result is due to output of soil K removed out of cropland with crop straw, which could be returned back to soil by organic material recycling in stubble retention treatments. It has been proposed that straw itself may be

Table 4: Soil available nutrients as affected by stubble management in different rotation sequence (mg kg^{-1}).

Depth (cm)	Treatment	P/W			W/P		
		Available N	Available P	Available K	Available N	Available P	Available K
0–5	T	37.37 ± 1.77	13.61 ± 0.84	211.41 ± 21.28	37.31 ± 0.94	14.22 ± 0.82	192.04 ± 13.02
	NT	33.94 ± 1.97	13.78 ± 0.55	247.13 ± 12.49	35.63 ± 0.90	14.25 ± 2.50	227.91 ± 21.18
	TS	37.97 ± 0.92	16.40 ± 1.34	263.76 ± 38.74	40.64 ± 1.82	16.23 ± 0.76	265.17 ± 16.01
	NTS	37.44 ± 1.02	18.96 ± 1.46	280.66 ± 47.11	40.76 ± 1.86	18.68 ± 2.29	286.98 ± 41.57
	Significant	ns	***	ns	*	*	*
5–10	T	35.13 ± 3.02	12.34 ± 1.10	190.74 ± 19.19	35.94 ± 2.25	14.95 ± 1.02	187.41 ± 37.75
	NT	34.54 ± 0.99	13.12 ± 1.45	202.43 ± 32.17	34.51 ± 5.88	15.09 ± 0.89	191.90 ± 26.75
	TS	36.77 ± 1.84	14.15 ± 1.38	259.75 ± 4.02	39.63 ± 0.98	16.30 ± 2.22	248.04 ± 34.31
	NTS	34.61 ± 2.03	16.04 ± 2.12	262.65 ± 24.37	37.95 ± 2.65	16.11 ± 0.58	265.27 ± 45.85
	Significant	ns	*	*	ns	ns	ns
10–30	T	41.91 ± 0.09	5.57 ± 2.34	159.45 ± 9.27	42.44 ± 2.61	5.15 ± 0.65	139.25 ± 5.39
	NT	37.43 ± 1.95	4.76 ± 0.72	163.82 ± 6.59	37.99 ± 1.00	4.74 ± 0.56	147.90 ± 16.71
	TS	41.38 ± 0.98	5.74 ± 0.39	195.07 ± 9.71	42.42 ± 2.46	5.24 ± 0.38	192.27 ± 6.81
	NTS	37.91 ± 2.50	5.57 ± 0.44	202.21 ± 20.53	40.77 ± 2.48	4.96 ± 0.61	202.03 ± 46.50
	Significant	*	ns	*	ns	ns	*

Significant level (ns: not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

considered an important K source, which could return back 70∼80% of soil K removed from cropland with crop straw [9].

3.2. The Distribution of Soil Available Nutrients under Different Stubble Management. Results showed that soil available N concentration was higher in the top 5 cm depth of stubble retention, while higher in the 10–30 cm depth of stubble removal treatment among residue management and tillage systems in either rotation sequences. However, there were no difference in soil available N concentration ($P > 0.05$) among stubble management and tillage systems, except for that in the top 5 cm of W/P rotation sequence and 10–30 cm of P/W rotation sequence (Table 4). In this experiment, available N referred to nitrate-N and ammonia-N. Residue retention could reduce gaseous loss by volatilization of ammonia-N arisen from soil alkalinity and increased temperatures [40, 41], while denitrification and leach of nitrate-N, on the other hand, could be very serious with a combination of no tillage due to improved soil moisture [42–45].

Available P concentration was significantly ($P < 0.001$) greater in the top 5 cm depth of stubble retention treatments in P/W rotation sequence, while significantly ($P < 0.05$) greater in the top 5 cm depth of stubble retention treatments in W/P rotation (Table 4). In the 5–10 cm depth, available P concentration was significantly ($P < 0.05$) greater in P/W rotation sequence, but not in W/P rotation sequence. In the 10–30 cm depths, there was no difference in soil available P concentration ($P > 0.05$) among residue management and tillage systems in either rotation sequences. Surface application of phosphate fertilizer, immobilization of phosphate fertilizer as well as stubble cover in surface soil may account for this result. P solubility is known to be enhanced by increasing SOM and decreasing pH in alkaline soils [46] by acidifying the rhizosphere soil. Where crop residues are returned to the soil, an increase in P availability may occur by decreasing the adsorption of P to mineral surfaces [47] which complements biologically mediated release of P to improve crop P status. With time, soil and crop residue management practices that promote organic matter accumulation would be expected to improve P nutrition of crops.

Available K concentration was only significantly ($P < 0.05$) greater in the top 5 cm depth of stubble retention treatments in W/P rotation sequence, but not in P/W rotation sequence (Table 4). In the 5–10 cm, available K concentration was only significantly ($P < 0.05$) greater in rotation P/W sequence, but not in W/P rotation sequence. In the 10–30 cm depth, there was significantly ($P < 0.05$) difference among stubble management in either rotation sequences. Intermediate or final products involving organic acids and CO_2 produced by SOM could improve availability of fixed K. Where crop residues are returned to the soil, an increase in K availability may occur by decreasing the adsorption of K to clay mineral surfaces [43]. In this case, quality of available nutrients for seasoning crop could be enhanced by residue management and tillage systems, thus ensuring improvement of grain yields [48].

3.3. Soil Nutrient Balance under Different Stubble Management. TS and NTS had higher total N input than T and NT due to extra N from previous straw (Table 5), but no difference between TS and NTS and between T and NT in either rotation sequences. However, the total N output was significantly ($P < 0.01$) different between treatments in either rotation sequences. The treatments with high harvest dry matter and higher grain yield had more N% output from the system. However, the most of N could be returned back to soil by organic material recycling in stubble retention treatments. The T treatment exported the greatest N from crop harvest, whereas TS treatment had the lowest over 4 years (Table 5). N fixation by field pea under different treatments was also significantly ($P < 0.001$) different in

Table 5: Nitrogen balance under different treatments over 4 years (kg N ha^{-1}).

Rotation	Item	T	NT	TS	NTS	Significant
P/W	Input	269.00	269.00	299.37	299.37	—
	Fixation	4.65	27.12	20.73	26.06	***
	Output	183.45	167.18	139.73	159.25	**
	Balance	90.20	128.94	180.37	166.18	***
W/P	Input	269.00	269.00	299.37	299.37	—
	Fixation	3.42	25.63	16.90	24.89	***
	Output	144.82	130.34	102.51	139.09	**
	Balance	127.60	164.29	213.76	185.17	***

Significant level (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

Table 6: Potassium balance under different treatments over 5 years (kg K ha^{-1}).

Rotation	Item	T	NT	TS	NTS	Significant
P/W	Input	4.93	4.65	40.15	49.27	—
	Output	41.88	33.01	52.48	60.52	**
	Balance	−36.95	−28.36	−12.32	−11.25	**
W/P	Input	0.68	0.68	14.68	19.03	—
	Output	27.82	31.38	35.99	39.87	**
	Balance	−27.14	−30.70	−21.31	−20.84	**

Significant level (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

either rotation sequences (Table 5). As a result, over 4 years all treatments accumulated N in soils, and there was also significant ($P < 0.001$) difference under different treatments in either rotation sequences (Table 5). Treatments, retained the crop stubble, had high N balance at the end of two rotation cycles. Therefore, stubble retention could improve soil N balance year by year. But as a whole, soil N increased from 2002 to 2005 for both phases, indicating total N fertilizer applied in the field were more than crop needed as evidenced by high N balance at the end of two rotation cycles. Therefore, it is recommended that stubble retention should be practiced to increase crops productivity and improve soil N storage and N fertilizer use should be reduced. High N input not only increased input costs, but also increased the risk of environmental contamination in the ground water system. In the current research, there was up to 185 kg ha^{-1} surplus N (i.e., W/P rotation sequence) accumulated after two rotation cycles under the current fertilizer regime over the 4 years. It appears to be excellent prospects for reducing current farmer fertilizer N inputs while maintaining spring wheat yields in all seasons. This is a significant finding as it will directly increase farm profitability with little risk. Considerable savings are likely by adopting optimum fertilizer N rates. There appear to be no other reports on estimates of N fixation for the Loess Plateau.

No K fertilizer and farm manure was applied in this experiment. In P/W rotation sequence, total K input was same for T and NT, but more K input for TS and NTS due to K input from previous straw. However, total K output was significantly ($P < 0.01$) different between treatments. The higher harvest dry matter and higher grain yield, the higher K output from the system. NTS treatment exported the greatest K from crop harvest, whereas NT treatment had the lowest K. As a result, there was also significant ($P < 0.01$) different in soil K balance under different treatments (Table 6). Soil K balance in W/P rotation sequence was similar to that in P/W rotation sequence. However, the average K balance under T treatment in W/P rotation sequence was higher than that in P/W rotation sequence, whereas the average K balance under TS, NT, and NTS treatments in W/P rotation sequence was lower than that in P/W rotation sequence. Unlike soil N balance being in surplus, soil K was indeficient for all treatments, ranged from 11.25 kg K ha^{-1} for the NTS treatment to 36.95 kg K ha^{-1} for the T treatment (Table 6). Therefore, it is suggested that fertilizer K should be applied to maintain soil K balance.

3.4. Crop Yield under Different Stubble Management. For spring wheat, the treatments with stubble retention produced more grain yields than the treatments with stubble removed in all 8 years. There were significant differences in grain yield among treatments, except that in 2005, 2006, and 2008 (Table 7). For field pea, there were significant differences among treatments, except that in 2004, 2005, 2008, and 2009. Averaged across 8 years, yield of wheat under the NTS and TS treatment was 21% (0.344 t ha^{-1}) and 9% (0.144 t ha^{-1}) higher than the T treatment. Similarly, yield of field pea under the NTS and TS treatment was 20% (0.227 t ha^{-1}) and 2% (0.026 t ha^{-1}) higher than the T treatment. A recent survey of farmers in the area surrounding the experimental site found average grain yield was just 1.0 t ha^{-1} for spring wheat and 0.8 t ha^{-1} for field pea in the 2003 season [49]. Corresponding results from the current study for T and NTS were 1.641 t ha^{-1} and 1.986 t ha^{-1} for

Table 7: Grain yield under different treatments (t ha^{-1}).

Crop	Year	T	NT	TS	NTS	Significant
Spring wheat	2002	1.816 ± 0.279	1.414 ± 0.362	1.736 ± 0.276	2.151 ± 0.246	***
	2003	1.416 ± 0.281	1.545 ± 0.356	1.646 ± 0.367	1.825 ± 0.132	*
	2004	2.189 ± 0.248	1.664 ± 0.219	2.162 ± 0.221	2.382 ± 0.304	*
	2005	2.900 ± 0.519	3.077 ± 0.292	2.988 ± 0.663	3.327 ± 0.060	ns
	2006	1.383 ± 0.210	1.317 ± 0.200	1.565 ± 0.235	1.549 ± 0.123	ns
	2007	0.562 ± 0.132	0.633 ± 0.169	0.666 ± 0.126	0.944 ± 0.187	**
	2008	1.632 ± 0.549	1.818 ± 0.899	1.851 ± 0.312	2.100 ± 0.329	ns
	2009	1.233 ± 0.371	0.985 ± 0.644	1.670 ± 0.325	1.607 ± 0.383	*
Field pea	2002	1.653 ± 0.177	1.416 ± 0.275	1.527 ± 0.313	1.790 ± 0.213	*
	2003	0.881 ± 0.206	0.803 ± 0.156	0.823 ± 0.101	1.269 ± 0.288	**
	2004	1.708 ± 0.145	1.496 ± 0.440	1.681 ± 0.349	1.668 ± 0.193	ns
	2005	1.686 ± 0.241	1.816 ± 0.268	1.911 ± 0.672	2.119 ± 0.534	ns
	2006	0.759 ± 0.129	0.552 ± 0.122	0.872 ± 0.123	0.890 ± 0.048	**
	2007	0.206 ± 0.023	0.277 ± 0.067	0.342 ± 0.053	0.553 ± 0.088	***
	2008	1.342 ± 0.196	1.306 ± 0.387	1.190 ± 0.486	1.649 ± 0.180	ns
	2009	0.762 ± 0.127	0.727 ± 0.087	0.857 ± 0.143	0.873 ± 0.249	ns

Significant level (ns: not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

spring wheat, and 1.125 t ha^{-1} and 1.351 t ha^{-1} for field pea, respectively. This represents a potential significant improvement in grain yield of 40%∼69% for field pea and 64%∼99% for spring wheat through better agronomic practices (e.g., stubble retention). These results were inconsistent with the findings of McCalla and Army [50] who reported that stubble retention decreased yields, especially in a humid climate, due to poor crop establishment under reduced tillage [51]. Graham et al. [52] found that in autumn-sown crops under direct drill, the yields were lower when straw residues were left on the surface than when residues were burnt. Incorporation of straw reduced the detrimental effect of straw on yields, but the yields were lower than those where the straw had been burnt.

4. Conclusions

Stubble retention on an alkaline soil in a semiarid areas for several years markedly improved fertility and increased crop productivity. Stubble retention resulted in significantly greater soil organic matter at the 0–30 cm depth. Stubble retention increased soil total N significantly at the soil surface 0–5 cm, but soil total N concentration remained similar at 10–30 cm for all treatments. Total P and total K were significantly greater in the 0–10 cm depth under stubble retention treatments. There were no difference in soil available N concentration among stubble management and tillage systems, except that in the top 5 cm of W/P rotation sequence and 10–30 cm of P/W rotation sequence. Available P concentration was significantly greater in the top 5 cm and 5–10 cm depth. Available K concentration was significantly greater at the 0–30 cm depth of stubble retention treatments. Soil N increased greatly from 2002 to 2005 for all treatment. The two stubble retention treatments had the higher N balance at the end of two rotations. Soil K was indeficient for all treatments with more deficit under two stubble retention treatments. As a result, the treatments with stubble retention produced more grain yields than the treatment with stubble removed in all 8 years. Grain yields were the highest under NTS, but the lowest under NT for both spring wheat (1.986 versus 1.557 t ha^{-1}) and field pea (1.351 versus 1.049 t ha^{-1}). It was concluded that stubble retention should be practiced to increase crops productivity, improve soil fertility as well as agriculture sustainability in the Loess plateau.

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Research Article

Soil Application of Tannery Land Plaster: Effects on Nitrogen Mineralization and Soil Biochemical Properties

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Tannery land plaster (TLP) is a byproduct of lime hydrolysis of leather shavings. Its use in agriculture (organic C ≈ 17%, N ≈ 6% dm) could represent an alternative to landfill or incineration, but the high Cr(III) content (≈5% dm) makes it necessary to evaluate the effect on soil biochemical properties. TLP was therefore added at the rates of 220 and 440 kg of N ha^{-1} to 2 agricultural soils and incubated for 56 days under controlled conditions. Extractable NH_4^+-N and NO_3^--N, CO_2-C evolution, microbial biomass-N, protease activity, and extractable Cr were monitored. The organic N was readily mineralized (>50% in the first week) and a significant increase in microbial activity was measured, regardless of soil type and addition rate. Extractable Cr(III) quickly decreased during the incubation. The absence of a negative impact on soil biochemical properties seems to support the use of TLP in agriculture, although further investigations in long-term field experiments are suggested.

1. Introduction

The production of biosolids from municipal solid waste, sewage sludge, and waste of agroindustrial origin is continually increasing [1]. Their potential use in agriculture as an alternative method to landfill or incineration has become an increasingly attractive option, due to current trends in European waste policy [2, 3]. The agricultural use of these byproducts could help in maintaining soil organic matter (OM) content and promoting the recycling of plant nutrients, thus reducing the use of chemical fertilizers [1] and increasing agricultural production sustainability [4]. However, in order to recycle biosolids in soil it is necessary to exclude any hazardous effects for humans, animals, plants, and soil microbial populations. Appropriate control of chemical and physical characteristics of the biosolids, nutrients, and heavy metal dynamics in soils is needed in order to guarantee the agronomical value of the products and environmental safety.

Tannery land plaster (TLP) is a by-product of lime hydrolysis of leather shavings, a residue of the leather production cycle classified as treated industrial sewage sludge [5]. After the alkaline hydrolysis of leather shavings, sulphuric acid is added to neutralize the suspension, and a calcium sulphate precipitate is then separated by filtration, obtaining the TLP. TLP is currently disposed of in landfill or, at best, used to correct soil acidity. However, due to the significant amount of N (≈6% dm) its soil application as a source of organic N may represent a more suitable recycling strategy.

TLP originates from Cr tanned animal hides, thus it contains large amounts of Cr(III) (≈5% dm), a human, and animal micronutrient but, at the same time, a potentially toxic element [6] that could represent a factor of concern in agriculture and the environment. To date European legislation on sewage sludge [5] does not set any threshold value for Cr, even though the introduction of such a limit is presently under discussion (total Cr in sewage sludge and

Table 1: Chemical characteristics of tannery land plaster (TLP).

Parameter	Units	Value (all data are expressed on a dry matter basis)
Water content	($g\,kg^{-1}$)	730*
Total solids (TS)	($g\,kg^{-1}$)	270*
Ash	($g\,kg^{-1}$)	660
Volatile solids (VS)	($g\,kg^{-1}$)	90*
pH (in water)	pH	10.6
Electrical conductivity (EC)	($dS\,m^{-1}$)	3.6*
Total organic carbon (TOC)	(%)	17
Total nitrogen (TN)	($g\,kg^{-1}$)	57
NH_4^+-N	($g\,kg^{-1}$)	0.7
NO_3^-N	($g\,kg^{-1}$)	0.3
C : N ratio	—	3
Total P	($g\,kg^{-1}$)	3.1
Total K	($g\,kg^{-1}$)	0.24
Total S	($g\,kg^{-1}$)	123
Total Ca	($g\,kg^{-1}$)	175
Total Mg	($g\,kg^{-1}$)	3.9
Total Na	($g\,kg^{-1}$)	2.3
Total Fe	($mg\,kg^{-1}$)	755
Total Cu	($mg\,kg^{-1}$)	3.3
Total Zn	($mg\,kg^{-1}$)	8.3
Total Mn	($mg\,kg^{-1}$)	36
Total Co	($mg\,kg^{-1}$)	9.6
Total Al	($mg\,kg^{-1}$)	373
Total Cd	($mg\,kg^{-1}$)	<1.0
Total Ni	($mg\,kg^{-1}$)	1.6
Total Pb	($mg\,kg^{-1}$)	<0.5
Total Cr	($g\,kg^{-1}$)	52.0
Cr(VI)	($mg\,kg^{-1}$)	<0.5

* Fresh matter.

soil). However, since the form in sewage sludge is Cr(III), characterized by very low mobility and translocation in both soil and plants [7], we believe that its available fraction, rather than its total concentration, is the key parameter determining its potential toxicity. Although Cr(III) is 3 orders of magnitude less toxic than Cr(VI) [8], at high concentrations it can be harmful to the microbial community. Liang and Tabatabai [9] found an inhibitor effect on soil N mineralization processes after the addition of sewage sludge at a concentration of Cr(III) of 550 $mg\,kg^{-1}$. Soil biological parameters can be used as effective indicators of modification in soil status due to pollution and variation in soil management [9]. Soil respiration, a good indicator of the oxidation of the soil OM to CO_2 by aerobic heterotrophic microorganisms, and enzyme activities related to the cycle of main nutrients could give important indications on the rate of nutrient turnover. These parameters can also be used as a measure of microbial activity and its level of disturbance by inhibiting effects of pollutants and particularly of heavy metals [10, 11]. Plant availability of organic N in a biosolid mainly depends on soil microbial processes of mineralization and immobilization whose turnover is mainly influenced by its C : N ratio, although the soil N turnover cannot be explained by this parameter alone. Soil chemical-physical characteristics and biosolid properties such as soluble C content, N biochemical quality, and phenolic content could play a crucial role in determining N mineralization [12–16]. Specific studies on the mineralization dynamic of the organic N in biosolids are therefore required in order to evaluate N supply to plants and to avoid leaching of N in the environment.

Table 2: Main physical and chemical characteristics of the two soils (M and P).

Parameter	Units	Soil	
		M	P
Sand	($g\,kg^{-1}$)	330	340
Silt	($g\,kg^{-1}$)	510	500
Clay	($g\,kg^{-1}$)	160	160
Water holding capacity (WHC)	($g\,kg^{-1}$)	310	280
pH (in water)	—	8.3	7.9
Total calcium carbonate ($CaCO_3$)	($g\,kg^{-1}$)	15	20
Cation exchange capacity (CEC)	($cmol_c\,kg^{-1}$)	26	25
Total organic C (TOC)	($g\,kg^{-1}$)	10.6	7.7
Total Kjeldahl N (TKN)	($g\,kg^{-1}$)	1.1	0.91
C : N ratio	—	10	8.5
Olsen-P	($mg\,kg^{-1}$)	18	42
Total Mn	($mg\,kg^{-1}$)	42	40
Total Cr	($mg\,kg^{-1}$)	25	23
Extractable Cr	($mg\,kg^{-1}$)	0.63	0.57
Cr(VI)	($mg\,kg^{-1}$)	<0.5*	<0.5*

* Detection limit.

At present little is known about TLP-Cr(III) behaviour in soil and, in particular, on the dynamics of its extractable fraction, likely to be available to soil microorganisms.

The aim of this research was to study the effects of applying agronomical rates of TLP on (i) C and N mineralization, (ii) soil microbial biomass N, (iii) protease activity, and (iv) extractable Cr(III) dynamics in two agricultural soils in a short-term laboratory experiment.

2. Materials and Methods

2.1. Chemical Characterization of Tannery Land Plaster. A chemical characterization of TLP was carried out (Table 1) to determine water and ash content, total (TS) and volatile solids (VS), pH, electrical conductivity (EC), total organic carbon (TOC), and nitrogen (TN), ammonium (NH_4^+-N), nitrates (NO_3^--N), water extractable hexavalent chromium [Cr(VI)], total nutrients, and the most representative trace heavy metal contents. Water content was determined after oven drying at 105°C for 24 h, pH and EC were measured according to the method reported by Trinchera et al. [17]. TOC was determined by the $K_2Cr_2O_7$ oxidation method described by Ciavatta et al. [18], TN using an elemental analyzer (Thermo Fisher Scientific) and mineral N according

to the method reported by Violante [19]. Other nutrients and heavy metals were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES, Spectro Ciros^{CCD}) after digestion of the sample with 65% HNO_3. The Cr(VI) was determined by 1,5-diphenylcarbazide colorimetric analysis after water extraction [17].

2.2. Soil Properties and Incubation Conditions. Two soils (Typic Udifluvent, USDA Soil Taxonomy) representative of an important agricultural area located in the southeast of the Po valley (Ravenna, Italy), hereinafter named M and P, were chosen for the experiment because they are involved in a larger study, in which the TLP is used for the N fertilization of maize and tomato under field conditions (Table 2). Soil samples from the top layer (0–20 cm depth) were collected in the early spring of 2007, wet-sieved at 4 mm, and then air-dried. Chemical analyses were carried out using the official Italian methods [19]. Water holding capacity (WHC) was determined as described by Agehara and Warncke [20]. Total Cr was determined by ICP-OES after digestion with 65% HNO_3 while Cr(VI) in the water extract by diphenylcarbazide colorimetric method [21]. Extractable Cr was evaluated using the rhizosphere-based method [22]. Briefly, 2 g of moist soils were mixed with 20 mL of combined solution of acetic, lactic, citric, malic, and formic acids in a 50 mL centrifuge tube (extraction ratio 1 : 10 w/v). The concentration of the organic acids was 10 mM, and their molar ratio was 4 : 2 : 1 : 1 : 1, respectively. The soil suspension was shaken by an end-over-end shaker for 16 h (60 rpm), centrifuged at 1000 g for 10 min and filtered with Whatman no. 42 filter paper. Five mL of supernatant were mixed with 5 mL of 2% HNO_3. The Cr content in soil extracts was determined by ICP-OES.

The soils were preconditioned for 14 days at 70% of their WHC at 25°C, to enable acclimatization to incubation conditions. At the end of this period, 3.16 and 6.32 mg g^{-1} dry soil of TLP (previously lyophilised and grounded) corresponding to 0.18 and 0.36 mg N g^{-1} of dry soil, respectively, were added to 150 g soil samples. The application rates were calculated on an agronomic basis and corresponded to 220 and 440 kg of N ha^{-1}, respectively. Unamended soil was taken as the control. The experiment was carried out in triplicate and soils were sampled after 0, 3, 7, 14, 28, and 56 days of incubation that was performed in the dark at the same conditions adopted in the pre-conditioning phase.

2.3. Extractable Mineral N. Aliquots of 5 g of soil samples were extracted with 50 mL of 0.5 M K_2SO_4 for 1 hour. Extractable NH_4^+-N and NO_3^--N were determined by the Bran Luebbe AACE 5.46 Auto Analyzer method. The NO_2^--N content was assumed to be negligible in comparison with NH_4^+-N and NO_3^--N.

The cumulative amount of mineral N released from TLP at time t (N_{TLP}) was calculated using the following equation:

$$N_{TLP} = N_m(\text{TLP-treated soil})_t - N_m(\text{control})_t - N_m\text{TLP}, \quad (1)$$

where N_m is the mineral N content (NH_4^+-N + NO_3^--N).

The percentage of organic N released from TLP at time t ($\%N_{TLP}$) was calculated using the following equation:

$$\%N_{TLP} = \left(\frac{N_{TLP}}{N_{0TLP}}\right) * 100. \quad (2)$$

N_{0TLP} is the amount of organic N in TLP sample calculated as follows: $N_{0TLP} = TN_{TLP} - MN_{TLP}$, where TN_{TLP} is the total N in TLP samples and MN_{TLP} is the mineral N in TLP sample.

The percentage of mineral N released was fitted to a first-order model [21] using a nonlinear curve-fitting procedure:

$$\%N_{TLP} = \%N_0 * [1 - \exp(-k_0 * t)], \quad (3)$$

where $\%N_0$ is the % of potentially mineralizable organic N added; k_0 is the first-order rate constant (day^{-1}).

The N_0 and k_0 values were deemed significantly different ($\alpha = 0.05$) if the 95% confidence intervals did not overlap.

2.4. Biochemical Analysis. Ninhydrin (2,2-dihydroxyindane-1,3-dione) reactive N content of the microbial biomass was determined on soil extracts obtained using the fumigation extraction method [23]. Moist soil portions, equivalent to 10 g of oven dried soil each, were fumigated with ethanol-free chloroform for 24 h, then extracted with 40 mL 0.5 M K_2SO_4 for 30 minutes. Unfumigated soil samples were similarly extracted. Microbial biomass ninhydrin reactive N was calculated by the difference between the ninhydrin N value extracted by K_2SO_4 from fumigated samples subtracted with the ninhydrin N value extracted by K_2SO_4 from unfumigated samples [24].

Protease activity was determined according to Ladd and Butler [25]. Moist soil (1 g oven dry basis) was mixed with 5 mL TRIS (2-Amino-2-hydroxymethyl-propane-1,3-diol) buffer (pH 8.1), and 5 mL of 2% Na-casein (suspended in the TRIS buffer). The soil mixture was incubated in a shaking water bath at 50°C for 2 h. Controls were performed by adding the substrate suspension after the incubation. The reaction was stopped with 5 mL of 15% trichloroacetic acid solution (TCA) and the suspension was centrifuged for 10 min at 5000 rpm. The clear supernatant (5 mL) was placed in tubes, treated with 7.5 mL of a 50 : 1 : 1 mixture of 0.06 M NaOH, 5% Na_2CO_3 0.5%, $CuSO_4 \cdot 5H_2O$, and 1% potassium sodium tartrate and incubated for 15 minutes. After the incubation, 5 mL of 33% Folin-Ciocalteu reagent (FCR) were added and after 1 h, the absorbance was determined at λ 700 nm.

Microbial respiration was measured using the method described by Isermeyer [26]. CO_2 evolution was measured after 1, 2, 3, 5, 7, 9, 14, and 21 days on aliquots of moist soils (10 g oven dry basis; 70% WHC) incubated at 25°C in glass jars by means of 10 mL 1 M NaOH traps. Three replicates were carried out as well as blank CO_2 traps without soil samples. CO_2 evolution was determined by adding 2 mL of 0.5 M $BaCl_2$ to CO_2 traps and titrating to 8.8 pH with 0.025 M HCl. The respiration rate (μg CO_2-C g^{-1} soil h^{-1}) and the cumulative evolved CO_2 (μg CO_2-C g^{-1} soil) were calculated.

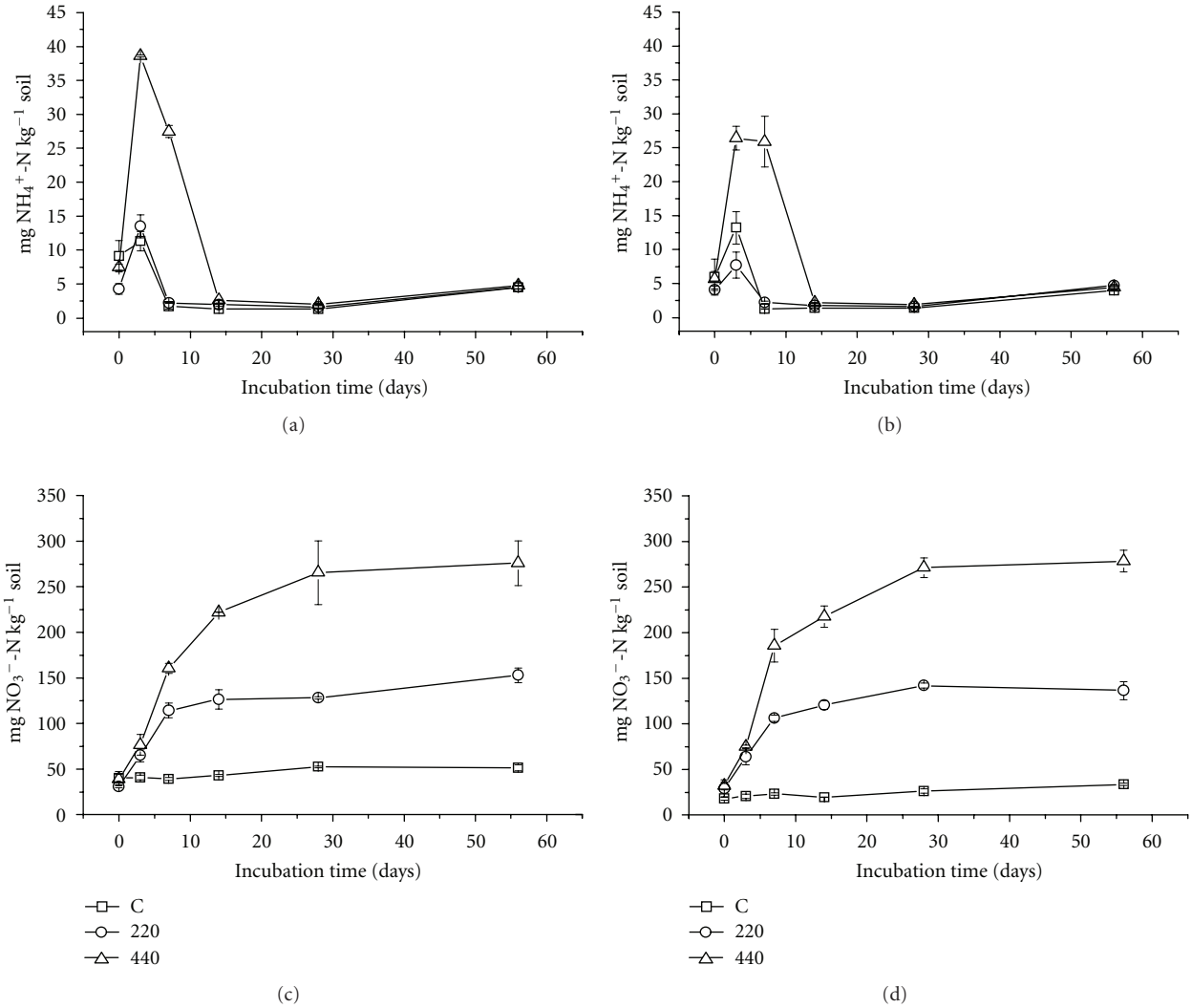

Figure 1: Concentrations of K_2SO_4 extractable NH_4^+-N in soil M (a) and soil P (b) and K_2SO_4 extractable NO_3^--N in soil M (c) and soil P (d) at different sampling times. C: controls; 220: TLP addition rate at 220 kg N ha^{-1}; 440: TLP addition rate at 440 kg N ha^{-1}; bars represent standard deviation ($n = 3$).

2.5. Data Analysis. An overall analysis of variance (ANOVA) was used for each dependent variable; the model included the main effects of soil type and amendment and their two-way interaction.

Data obtained at each sampling time and from each soil type were subjected to one-way analysis of variance and to mean separation by *Bonferroni* test at $^*P \leq 0.05$ significant levels.

3. Results and Discussion

3.1. Pattern of Mineral-N Release. The mineralization process started immediately after TLP addition and a significant accumulation of NH_4^+-N was observed in both soils treated with 440 kg N ha^{-1} during the first week of incubation (Figure 1). The concentration of NH_4^+-N then rapidly decreased and from the 2nd week of incubation was very similar to the other treatments. On the contrary, both soils treated with 220 kg N ha^{-1} showed values of extractable NH_4^+-N similar to the controls.

Amendment with TLP caused a significant increase of NO_3^--N in both soils (Figure 1), detectable from the 3rd day of incubation. After a week, a significant effect of the application rate was detected in both soils and at the end of incubation the NO_3^--N concentration a stabilization was shown.

The percentages of net cumulative mineral N (NH_4^+-N + NO_3^--N), fitted to a first kinetic order model, are reported in Figure 2. After 14 days of incubation, from 45 to 55% of the N added with TLP was mineralized and the values reached 57 to 68% at the end of the experiment.

The organic N added with TLP was readily mineralized to N-NH_4^+ and further converted into N-NO_3^-, regardless of the soil type and the addition rate. The extent and dynamics of N mineralization observed in this study are in agreement with other results obtained with different organic fertilizers

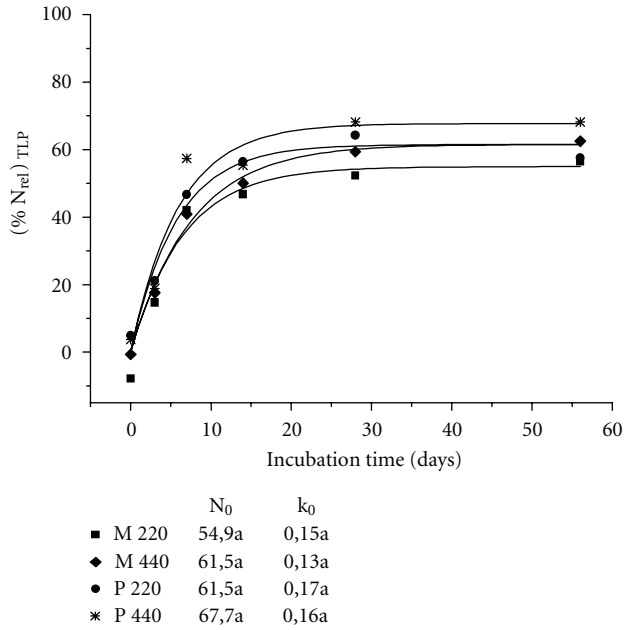

Figure 2: Percentage of the net cumulative N released by TLP into M and P soils. The N_0 or k_0 values followed by the same letter are not significantly different at $\alpha = 0.05$.

such as meat and bone meal [27], and with blood meal [21]. It is generally accepted that the main factors affecting N mineralization in soil treated with organic materials are the C : N ratio and the biochemical quality and N content [15, 28].

3.2. Biochemical Properties. Dynamics of C mineralization, measured as CO_2-C evolution rate, were characterized by a peak occurring after 3 days of incubation, followed by a progressive decrease (Figure 3). The amount of evolved CO_2-C was clearly affected by the application rate; soils treated with larger amounts of TLP were characterized by a significantly higher rate of CO_2-C evolution versus the soils treated with the lowest amount of TLP. On the contrary, the soil type did not affect the CO_2-C evolution rate (Table 3).

In both soils and for both rates of application, TLP induced a significant increase in B_{NIN} versus the control soils, 3–7 days after the amendment (Figure 4). However, the turnover of the microbial biomass N in soils treated with TLP was quite rapid with a tendency to decrease towards values approaching the controls by the end of the incubation. The ANOVA showed a significant influence of both the soil type and the amendment, but did not detect a significant effect of the TLP application rate (Table 3).

Protease activity (Figure 4) showed a marked increase with a maximum occurring 7–14 days after the TLP addition; in soil P the increase was faster than in soil M. The protease activity of treated soils remained significantly higher than the controls until the end of the incubation period, and the ANOVA revealed a significant effect of both the soil type and the TLP application rates (Table 3).

Dilly and Nannipieri [29] reported that the increase in soil respiration, enzyme activities, and microbial biomass

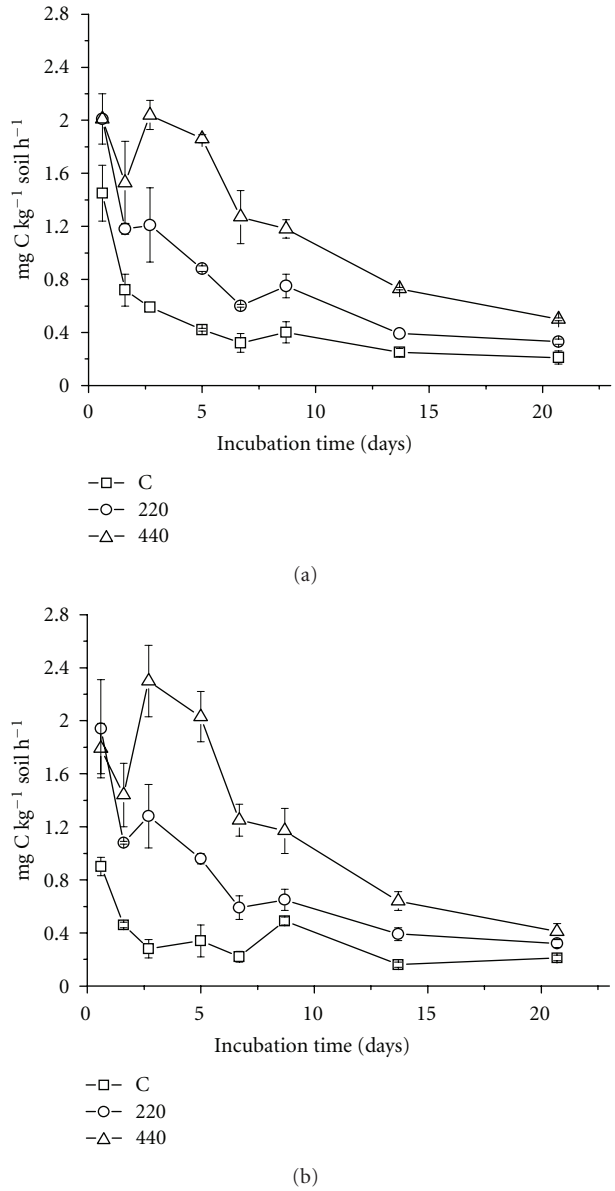

Figure 3: Dynamics of CO_2-C evolution rate in soil M (a) and soil P (b) at different sampling time. C: controls; 220: TLP addition rate at 220 kg N ha^{-1}; 440: TLP addition rate at 440 kg N ha^{-1}; bars represent standard deviation ($n = 3$).

after the addition of easily decomposable substrates to the soil are clear indicators of an increased microbial activity. In our experiment, these parameters were positively influenced by the TLP addition, pointing out to the absence of a significant action of potentially toxic or detrimental substances that could hamper microbial growth or activities. Dynamics of CO_2-C evolution showed that TLP addition caused an increase in soil respiration rate, indicating the presence of readily available substances that soil microorganisms can use as sources of C and energy. The extra cumulative CO_2-C that evolved after 21 days of incubation with TLP (23–32% of the added C) was higher with respect to the values recorded with other substrates of animal origin, such as meat and

Table 3: Results of the F-test from an overall ANOVA for extractable NH_4^+-N, extractable NO_3^--N, protease activity, respiration rate, microbial biomass, and extractable Cr.

Parameter	Soil	Fertilization rate			Soil type fertilization rate
		Control versus 220	Control versus 440	220 versus 440	
Extractable NH_4^+-N	ns	ns	<0.05	<0.05	ns*
Extractable NO_3^--N	ns	<0.05	<0.05	<0.05	ns
Protease activity	<0.05	<0.05	<0.05	<0.05	ns
Respiration rate	ns	<0.05	<0.05	<0.05	ns
Microbial biomass	<0.05	<0.05	<0.05	ns	ns
Extractable Cr	ns	<0.05	<0.05	<0.05	ns

* ns: not significant at $P > 0.05$.

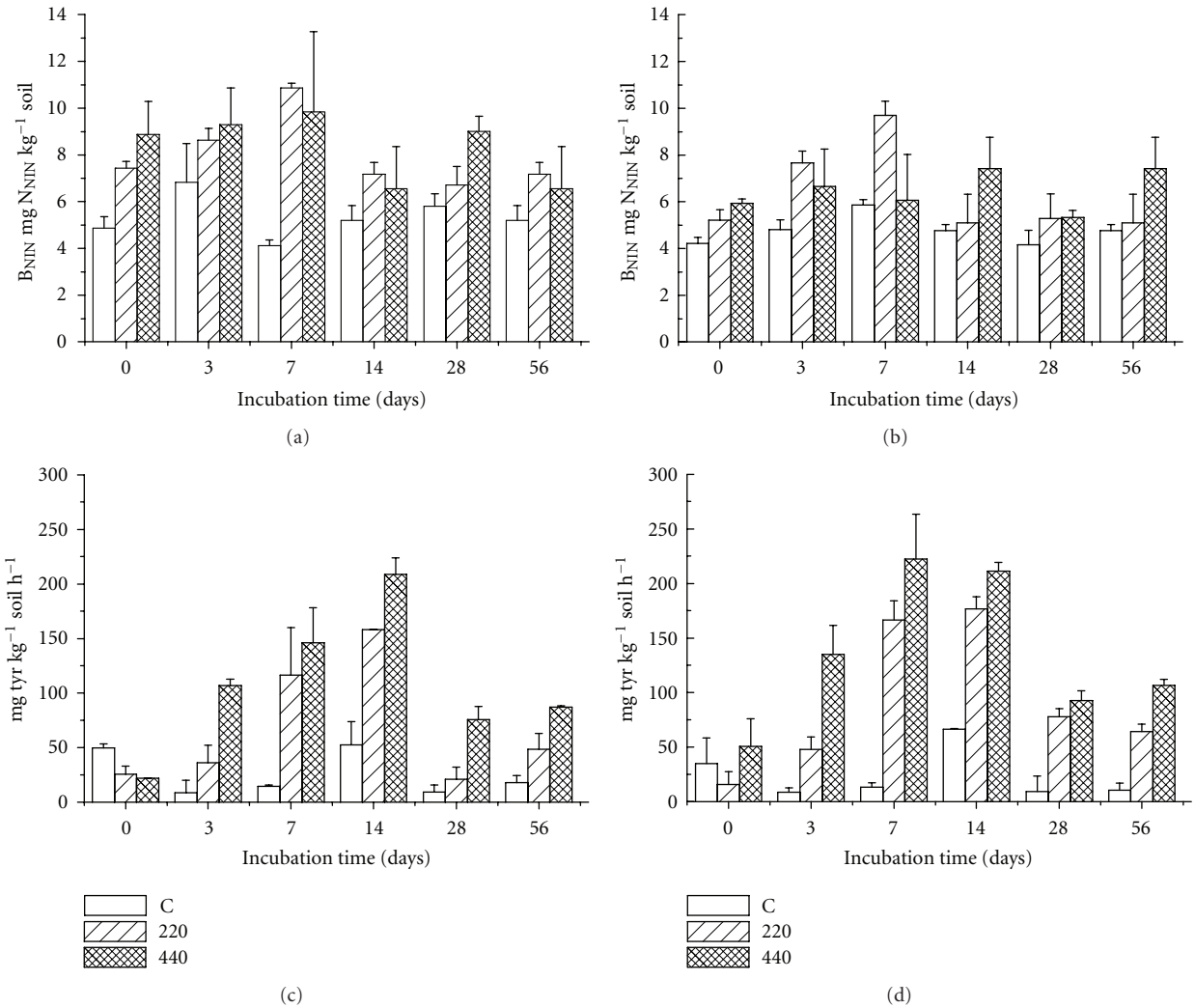

Figure 4: Microbial biomass ninhydrin-reactive N (B_{NIN}), in soil M (a) and soil P (b) and protease activity in soil M (c) and soil P (d) at different sampling time. C: controls; P. 220: TLP addition rate at 220 kg N ha^{-1}; 440: TLP addition rate at 440 kg N ha^{-1}; bars represent standard deviation ($n = 3$).

bone meal (10–16%) [27], poultry manure, and pig slurry (16 and 19% resp.) [30]. This could be due to differences in the microbial use efficiency of the added C and could have caused the limited and transitory increase of microbial biomass shown by the B_{NIN} parameter.

Soil extracellular proteases are produced by a wide range of bacteria, especially actinomycetes, and fungi, are stimulated by the addition of organic residues containing N in proteinaceous forms through mechanisms of substrate induction and are positively correlated with the amount

of added substrates [31]. On the other hand, when high levels of end products such as amino acids, NH_4^+-N and easily available C sources are abundant, the allocation of resources to protease production may be repressed. However, a fraction of soil proteases can be stabilized by interaction with the soil matrix and may be insensitive to environmental conditions that affect microbes. TLP addition caused a marked increase in protease activity and this increase was positively influenced by the TLP addition rate. The presence of end products apparently did not repress proteases. During the rapid phase of organic N mineralization, the lack of evident protease repression could be due to the fact that substrate induction was the dominant mechanism regulating enzyme synthesis and release and dwarfed the repression mechanisms caused by directly available C and N sources [31]. After 8 weeks of incubation, the levels of protease activity in the amended samples remained relatively constant and significantly higher than the controls. We hypothesize that stabilization processes of extracellular protease had occurred [27, 29], preventing the enzymatic activity from falling below basal levels [32].

The dynamics of CO_2-C evolution, B_{NIN} and protease activity suggest a tendency of soils microorganisms to use the substrate to produce energy, with a reduced ability to promote a stable microbial growth. The energy obtained by the intense processes of C and N mineralization could have been used to sustain the intense enzymatic synthesis observed in the treated soils.

3.3. Chromium Oxidation and Extractable Fraction. The high concentration of Cr(III) in TLP is a matter of great concern for its agronomical use. As known, Cr(III) is characterized by a scarce mobility in both soil and plants, and generally only a small fraction of the Cr in soil is available to plants and microorganisms [6, 33]. On the contrary, Cr(VI) shows high mobility in soils and toxicity to plants and animals. In order to exclude the oxidation of Cr(III) to Cr(VI) in soil, the determination of water extractable Cr(VI) was carried out at each sampling time: Cr(VI) was never detected throughout the incubation experiment (data not shown).

Since the extent of the extractable fraction is the main element determining the effect of Cr on microorganisms and plants, we monitored its dynamics during the TLP mineralization. Figure 5 shows the dynamics of the extractable Cr fraction, using the extraction method described by Feng et al. [22]. At the beginning of the incubation period from 12.6 to 16% of the total Cr added to the soil with TLP was extractable by the organic acid solution. After 3 days of incubation, the percentage of extractable Cr decreased to 50%, regardless of the soil type and the TLP addition rate. The extractable Cr fraction then steadily decreased and at the end of the incubation period from 3.9 to 4.3% of the total Cr added was still extractable, although it was not influenced either by the soil type or TLP application rate (Table 3). The values of the controls were significantly lower (Table 2) and stable during the incubation period.

The extractable Cr fraction was clearly reduced during the period of incubation. This could be due to the precipitation of Cr(III) in insoluble forms (e.g., oxide and hydroxide Cr forms) in these alkaline soils relatively rich in carbonates [34].

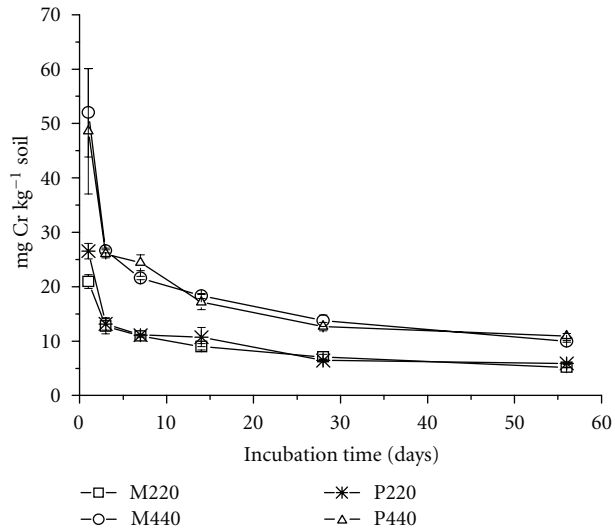

Figure 5: Dynamics of extractable Cr in soils M and P. 220: TLP addition rate at 220 kg N ha^{-1}; 440: TLP addition rate at 440 kg N ha^{-1}; bars represent standard deviation ($n = 3$).

4. Conclusions

TLP added to the soil was characterized by a fast mineralization. The net potential mineralization accounted for more than 50% of the added TLP-N during the first two weeks of incubation, indicating that TLP is a good source of readily available N. The addition of TLP caused a significant increase in the size and activity of microbial biomass, showed by soil respiration, N microbial biomass and protease. The increase of these parameters indicates no toxic or detrimental substance that could hamper microbial growth or inhibit microbial activity like protease at least in the short-term period. The extractable Cr(III) fraction was quickly reduced during the incubation and was probably involved in an intense precipitation process. Assuming that the rate of N mineralization and the effect on biological and microbiological soil properties represent some of the most important factors that we have to consider in the evaluation of the agronomical value of biosolids of industrial origin, we can conclude that TLP should be recycled in agriculture. However, the effect of repeated applications of TLP should be tested in order to evaluate the possibility of long-term accumulation of Cr in soil and its effect on biochemical parameters, in order to confirm the results obtained here.

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Research Article

Mineralization and Crop Uptake of Nitrogen from Textile Manufacturing Wastewater Sludge Cake

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Wastewater sewage sludge cake from textile manufacturing was evaluated by soil incubation experiments and a greenhouse experiment for use as a soil amendment to provide crop N. Although the sludge had 96% of N in organic combination, 20% of total sludge N was released to soil as mineral N over 28 days. N mineralization from the sludge was indistinguishable from that seen for alfalfa shoot in parallel incubations. However, nitrification inhibition was seen for the alfalfa amendment. Soil respiration was low for the sludge treatments compared to the alfalfa treatments, suggesting that carbon substrates in the sludge were less easily broken down. A second incubation experiment indicated that fine fragmentation of the sludge is not necessary to ensure mineralization proceeds. In a greenhouse experiment, sludge N was approximately 25% as available to *Zea mays* L. as NH_4NO_3. The textile manufacturing sludge offered potential to offset N fertilizer requirement.

1. Introduction

Sewage sludges are generated by the treatment of wastewater streams in municipal and industrial installations [1]. The composition of these materials varies considerably. Sewage sludge from water treatment plants in Ontario, Canada, were reported [2] to have 4%–8% solids by mass ($n = 4$), whereas mechanically dewatered sludge normally contains up to 30% solids by mass [3]. A comparison of aerobic sewage sludges [4] gave ranges of values for total N from 5–76 g kg^{-1} ($n = 38$) and NH_4–N from 0.03–11.3 g kg^{-1} ($n = 33$). Corresponding ranges given by [5] are organic N from 13–65 g kg^{-1} and NH_4–N from 0.4–42.8 g kg^{-1} ($n = 39$).

More than 50% of sewage sludge is applied to land for disposal [6], and sludge is often considered for use on farmland to supply N for crops [7–10]. N-release varies both within and among sludge materials. Between 4 and 48% of organic N was mineralized from anaerobically digested municipal sewage sludge in a soil incubation lasting 16 weeks [11]. Similarly, between 0% and 59% of organic N was mineralized over 58–74 days of incubation with soil in the laboratory for 16 municipal sludges [5]. Field trials also show variability. In the first year after application, 55% of organic N in liquid sludge from secondary municipal sewage treatment was mineralized in fields of corn (*Zea mays* L.) and hay [12]. In one season, 16% of applied N in the form of swine lagoon sludge was recovered in grass harvested, with 24% recovered from municipal sludge in a similar trial [13]. Sludge from lagoon sources was also found elsewhere to have less plant-available N than sludge from nonlagoon sources [14]. Residual effects of sludge application are also possible [2]. Nitrification in sludge is often pronounced [15, 16] and can lead to delivery of nitrate to 75 cm depth within a single season [17].

The sludge investigated here was prepared by dewatering of textile manufacturing wastewater that changed total solids to 43% by mass, corresponding to 1.32 g H_2O g^{-1} dry mass of sludge cake. Extraction of water from sewage sludge is expected to remove much of the mineral N, so that knowledge of the rate of N mineralization from this sludge was needed in addition to data for mineral N in order to evaluate sludge utility for land application. Leachate of saponin secondary metabolites from alfalfa roots can inhibit nitrification

[18]. If similar inhibition is caused by alfalfa shoot, then, compared to alfalfa, sludge may offer an alternative solid-form organic source of N without such inhibition.

The first aim of this study was to determine N mineralization from textile manufacturing wastewater sludge to evaluate possible use of the material to offset crop N demand. To further explore nitrification inhibition for mineralized ammonium, experimental comparison to alfalfa shoot amendment was undertaken in parallel soil incubations. A second aim of this study was to predict mineralization of sludge cake when applied as coarse material. For this objective, a separate incubation experiment was undertaken to investigate of the impact of particle size of the sludge cake on N mineralization. The third aim of the study was to compare availability for crop uptake of N from the sludge to that of inorganic N fertilizer. To this end, a greenhouse experiment was conducted with sludge application to soil that was then sown to corn with separate corn pots receiving only NH_4NO_3 fertilizer.

2. Materials and Methods

2.1. Collection of Materials. Conostogo silt loam soil was collected for use in incubation and greenhouse experiments from the top 15 cm of the profile of low-N fertility plots at the Elora Research Station at 43°41′ N and 80°14′ W in Ontario, Canada. To minimize N availability, the field soil had been cropped to barley without N fertilizer in the previous year. This soil is mapped as a gleyed melanic brunisol within the Canadian system of soil classification [19], with 300 g sand kg^{-1}, 500 g silt kg^{-1}, and 200 g clay kg^{-1} [20]. Soil was partially air-dried, passed through a 5 mm screen, and stored in polythene bags until use. Using standard forage management for the region, alfalfa tops were collected from plots at the Elora Research Station, dried at 70°C, and ground to pass a 2 mm screen. Alfalfa shoot had 29.5 mg N g^{-1} dry mass. Sludge was supplied as a dewatered cake from the sewage treatment plant of Du Pont Canada Inc. at Maitland, ON, Canada. Wastewater from textile manufacture generates this sewage sludge at the DuPont installation. The wastewater sludge was prepared at the Maitland site of textile manufacture from a well-mixed aerobic wastewater treatment lagoon. The sludge cake was prepared by partial water removal by application of physical pressure following bulking with $FeCl_3$. The sludge retained 1.32 g H_2O g^{-1} dry mass when delivered by refrigerated truck for study and was kept in polythene bags to stabilize this moisture level until use.

2.2. Analysis of Soil and Amendments. While at the moisture level as supplied, the sludge was passed through a 2 mm sieve prior to all analyses. Moisture contents of sludge and soil were determined by drying to constant mass at 105°C. Total N and C for amendments were determined by combustion using a Leco FP-428 furnace. Sludge and soil samples were extracted in 2.0 M KCl with a 5 : 1 ratio of extracting solution to sludge or soil for mineral N determination. Ammonium and nitrate concentrations in extracts were determined spectrophotometrically using a standard autoanalysis system [21]. Elemental analysis of the textile wastewater sludge was determined for triplicate 0.5 g samples that were heated in 5 mL 65% nitric acid in the following microwave sequence: 5 minutes at 250 W, 1 minute at 0 W, 4 minutes at 250 W, and 7 minutes at 400 W. Leachate was filtered using Whatman no. 42 paper and brought to a final volume of 100 mL using ultrafiltration-purified water. Elemental composition of the sludge was determined by analysis of leachate using ICP optical atomic emission spectrometry (ICP-OES) at the Ministry of Northern Development of Mines, Sudbury, ON, Canada.

2.3. Incubation Experiment One. Sludge was passed moist through a 2 mm sieve and incubated in glass Mason jars with screw caps tightly sealed and jars placed in the dark for 28 days at 25°C and 0.23 g H_2O g^{-1} dry soil at rates of 100, 200, 400, and 800 mg N kg^{-1} dry soil. All jars were mixed immediately following amendment on the day of setup. As well as controls, amendments of alfalfa shoot at the same rates of N addition gave nine amendment treatments. There were four replicates for each combination of amendment and time, giving 216 incubations in all. Available ammonium and nitrate were determined as described above for separate incubations on the day of setup and after 1, 4, 7, 14, and 28 days of incubation. Prior to harvest of each jar, headspace was sampled by gas syringe through a rubber port in the jar lid. Concentrations of CO_2 were determined for syringe samples using a Gow Mac gas chromatograph with infrared detector.

2.4. Incubation Experiment Two. Mineralization of inorganic N from moist sludge fractions able to pass 2 mm, 4 mm, and 9.5 mm screens was assessed, giving three amendment treatments. Screen fractions and the nonsieved sludge were applied at 400 mg N kg^{-1} dry soil and compared to controls with no sludge added. There were four replicates of each treatment combination of amendment and time, giving 48 incubation units in all. Incubations were maintained at room temperature and 0.23 g H_2O g^{-1} dry soil in Mason jars in the dark. Ammonium and nitrate mineralized was determined as above for separate incubations immediately after setup and after 4 and 14 days of incubation.

2.5. Greenhouse Experiment. Sludge was passed moist through a 2 mm sieve before use. Soil was mixed with sludge at 100, 200, and 400 mg N kg^{-1} dry soil. As well as controls, NH_4NO_3 fertilizer was mixed with soil in separate incubations at rates of 25, 50, and 75 mg N kg^{-1} dry soil, which gave seven treatments in all. There were four replicates of each treatment, giving a total of 28 20-cm diameter pots. Maize was grown for 24 days from presoaked seed in pots in the greenhouse. Six seeds were sown per pot, with thinning to four seedlings per pot after one week. Pots were watered daily to gravimetrically maintain 0.23 g H_2O g^{-1} dry soil. Plants were at the six- or seven-leaf stage at harvest. Shoots were removed by cutting 5 mm above the soil surface at harvest and dried at 70°C. Two 33 mm soil cores were then taken from each pot approximately mid-way between shoot bases, about half the distance from the center to the edge of the pot, and to the full pot depth. One core was selected at random

Table 1: Comparison of determinations for the textile wastewater sludge ($n = 4$) to wastewater sludge materials ($n = 17$) reported [24] for analyses on a dry-mass basis for total C and N, C/N ratio, ammonium-N, and nitrate-N; s.d. = standard deviation; n.d. = not determined.

Property	Units	Sludge in present study		Published data		
		Mean	s.d.	Minimum	Maximum	Median
Total N	g kg^{-1}	40.0	5.0	12	100	36
C	g kg^{-1}	261	19	175	531	290
C/N ratio	none	6.6	1.2	5.3	18.8	8.3
NH_4–N	g kg^{-1}	1.49[a]	0.04	0	20.1	1.7[b]
NO_3–N	g kg^{-1}	0.020[a]	0.003	n.d.	n.d.	n.d.

[a] 2.0 M KCl with 5 : 1 extract-to-sludge ratio.
[b] 1.0 M KCl with 100 : 1 extract-to-sludge ratio.

from each pot and used to wash roots on a 0.1 mm screen. Roots were then scored for root length [22] and dried at 70°C. Dried shoot and root was analyzed for total N by combustion as above. The soil from the second core from each pot was sieved on a 2 mm screen, discarding roots, stones, and organic debris. This sieved soil was used for analysis of total N and levels of ammonium and nitrate as above.

2.6. Statistical Design and Analysis. All experiments were arranged in a randomized complete block design with random arrangement of treatments within a block. Treatment effects were investigated using analyses of variance with separation of means using the Tukey system [23]. Comparison of rates of CO_2 production between incubations with amendments of sludge and alfalfa was made by the Wilcoxon nonparametric test [23] for samples paired according to rate of N addition and time of sample analysis.

3. Results

Analyses of 17 sludge materials [24] were compared to the textile wastewater sludge (Table 1). The textile wastewater sludge was found to be moderate, in terms of similarity to median values among the 17 sludge materials from the literature, for total N, total C, C/N ratio, and KCl-extractable NH_4–N (Table 1). Compared to the alfalfa amendment of 29.5 mg N g^{-1}, for which all N was assumed to be in organic combination, the sludge contained 1.5 g NH_4–N kg^{-1} and 40 g N kg^{-1} (Table 1) so that approximately 4% of N in the sludge was in the form of ammonium. The sludge was similar in elemental composition to municipal sludge (Table 2) although Mn at 838 mg kg^{-1} dry mass and B at 498 mg kg^{-1} dry mass were 2- and 6-times greater, respectively, than the concentration means for a wide range of sludges from earlier survey [4]. However, these values for Mn and B for the textile wastewater sludge were still within the wide ranges of concentrations in municipal sludges for these elements [4]. More recent data also show wide variability in concentration of B and Mn. Environmental Protection Agency sewage sludge analyses fell in the range 6–204 mg kg^{-1} for B with $n = 80$ and in the range 35–14.900 mg kg^{-1} for Mn with $n = 84$ [25].

3.1. Incubation Experiment One. Ammonium (Figure 1) and nitrate (Figure 2) were released from both the sludge and from the alfalfa over the period of incubation. Almost all mineral-N in the sludge treatments was in the form of nitrate at 28 days (Figures 1 and 2), indicating that the sludge did not interfere with nitrification. In contrast, ammonium accumulated transiently for the 400 mg N kg^{-1} alfalfa treatment (Figure 1(c)) and persistently following alfalfa addition at the 800 mg N kg^{-1} level (Figure 1(d)), indicating that nitrification was inhibited by these higher rates of alfalfa amendment. Where mineral N is the sum of ammonium and nitrate forms, the slope of the regression for mineral N released after subtracting controls, as a function of N added, was 0.20 (Figure 3). This slope indicates that on average, 20% of added N was recovered as mineral N after 28 days of the experiment, irrespective of whether the addition was in the form of alfalfa or sludge.

Table 2: Concentration in the sludge of the 20 elements exceeding 10 mg kg^{-1} in addition to C, H, O, N, K, and S. Means and standard deviations (s.d.) are for $n = 3$.

Element	Mean	s.d.
	mg kg^{-1} dry mass	
Fe	8214	279
P	5254	380
Ca	3544	474
Mg	1538	250
Cu	1004	43
Mn	838	143
Al	711	15
B	498	79
Ti	190	14
Zn	129	16
V	117	3
Cr	101	11
Ni	70	8
Zr	40	7
Ba	40	6
Pb	31	11
Ta	26	23
Sr	22	2
As	13	15
Co	13	1

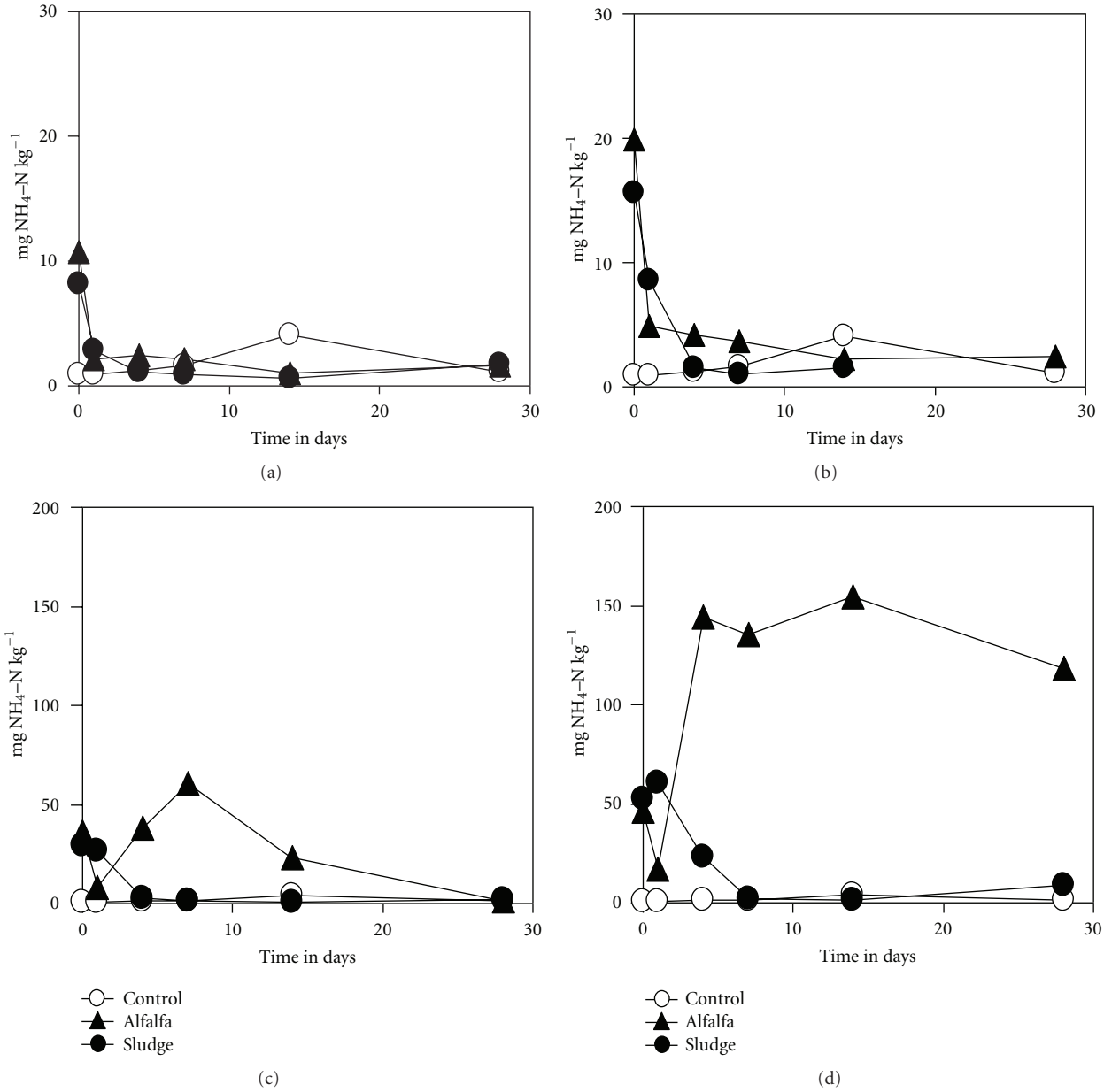

Figure 1: Incubation experiment one. Changes with time for ammonium extracted from incubations of sludge and alfalfa with soil at rates of (a) 100, (b) 200, (c) 400, and (d) 800 mg N kg^{-1} dry soil. Controls received no amendment.

A pulse of CO_2 production with maximum after four days was seen for the highest rate of alfalfa addition, whereas CO_2 release from the 200 mg N kg^{-1} and 400 mg N kg^{-1} alfalfa treatments were highest after one day (Figure 4(a)). Rates of CO_2 released from the sludge incubations (Figure 4(b)) were lower ($P = 0.002$) than for the corresponding alfalfa treatments (Figure 4(a)) for most combinations of amendment rate and time of sampling.

3.2. Incubation Experiment Two. Sieving the sludge did not affect nitrogen mineralization, with similar low values for ammonium and similar values for increased nitrate, relative to controls, for all screen sizes at both 4 and 14 days (Table 3). A decreased level of ammonium for the two finest screen sizes immediately after setup, relative to the coarsest screen (Table 3), was likely caused by more ammonium diffusion to smaller particle surfaces and gaseous losses from the more finely screened material.

3.3. Greenhouse Experiment. Plants in all treatments were developmentally advanced by one leaf stage over controls ($P < 0.001$), with 5.9 leaves plant^{-1} for controls, and means ranging from 6.8 to 7.0 leaves plant^{-1} for the other treatments. No negative impact of sludge treatment was evident on inspection of the plants in this study. Ammonium nitrate fertilization and sludge amendment both caused shoot growth

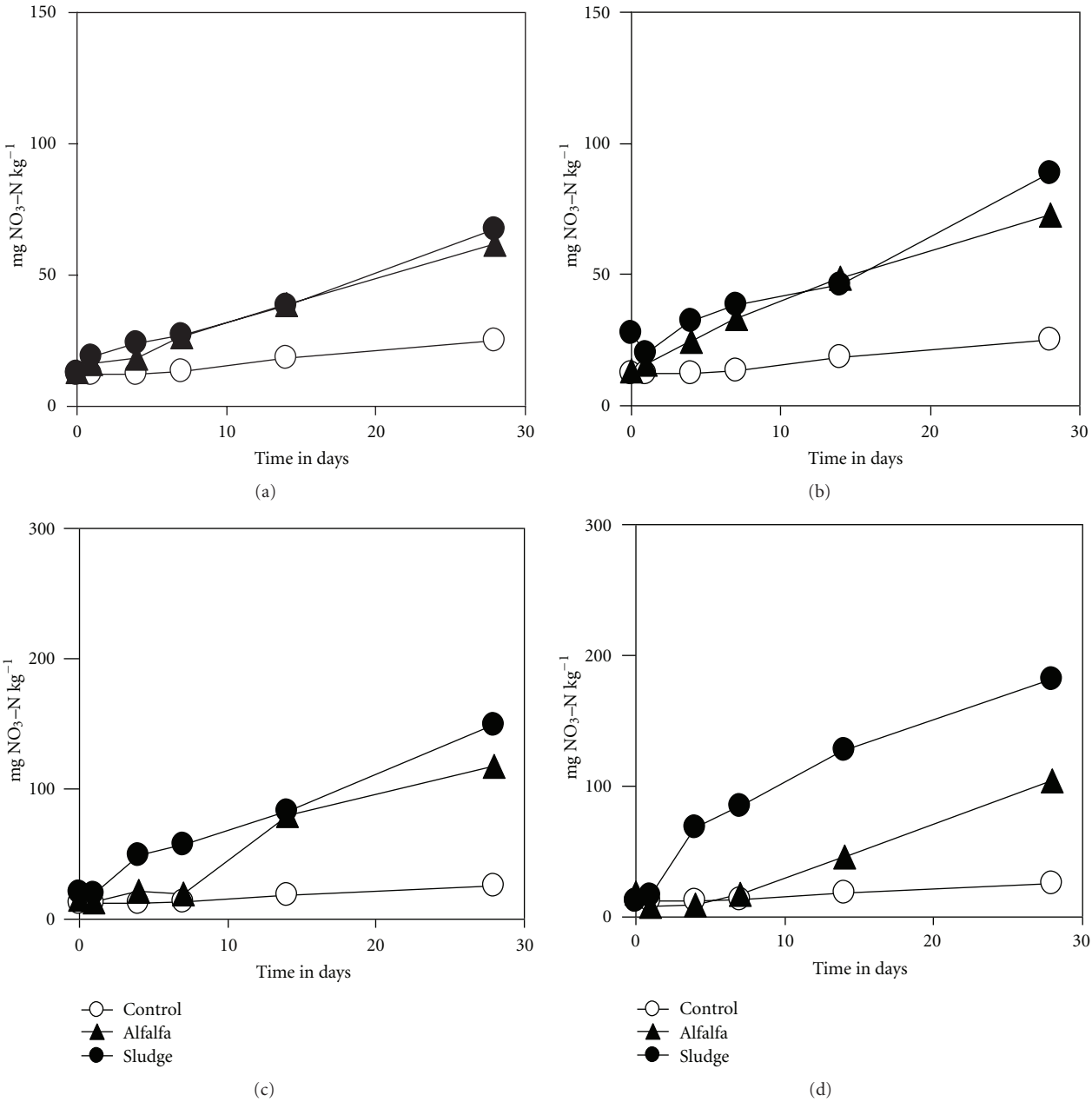

Figure 2: Incubation experiment one. Changes with time for nitrate extracted from incubations of sludge and alfalfa with soil at rates of (a) 100, (b) 200, (c) 400, and (d) 800 mg N kg^{-1} dry soil. Controls received no amendment.

responses ($P < 0.001$) as determined at 24 days (Figure 5(a)). Shoot N concentration responded in a stepwise manner to increments of fertilizer and sludge treatment (Figure 5(b)). Shoot N concentration in control pots was markedly deficient at 15.4 mg g^{-1} but increased to as high as 49.1 mg g^{-1} and 40.9 mg g^{-1} with the highest rates of addition of sludge and fertilizer, respectively (Figure 5(b)). Root length density was significantly ($P = 0.03$) greater for plants with the lowest amendment of sludge compared to those with the lowest amendment of ammonium nitrate, but no amended treatment was different from the control (Figure 5(c)). Root mass density ($P = 0.07$) was similar among treatments, with overall mean ± standard deviation = 0.36 ± 0.08 g root L^{-1} soil for $n = 28$. Specific root length ($P = 0.11$) was also similar among treatments, with overall mean ± standard deviation = 10.4 ± 2.9 cm root mg^{-1} root for $n = 28$. Root N concentration increased from 9.9 mg g^{-1} in controls to as high as 24.8 mg g^{-1} and 20.5 mg g^{-1} with the highest rates of addition of sludge and fertilizer, respectively (Figure 5(d)).

Ammonium in NH_4NO_3 fertilized soil did not differ from controls at harvest, but soil ammonium did increase in a stepwise manner (Figure 6(a)) in response to sludge amendment. Despite these differences among treatments, the concentrations of ammonium in soil at harvest were low in

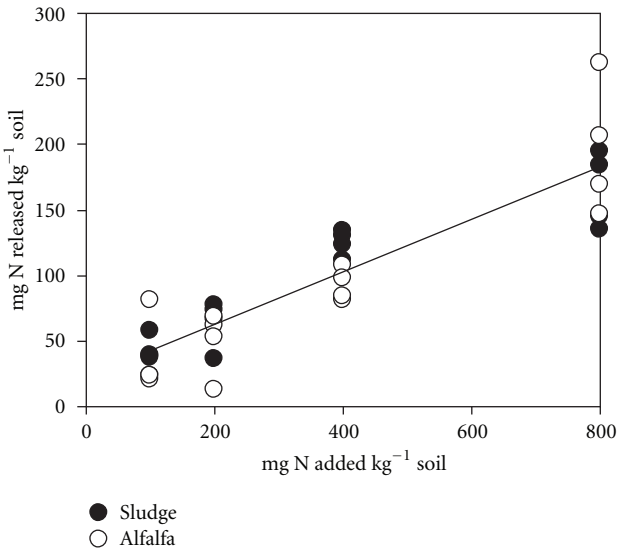

Figure 3: Incubation experiment one. Relation of N added to total mineral N released after 28 days for incubations of sludge and alfalfa with soil calculated with subtraction of mineral N in control incubations. The fitted regression line $y = 20.0 + 0.20x$ of $r^2 = 0.81$ for the data pooled across sludge and alfalfa amendments is significant ($P < 0.001$).

Table 3: Incubation experiment two. Ammonium-N and nitrate-N mineralized from different screen fractions of sludge applied at 400 mg N kg^{-1} dry soil, as compared to controls with no sludge added. Means in a column followed by different letters (a, b, and c for NH_4–N; x, y, and z for NO_3–N) are significantly different at the 5% probability level.

Screen size (mm)	Day		
	0	4	14
	mg NH_4–N kg^{-1} dry soil		
Control	0.5 a	0.1 a	0.2 a
2	31.7 b	3.7 a	0.6 a
4	36.6 b	8.5 a	0.8 a
9.5	57.9 c	11.5 a	0.8 a
	mg NO_3–N kg^{-1} dry soil		
Control	12.0 x	12.6 x	16.8 x
2	12.1 x	52.6 y	85.9 z
4	12.4 x	58.2 y	80.4 z
9.5	11.7 x	49.6 y	79.5 z

all treatments relative to plant demand and did not exceed 2.4 mg kg^{-1}. Nitrate in soil was low relative to crop demand and less than 3 mg kg^{-1} in all treatments, with the exception that nitrate accumulated to 30.4 mg kg^{-1} in pots with the highest rate of sludge amendment (Figure 6(b)).

4. Discussion

The sludge cake from textile manufacturing wastewater that was studied here released mineral N in soil incubations and provided adequate N to support maize growth in soil in pots without signs of damage to the crop. Mineralization of N was indistinguishable to mineralization from alfalfa shoot. The 20% of organic N released as ammonium or nitrate from the sludge over 28 days indicated that mineralization rates for the textile wastewater sludge were similar to those known for municipal sludges. In the first year following application of anaerobically digested municipal sludge biosolids, 40% of organic N was recovered by a field crop of maize [26]. Further recoveries of 20% and 10% of organic N applied were estimated [26] for the second and third years, respectively. Slightly lower rates of recovery have been recorded elsewhere, with 50% of sludge N recovered cumulatively over four successive field crops following land application of anaerobically digested municipal liquid sludge [7]. With the same sludge material as used by [7], much of the release of mineral N from organic N in sludge occurred within three weeks following field amendment [8]. Thus, the mineralization of 20% of organic N from the textile wastewater sludge cake within 28 days compares well to municipal sludge in terms of initial rate of N release.

Although similar to alfalfa shoot as an N source, the sludge offered freedom from nitrification inhibition at the highest rates of application, whereas nitrification inhibition appeared to occur for higher rates of alfalfa amendment. Ammonium accumulated transiently in the 400 mg N kg^{-1} alfalfa treatment and persistently in the 800 mg N kg^{-1} alfalfa treatment. Nitrification inhibition has been attributed to glucosinolates in shoot material of Brassicaceae [27], but the polyphenols of wider occurrence among plants, such the Fabaceae, do not inhibit nitrification [28]. Saponins in alfalfa root exudates are thought to inhibit nitrification [18], and so, saponins are a likely explanation for the nitrification inhibitory activity of alfalfa shoot.

Respiration was lower in the sludge amendments compared to the alfalfa treatments. Less respiration in the sludge compared to the alfalfa treatments probably relates to reduced availability of carbon substrates in the sludge. Soil respiration data found here are in keeping with expected values, as follows. A base reference value of oxygen demand for soils can be taken as 20 g O_2 m^{-2} day^{-1} [29]. Assuming 1 : 1 equivalency of O_2 consumed to CO_2 released, this oxygen demand converts to 1.6 mg CO_2–C kg^{-1} h^{-1} respired for a 15 cm depth of generalized field soil of bulk density 1.33 g kg^{-1}. Control values in incubation experiment one ranged from 0.9–5.6 mg CO_2–C kg^{-1} h^{-1}, with values following amendment varying widely but not exceeding 83.5 mg CO_2–C kg^{-1} h^{-1}.

Application of sludge cake to fields would likely be undertaken by manure spreader. Therefore, the impact of sludge particle size on reaction rates could, in theory, bring about differences between field responses and laboratory or greenhouse studies. However, the sieve-size experiment conducted here shows that at least over the range from 2 mm to 9.5 mm sludge particle diameter, cake size dimension has no influence on the reaction of the sludge with soil in terms of rate of nitrate released. Fine-screened fractions of sludge cake studied here lost ammonium relative to the coarsest fraction at incubation establishment, likely because of gaseous loss

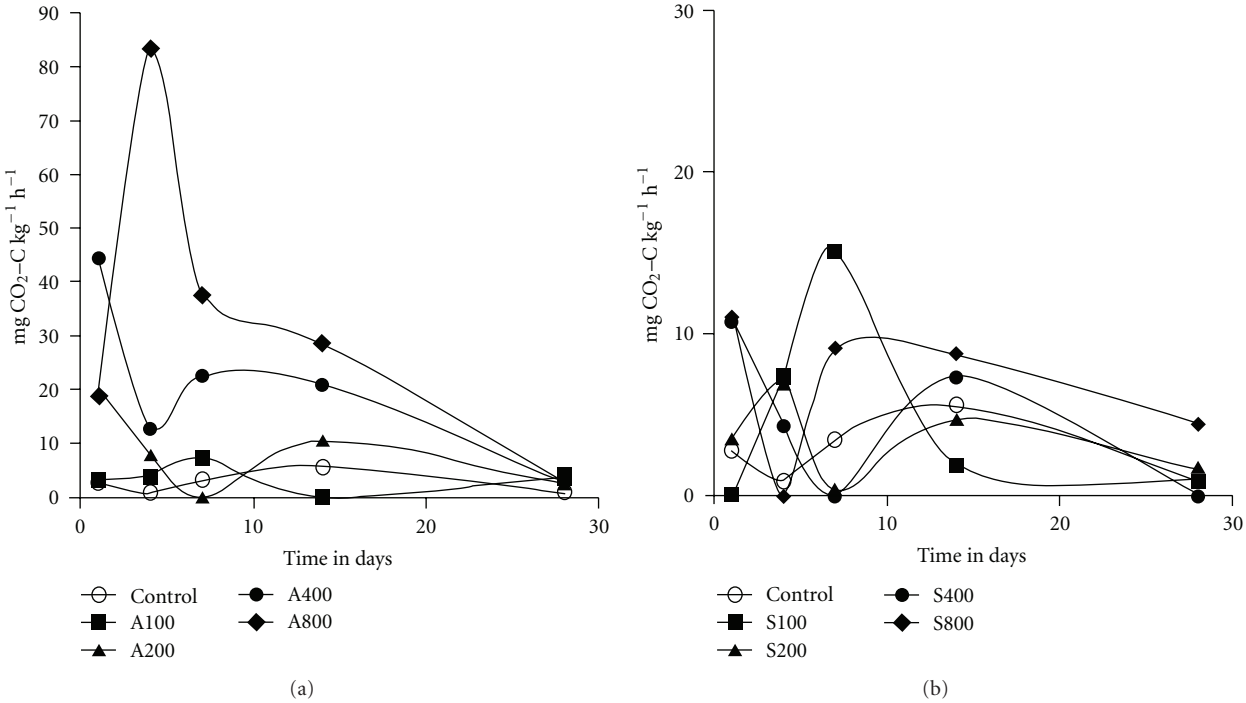

Figure 4: Incubation experiment one. Specific rates of carbon dioxide released from incubation of soil with (a) alfalfa and (b) sludge. Treatment codes refer to amendment rates of alfalfa (A100–A800) and sludge (S100–S800) added in the range 100–800 mg N kg^{-1} dry soil.

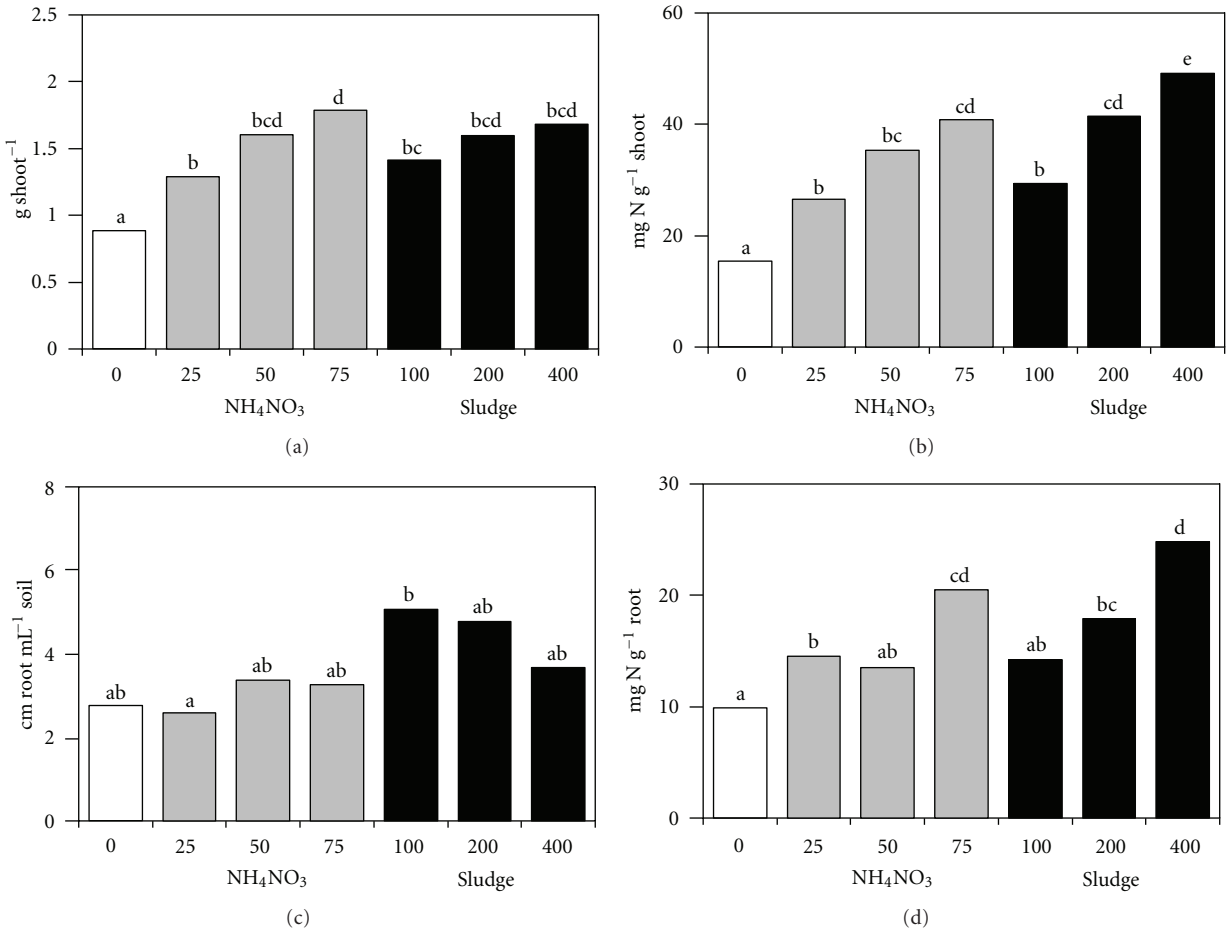

Figure 5: Greenhouse experiment. (a) Shoot dry mass, (b) shoot N concentration, (c) root length density, and (d) root N concentration after 24 days of growth for pots amended with ammonium nitrate or sludge at rates shown in mg N kg^{-1} dry soil. Controls received no amendment. Means with different letters (a, b, c, d, and e) are significantly different at the 5% probability level.

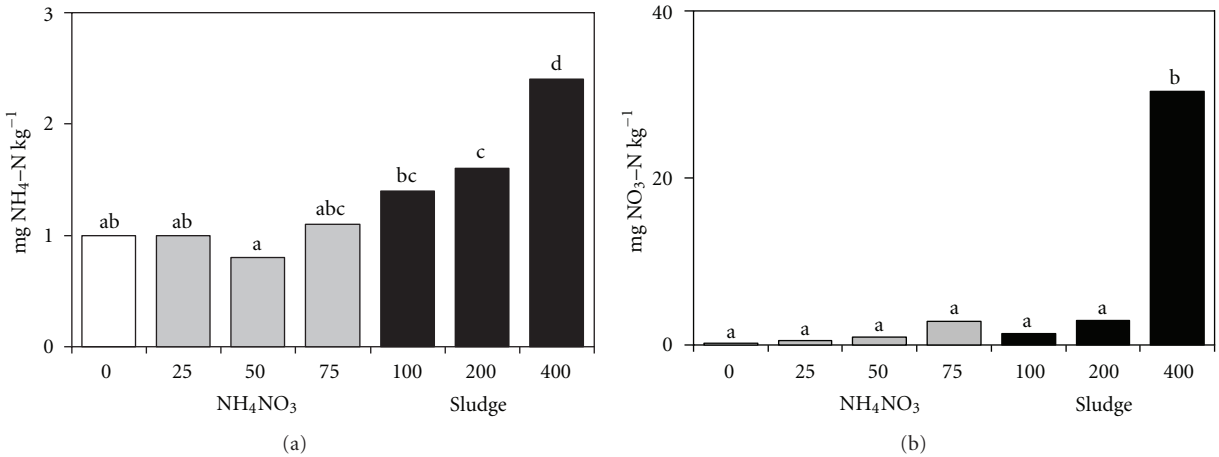

Figure 6: Greenhouse experiment. Soil ammonium-N and nitrate-N extracted from pots after 24 days of growth following amended with ammonium nitrate or sludge at rates shown in mg N kg^{-1} dry soil. Controls received no amendment. Means with different letters (a, b, c, and d) are significantly different at the 5% probability level.

(Table 2). Use of coarse sludge cake material for land application, followed by incorporation where possible, would be expected to limit such gaseous loss of N.

The N sufficiency range for maize during early growth is given as 35–50 mg g^{-1} [30]. Control treatment plants in the greenhouse experiment had 15 mg g^{-1} and were N deficient, and N concentrations of shoots across treatments ranged up to 50 mg g^{-1}. The absence of a pronounced effect of fertilizer and sludge amendment on root growth, together with significant stimulation of shoot growth, was in keeping with established response patterns of increased shoot-to-root ratio following N addition [31]. Overall mean for root length density in the greenhouse study of 3.6 cm cm^{-3} was high compared to the season maximum of 2.0 cm cm^{-3} for maize in the field [32], but a relatively high root length density was not unexpected, given pot confinement. Specific root length of 10.4 cm root mg^{-1} root is slightly lower than values available for other grasses, but it is of the same order [33]. In contrast to fertilizer additions, however, the highest level of sludge amendment left residual soil nitrate in excess of that utilized by the crop. Sludge amendments at 100, 200 and 400 mg N kg^{-1} were indistinguishable from NH_4NO_3 amendments at 25, 50, and 75 mg N kg^{-1}, respectively, in terms of values for shoot N uptake, which indicated that sludge N was approximately 25% as available as fertilizer N.

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Research Article

Effect of ISPAD Anaerobic Digestion on Ammonia Volatilization from Soil Applied Swine Manure

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Swine manure subjected to In-Storage Psychrophilic Anaerobic Digestion (ISPAD) undergoes protein degradation but limited NH_3 volatilization, producing an effluent rich in plant-available N susceptible to more volatilization during land application. This study therefore measured NH_3 volatilization from both ISPAD and open tank (OT) swine manures when applied to 5 different soils (washed sand, Ste Rosalie clay, Upland sandy loam, St Bernard loam and Ormstown silt) within laboratory wind tunnel simulations. After 47 h, the NH_3 volatilized varied with both manure and soil type. For all soils, the ISPAD manure lost less NH_3 compared to OT manure, averaging 46% less. The lower solids content and higher buffering capacity of the ISPAD manure explain the advantage. The Ormstown loam maintained the same NH_3 volatilization rate after 47 h because of higher capillary effect and the St Bernard sandy loam lost the same N mass for both manures, because of a higher pH and buffer pH, with an intermediate CEC resulting in more soil solution NH_3. Within each manure type, % TAN volatilized was highest for washed sand and lowest for the clay soil. Thus, ISPAD manure can offer up to 21% more plant-available N especially when not soil incorporated following its application.

1. Introduction

In-storage psychrophilic anaerobic digestion (ISPAD) occurs in manure storage tanks with an air-tight cover when its anaerobic microbial community acclimates to ambient conditions [1]. Developed for livestock operations in temperate climatic zones, ISPAD can release 65% of the manure's potential methane while lowering volatile solids by 24% [1]. As opposed to mesophilic, psychrophilic anaerobic digestion limits biogas ammonia (NH_3) levels, despite the breakdown of proteins [2, 3]. However, when land spread, the treated effluent may lose to the atmosphere the conserved total available nitrogen (TAN or NH_4^+ and NH_3), resulting in a net loss of nitrogen lowering its fertilizer value.

Following land spreading, the volatilization of NH_3-N from swine manure depends on several factors. Some are independent of manure storage and treatment, such as pig diet [4], method of soil application [5], application timing [6], and subsequent weather conditions [7–9]. Diet manipulations reducing protein intake result in lower manure TAN and less volatilization losses following land application [10]. A data base analysis from field measurements concluded that NH_3 volatilization was proportional to the manure TAN and the percentage released remained constant [7].

The concentration and thus volatilization of NH_3 are also influenced by the manure characteristics and their interaction with the soil properties. The speciation of TAN into NH_3 depends not only on the pH and temperature of the manure [11, 12] but also on the presence of other ionized species [13]. The soil alkalinity and buffering capacity can change the manure pH [11, 14, 15]. The NH_3 speciation equilibrium is further affected by the soil cation exchange capacity (CEC) affecting the level of free NH_4^+ [16]. Furthermore, NH_4^+ can be precipitated by soil solution cations, particularly Ca^{2+} and Mg^{2+}, producing calcite and struvite [12].

Physical aspects of the soil/manure system also intervene. Lower NH_3 volatilization results from higher manure infiltration into the soil, especially when the manure has a low volatile solids content [8]. Nevertheless, manure applied on a dry soil loses its water content releasing NH_3 [17].

Anaerobic digestion can change the physicochemical characteristics of swine manure thereby influencing several of the above-mentioned factors. Untreated and anaerobically digested under mesophilic conditions, swine manure offered the same rate of NH_3 volatilization after application to grassland [18]. In a similar comparison, soil pH and NH_4^+-N content were found to be more influential following the application of both treated and untreated swine manures to bare soil [19]. In comparison to untreated manure, NH_3 volatilization was 22% less for anaerobically digested manure applied to a bare silty clay loam [20].

While similar nutrient values (N, P, and K) are found for swine manures from all types of operations and in several countries [21–23], plant availability depends on best management practices [24]. Anaerobic digestion degrades organic N releasing TAN [2, 25, 26] readily available for plant uptake. Once soil applied and as compared to untreated manure, digested effluents were found to offer higher plant available nutrients for wheat [27], corn [28], and timothy [29].

Accordingly, ISPAD can enhance the manure N fertilizer value by minimizing biogas NH_3 content as compared to mesophilic systems, while still providing all benefits of anaerobic digestion and higher plant-available N. The objective of the present study was therefore to compare the extent of NH_3 volatilization between ISPAD treated and raw (untreated or open tank) swine manures. This was done by simulating land application of both manures using the wind tunnel technique and monitoring NH_3 release with boric acid traps. Five different experimental soils were used, each offering a similar pH but different cation exchange capacity (CEC), cation saturation level, organic matter content, and water holding capacity.

2. Materials and Methods

2.1. Experimental Manures and Soils. In 2004, a full-scale swine manure ISPAD was established in St. Francois Xavier, Quebec, Canada, using a circular concrete tank, 30 m in diameter and 3.66 m deep, covered with an air-tight membrane (GTI, Fredericton, NB, Canada). The ISPAD was fed swine manure weekly and was emptied in the spring and fall of each year, except for a 0.3–0.6 m depth. In 2010 for the present experiment, manure from this facility was compared to 12-month-old swine manure from the open tank of the Experimental Swine Facility of McGill University at its Macdonald Campus, Montreal, Canada. Produced by hogs fed a standard corn and soybean ration, these two manures were considered comparable in terms of solids and nutrients [21]. All manure samples were collected in March 2010 using a sludge-judge apparatus to obtain a composite sample representing the average of depths and locations within the tanks [1].

Manure samples were analyzed according to standard methods [30] for solids, pH, total Kjeldahl nitrogen (TKN), total ammonia nitrogen (TAN), and phosphorus (P) and potassium (K) content. Total solids (TSs) were determined by drying whole samples at 103°C overnight (*VWR*, Sheldon Manufacturing, model 1327F, OR, USA). Volatile solids (VS) were determined by incineration of dried samples at 500°C for two hours (*Barnstead Thermodyne*, model 48000, Iowa, USA). Fixed solids (FSs) were computed as the difference between VSs from TS. The pH of all samples was determined using a pH meter (*Corning*, model 450, NY, USA). The TKN, P, and K were determined by digesting samples of each manure with sulphuric acid and 50% hydrogen peroxide at 500°C for 15 minutes (*Hach Canada*, Digesdahl model 23130-20, Mississauga, ON). Subsamples of digestate were used to quantify P and K colorimetrically at a pH of 7, using a spectrophotometer (*Hach*, model DR 5000, Loveland Clo, USA). For TKN, the pH of subsamples was adjusted to 13 using NaOH, and the NH_3-N content was measured with an NH_3-sensitive electrode (*Orion*, Boston Mass, USA) connected to a pH meter (*Corning*, model 450, NY, USA). Total ammonia nitrogen was measured in the same way using undigested samples, after adjusting the pH to 13.

The five experimental soils were washed sand (S. Boudrias Inc., Laval, QC, Canada), Ste Rosalie clay from Howick, Canada, 50 km west of Montreal; Upland sandy loam from Ste Anne de Bellevue, Canada at the western tip of the Montreal Island, St Bernard loam also from Ste Anne de Bellevue at the western tip of the Montreal Island, and Ormstown loam from Ormstown, Canada, 70 km west of Montreal. Except for the washed sand, all soils were collected from the ground surface for a minimum organic matter content of 3.9%. All soils were dried and ground before sieving to remove organic particles larger than 6 mm.

All experimental soils were analyzed using standard methods. After soaking for 24 hours in equal amounts of distilled water, pH was determined using a pH electrode [31]. Organic matter content was determined on samples dried at 150°C for 16 hours, using a muffle furnace at 375°C for 16 hours [32]. Minerals and trace elements were measured first by extracting with a Mehlich III solution and then by plasma emission spectrometry [33]. The CEC was determined by saturating the samples with NH_4^+ using an ammonium acetate solution at pH 7 and then quantifying the NH_4^+ released after adding an NaCl solution [34]. Total Kjeldahl nitrogen was measured after sulphuric acid/peroxide digestion using an NH_3-sensitive probe connected to a pH meter (Orion, Boston, USA). Soil particle size distribution was determined using the hydrometer method [35]. The gravimetric water holding capacity was measured by soaking previously dried subsamples in distilled water for 24 hours and draining under cover to prevent evaporation.

2.2. Experimental Wind Tunnels. Ammonia volatilization tests were conducted using five wind tunnels designed for manure spreading simulations [36]. Measuring 2.0 m in length, with an inlet diffuser and outlet reducer of 0.3 and 0.15 m, respectively, each tunnel sat on a soil pan measuring 1.5 m long × 0.1 m wide × 0.05 m deep (Figure 1).

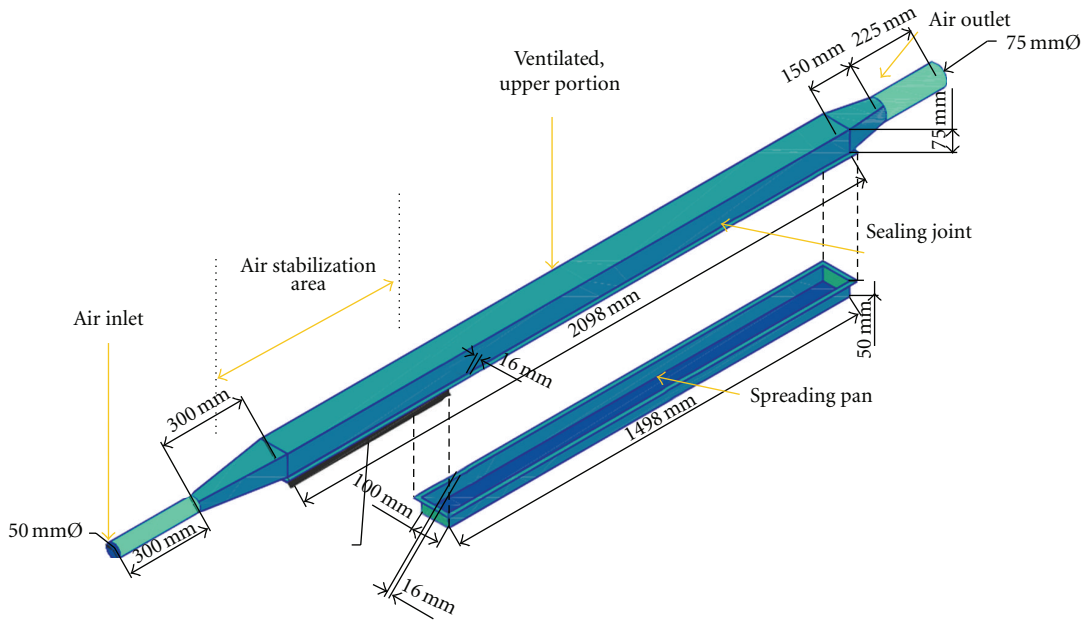

Figure 1: Wind tunnels used for the laboratory manure land application simulations to measure NH_3 volatilization. The wind tunnel is set on a spreading pan holding 50 mm of experimental soil. Fresh air is introduced through the bottom left hand pipe, circulated through the body at a speed of 3 m s^{-1} and exited through the top right hand pipe. To measure NH_3 volatilization, a known portion of the outlet air was bubbled through an acid solution.

Connected in parallel, each wind tunnel received the same fresh air at a rate maintaining a consistent inside air speed of 0.3 ms^{-1}. Exhaust air from each wind tunnel was routed through 3 mm Teflon tubing (Laurentian Valve & Fittings ltd., Saint-Laurent, Canada) into NH_3 traps consisting of 250 mL of 0.32 M HBO_3 indicator solution in a sealed 500 mL flask [37], at a rate of 6 L min^{-1} using a 0.5 kW vacuum pump (*Gast*, model 0823, *Wainbee ltd.*, Pointe-Claire, QC, Canada). Flow meters (*Rate-Master*, model RMA-21-BV, *ITM*, Ste-Anne-de-Bellevue, QC, Canada) adjusted the air flow of each wind tunnel line.

2.3. Land Application Simulation. Land application simulations for the ISPAD and the open tank swine manures were tested in triplicates on all five soil types, resulting in 30 simulations. For each simulation using all five wind tunnels, 7.5 L of prepared soil was spread in each soil pan and water was added to bring its water content to 25% of its gravimetric holding capacity. Manure samples were weighed and then quickly but uniformly applied to the soils by hand at a simulated rate of 115 kg TKN ha^{-1}. The tunnels were quickly placed on the pans and airflow was started immediately.

Volatilized NH_3 was measured after 2, 4, 6, 8, 24 and 47 hours, by replacing the acid trap with a fresh one. The removed acid traps were chilled to 4°C before analysis for NH_4^+-N by titration with 0.1 M HCl [37]. Ammonia volatilization was computed from the air flow ratio of the acid trap and wind tunnel, at a constant ambient air temperature of 21°C.

2.4. Statistical Analysis. All statistical analyses were performed using SAS 9.2 (2010, SAS Institute inc., Cary NC, USA). The triplicate manure and soil characterization was used to determine the significance between measured values using the Student-Newman-Keuls method in a simple analysis of variance based on a completely randomized design. The NH_3 volatilization wind tunnel tests used a randomized complete block design, considering manure type as the treatment factor and soil type as the block factor. The dependant variable was NH_3 volatilization. Treatments were assigned randomly to experimental units (wind tunnels) and all treatments-block combinations were completed in triplicate.

3. Results and Discussion

3.1. Experimental Manure and Soil Characteristics. The experimental manures are characterized in Table 1. Despite similar contents, the VS : TS and VS : FS ratios were significantly different ($P = 0.0059$), reflecting the lower organic matter content of the ISPAD manure as a result of anaerobic digestion. Considering that suspended solids are volatile solids by nature and that both manure had similar level of dissolved solids, it can therefore be concluded that the ISPAD manure had less suspended solids as compared to that from the open tank.

Both the ISPAD and open tank manures offered a similar pH and TKN, but their VS : TS and TAN : TKN ratios were significantly different ($P = 0.0096$ and 0.0407, resp.) at 0.69 and 0.49, respectively, indicating greater protein breakdown for the ISPAD manure [2]. The total P and K contents were

Table 1: Characteristics of experimental manures where both the ISPAD or anaerobically digested manure and the open tank or untreated manure are one year old.

Property	Open tank manure	ISPAD manure
TS (gL^{-1})	37.7 (1.93)	33.5(4.12)
VS (gL^{-1})	27.7 (1.75)	23.0 (3.24)
VS : TS	0.73 (0.01)	0.69 (0.01)
VS : FS	2.8	2.2
pH	7.54 (0.01)	7.60 (0.05)
TAN (gL^{-1})	1.31 (0.06)	1.63 (0.08)
TKN (gL^{-1})	2.66 (0.20)	2.35 (0.30)
TAN : TKN	0.49 (0.08)	0.69 (0.12)
TKN : FS	2.66	2.33
P (gL^{-1})	2.29 (0.49)	2.82 (0.48)
K (gL^{-1})	1.42 (0.39)	1.04 (0.16)

The standard deviation is in the parenthesis ($n = 3$). TS: total solids; VS: volatile solids; FS: fixed solids.

similar. The ISPAD manure demonstrated some loss of NH_3 during sampling and transportation, based on its TKN : FS ratio compared to that of the open tank manure.

This combination of similarities and differences provided a wind tunnel comparison based on the significantly different characteristics of the two manures for the 115 kg TKN ha^{-1} applied. The similar TKN resulted in the same volume of manure being applied, thus conserving similar soil water holding capacity levels. Similar TS but different VS levels were applied. The TAN was the most important difference between manures, which was reported not to affect % N volatilization [7, 19]. Accordingly, the application of ISPAD and open swine manures provided a TAN level of 79 and 56 kg ha^{-1}.

The experimental soils (Table 2) were selected to offer a range of properties, illustrated by the groupings produced by the Student-Newman-Keuls test. Organic matter was similar only between the St Bernard loam and Ste Rosalie clay; gravitational water holding capacity was similar for the Upland sandy loam, St Bernard loam, and Ormstown loam; CEC, particle size distribution, especially clay content, and TKN differed among all soils. The pH was similar between the St Bernard loam and Ste Rosalie clay at 6.9 and 6.8, respectively, but different for the rest of the soils offering a pH of 6.3 to 6.5. The mass balance attempted between the initial soil and final soil manure TKN levels were inconclusive because the applied manure TKN was much smaller.

3.2. Ammonia Volatilization. The NH_3 volatilized from all five soils and the two experimental swine manures are compared in Figures 2(a) and 2(b). After 47 hours, the ISPAD swine manure had lost significantly less TAN, from 8 to 37% of that applied, while the open tank manure had lost from 25 to 61%. Accordingly, the ISPAD manure suffered from 75 to 50% less TAN losses as compared to the open tank manure, depending on the soil type. Considering that the two experimental manures offered similar properties except for their treatment, reflecting VS : TS and TAN : TKN ratio, anaerobic digestion was found to reduce NH_3volatilization. Similar results were obtained in a field experiment using different crops grown on a loamy soil in Germany [38]. As compared to untreated manure, anaerobically digested manure lost volatilized NH_3 equivalent to 14.2 as opposed to 13% of its total N applied, which represents 20 as opposed to 26% of its TAN.

The ISPAD manure not only suffered significantly less NH_3 volatilization losses over 47 hours but also exhibited a rate of loss which leveled off at 47 hours as opposed to that of the open tank manure. After 47 hours, the ISPAD manure was losing TAN at a rate of 0.25 to 0.05% h^{-1} for all five experimental soils, as compared to the open tank manure loosing TAN at a rate of 1.7 to 0.25% h^{-1}.

The statistical analysis using both mg NH_3-N and % TAN volatilized revealed that the effect of both manure and soil types was significant ($P < 0.003$ for mg N and $P < 0.0001$ for % TAN), with no significant interaction between the factors in either case. The significance of manure effect in mg N confirms that the results were not limited by air saturation in NH_3 inside the wind tunnels, which would have resulted in equal values among tunnels despite higher % TAN losses. Therefore, the remaining results will be presented in terms of % TAN volatilized.

Past research demonstrated a consistent % TAN volatilized for different application rates, with other factors held constant. For the present study, the significant effect results from the manure treatment as both manures exhibited similar properties. For anaerobically digested manure, the specific properties reducing NH_3 volatilization are lower volatile solids allowing for an improved infiltration into the soil and more complex ionic solution lowering TAN speciation into NH_3 [8, 13].

Comparing soil type, the % TAN volatilized results were grouped using the Student-Newman-Keuls method, indicating that, for each manure type, volatilizations from the Upland sandy loam, St Bernard loam, and Ormstown loam soil series were statistically similar while those from the Ste Rosalie clay and the washed sand were each significantly different. For each manure type, volatilization was highest for the washed sand, intermediate for the three similar soils, and lowest for the Ste Rosalie clay, the same grouping observed for H_2O holding capacity and CEC.

Exploratory plots of % TAN volatilized versus each individual soil property also revealed patterns suggesting linear relationships for water holding capacity, CEC and cation saturation. These relationships were examined using a two-factorial rather than a blocked experimental design, which compared the qualitative rather than quantitative soil type values. A variety of combinations revealed a significant effect of the water holding capacity and CEC ($P < 0.0001$), but only when considered separately. The use of only two manure types limited the scope of analysis available, but interesting leads for future research suggest extracting a numerical equation relating the appropriate manure and soil characteristics to % TAN volatilized.

Table 3 summarizes the % and mass (kg ha^{-1}) TAN volatilized in 47 h for each manure-soil combination. The ISPAD manure was found to lose 46% less TAN than the

Table 2: Characteristics of the five experimental soils used to measure NH_3 volatilization for the two experimental manures, untreated (open tank) and anaerobically treated (ISPAD).

Property	Washed sand	Ste Rosalie clay	Upland sandy loam	St Bernard loam	Ormstown loam
Organic matter (%)	0.1 (0.0)	6.2 (0.5)	5.0 (0.3)	6.5 (0.4)	3.9 (0.2)
H_2O capacity (%)	14.3 (0.0)	32.8 (1.6)	24.8 (1.7)	25.4 (1.5)	23.8 (2.5)
Particle size (%):					
Sand	82.9 (0.9)	7.4 (1.7)	71.2 (1.8)	38.6 (1.5)	1.6 (3.4)
Silt	0.5 (0.9)	25.7 (1.5)	6.8 (1.7)	31.3 (1.9)	62.2 (1.3)
Clay	16.6 (0.9)	66.9 (2.2)	22.0 (0.1)	30.1 (0.4)	36.3 (2.2)
pH	6.3 (0.1)	6.8 (0.1)	6.5 (0.1)	6.9 (0.3)	6.5 (0.9)
Buffer pH	7.3 (0.1)	7.2 (0.1)	6.9 (0.1)	7.3 (0.3)	7.1 (0.4)
CEC (cmol kg^{-1})	2.0 (0.5)	36.8 (2.8)	18.3 (1.0)	23.3 (2.2)	20.6 (1.1)
Cation saturation (%)	26.0 (5.9)	92.0 (2.6)	73.0 (3.5)	90.6 (8.8)	81.8 (17.3)
TKN (g kg^{-1})	2.4 (0.6)	5.4 (0.8)	4.4 (0.2)	3.5 (0.9)	3.7 (1.3)
TAN (mg kg^{-1})	3.0 (2.0)	3.0 (0.4)	6.0 (1.0)	5.0 (3.0)	8.0 (3.0)

The standard deviation is presented in the parenthesis ($n = 3$).

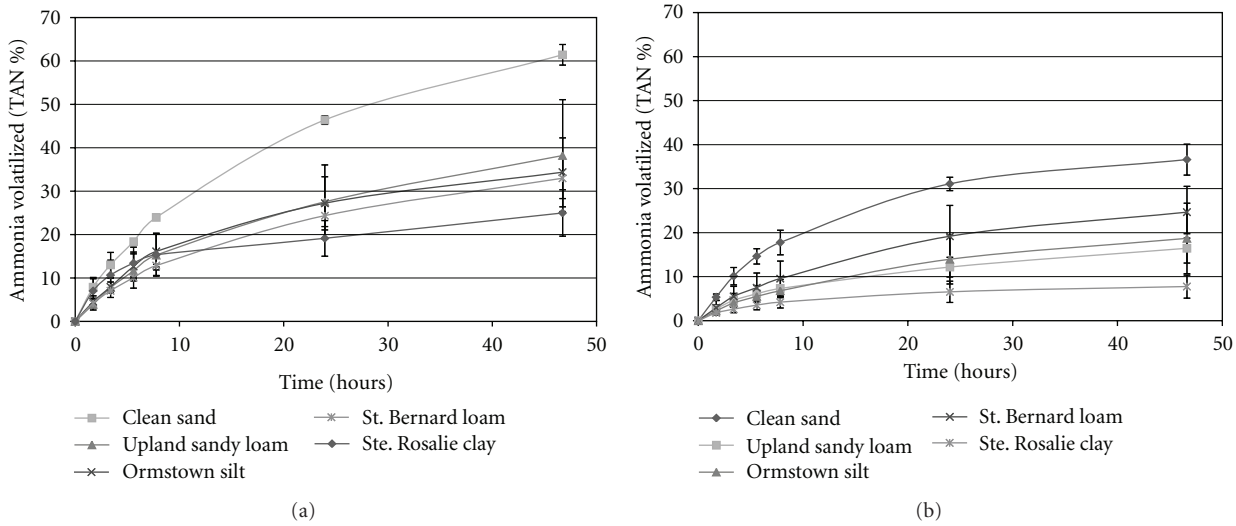

Figure 2: Cumulative NH_3 volatilized during 47 hours following the land application of untreated swine manure from the open tank (a) and anaerobically treated swine manure ISPAD (b). The NH_3 volatilization was measured using the laboratory wind tunnels. The data points are the average of $n = 3$ and the error bars represent ± one standard deviation.

Table 3: Fraction of the manure TAN volatilized in 47 hours of wind tunnel simulations for the five experimental soils and two manures, untreated (open tank) and anaerobically digested (ISPAD).

Soil type	Open tank manure		ISPAD manure		Average	
	% manure	kg ha^{-1}	% manure	kg ha^{-1}	%	kg ha^{-1}
Washed sand	61.4 (2.4)	34 (1.3)	36.6 (3.5)	29 (2.8)	49.6	31.5
Upland Sandy Loam	38.2 (12.9)	21 (7.1)	16.4 (3.3)	13 (2.6)	27.3	17.0
Ormstown Loam	34.3 (7.9)	19 (4.4)	18.7 (8.0)	15 (6.4)	26.5	17.0
St Bernard Loam	33.0 (4.7)	19 (2.7)	24.7 (5.9)	20 (4.8)	28.8	19.5
Ste Rosalie Clay	25.0 (5.4)	14 (3.0)	7.8 (2.6)	6 (2.1)	16.4	10
Average	38.6	21.4	20.8	16.6		

The standard deviation is presented in the parenthesis ($n = 3$).
The % value indicated the percentage of the TAN in the applied manure while the kg ha^{-1} value indicates the mass volatilized.

Table 4: Manure N fertilizer value for similar P and K applications for the two experimental manures, untreated (open tank) and anaerobically digested (ISPAD).

	Open tank manure	ISPAD manure
Case 1: Ideal, $N_{\text{available}}$ (gL^{-1})	1.78	1.88
Case 2: Average, $N_{\text{available}}$ (gL^{-1})	1.25	1.48
Case 3: Uncontrolled, $N_{\text{available}}$ (gL^{-1})	0.80	0.97

Case 1 refers to immediate soil incorporation for a spring application, Case 1 refers to soil incorporation after 48 hours for a summer application, and Case 3 refers to leaving the manure at the soil surface for a fall application.

open tank manure. The washed sand lost the most % TAN while the Ste Rosalie clay lost the least at 33% less than the washed sand. The 3 intermediate soils lost approximately 54% of the TAN compared to the washed sand. Interestingly enough, the St Bernard loam lost the same mass of NH_3-N for both manure types, likely because of its higher pH and buffer as compared to the other soils and average CEC, resulting in more soil solution NH_3. Accordingly, the ISPAD manure benefited from the combined effect of its lower volatile solids (VSs) and a more complex ionic solution lowering TAN speciation into NH_3 resulting in lower volatilization for higher levels of plant-available nitrogen [39].

3.3. ISPAD Manure N Fertilizer Value. Because of stringent requirements for nutrient management planning all over North America, the Quebec Ministry of Environment has produced detailed methods for calculating the fertilizer value of manures [34, 40]:

$$N_{\text{available}} = \left(\text{TAN} + \left(N_{\text{organic}} \times \text{CEFO}\right)\right) \times \text{CV}^{-1} \times \text{CA}^{-1} \times \text{CP}, \quad (1)$$

$$N_{\text{organic}} = \text{TKN} - \text{TAN},$$

where CEFO is the organic fraction efficiency factor based on C : N ratio, CV is the volatilization factor based on land application method, CA is the availability factor based on application date (spring or fall), and CP is the previous application factor based on years of manure applied.

Using (1), $N_{\text{available}}$ was calculated for the two experimental manures, assuming three possible cases selected to illustrate a full range of situations: Case 1 pertained to an immediate incorporation of the manure into a clay soil supporting row crops, in the spring; Case 2 pertained to the incorporation within 48 hours of the manure into a sandy loam soil in row crops, during the early summer; Case 3 pertained to manure left at the surface of a sandy loam soil supporting a hayfield, in the autumn. In all cases and for simplicity, no manure was presumed previously applied to the fields, thus CP = 1. For both manures, a C : N ratio in the range of 6 to 10 : 1 was assumed, based on the VS and TKN values (Table 1). The CV value for the ISPAD manure was modified using the ratio of 0.53 obtained by comparing NH_3 volatilized between the ISPAD and open tank manures in Table 3:

$$\text{CV}_{\text{ISPAD}} = 1 + ((\text{CV} - 1) \times 0.53). \quad (2)$$

The resulting N fertilizer values summarized in Table 4 demonstrate that in Case 1, representing the ideal manure land-application conditions, the ISPAD manure has a modest advantage of 6% over the open tank manure. This advantage increases to 18% under Case 2 where manure is spread on land and then incorporated within 48 hours. For Case 3, where no soil incorporation is practiced, the ISPAD manure offers the highest advantage of 21%. Accordingly, for the same P content, ISPAD manure can provide up to 21% more plant-available N than open tank manure, thus reducing the mineral nitrogen required for top-up by an equivalent amount.

4. Conclusions

The objective of this study was to compare the NH_3 volatilization potential of swine manure treated by a five-year-old in-storage psychrophilic anaerobic digestion system (ISPAD) against that from an open tank. The study simulated field spreading in the laboratory using wind tunnels and five soils offering a different texture and chemical properties.

The study revealed the following.

(i) For all experimental soils, the % TAN volatilized from the ISPAD manure was on the average 46% lower than that from an open tank indicating the beneficial effect of anaerobic digestion, such as lowering the volatile solids reflecting the suspended solids concentration, to improve infiltration and producing a more complex ionic solution lowering TAN speciation into NH_3.

(ii) Water holding capacity and cation exchange capacity were the most important soil parameters influencing NH_3 volatilization.

(iii) The ISPAD and open tank manures offered a TAN : TKN ratio of 69 and 49%, respectively; coupled with a lower NH_3 volatilization, ISPAD manure can thus increase by up to 21% the plant-available N for the same P and K value, as compared to open tank manure.

Further research is required to identify more clearly the parameters of ISPAD manure which facilitate its lower NH_3 volatilization during land spreading.

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Research Article

Influence of Surface Biosolids Application on Infiltration

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Biosolids from waste water treatment facilities applied to soils not only add plant nutrients, but also increase infiltration and decrease runoff and erosion. Wet biosolids from New York, NY, were surface applied at 0 to 90 Mg ha^{-1} dry weight to soils near El Paso, Tex. Simulated rainfall intensities of 16.4 cm hr^{-1} for 30 minutes applied to 0.5 m^2 soil plots yielded initial infiltration rates of ~16 cm hr^{-1} for all plots. Biosolids applications extended the duration of the initially high infiltration rates. After 30 minutes, infiltration rates for bare soil were 3 cm hr^{-1} without and 10 cm hr^{-1} with 90 Mg biosolids ha^{-1}. Applied biosolids, plant litter, surface gravel, and plant base contributed surface cover, which absorbed raindrop energy and reduced erosion. Biosolids increased cumulative infiltration on the vegetated, wet soils more than for the dry or bare soils. Biosolids increased cumulative infiltration from 2 to 6 cm on a bare gravelly soil and from 9.3 to 10.6 cm on a vegetated soil.

1. Introduction

Throughout history, human waste from raw to highly treated waste has been applied to soils by various methods. In the United States, Congress passed the Federal Water Pollution Control Act in 1948, which governed the release of waters and solids into the environment. This act codified raw sewage treatment in publicly owned treatment works (POTWs), where water is removed and residual solids yield sewage sludge. Industrial waste streams are separated from domestic waste to produce much cleaner wastewater solid residuals. The current term for wastewater solid residuals is "biosolids", which is a term coined by the Water Environment Federation. While biosolids have positive nutrient and hydrologic properties, biosolids were often considered a waste product to be discarded. One method of discarding biosolids was by ocean dumping. The Ocean Dumping Ban Act of 1988 as the name implies, forbid biosolids disposal in the ocean and increased biosolids competition for landfill disposal.

Changing the name from "sewage sludge" to the more benign term "biosolids" eased the way for beneficial land applications of biosolids. Biosolids are composed of water, organic matter, and inorganic matter. Water accounts for 60% to 80% of the mass in dewatered biosolids. The dry solids of anaerobically digested biosolids are about 60% organic matter [1]. The biosolids organic fraction is composed of relatively stable organic compounds that resist oxidation in the anaerobic digestion process. The organic matter in biosolids is a source of slow release nitrogen from the breakdown of amino acids. The clean biosolids produced by POTWs are now being used for beneficial purposes, such as land application for nutrients [2]. Proper biosolids applications can improve soil quality and plant production. With recent increases in biosolids land application, a better understanding of the impact of biosolids on soil physical properties is needed.

Biosolids have been used for years as a soil amendment in mine and disturbed land reclamation and in crop, pasture,

forest, and range production. Biosolids application not only adds nutrients, but also affects hydrology. Soil quality is appreciably improved in a number of land use settings. Mine and disturbed land reclamation pose a significant challenge in transforming spoils into soil. Biosolids have generally improved reclamation efforts across the USA [3]. Meyer et al. [4] reported increased plant cover and less erosion due to biosolids applications after a forest fire.

The hydrologic effects of biosolids on infiltration and erosion have been documented in numerous rangeland settings worldwide. In central Spain, Walter et al. [5] reported favorable soil and vegetation resulted from the decreased erosion and enhanced water relations that resulted from applications of 40 to 80 Mg biosolids ha^{-1}. Ojeda et al. [6] reported similar results in north-east Spain using fresh biosolids, composted biosolids, and thermally dried biosolids.

Interception is water absorbed by vegetation or litter (biosolids included), which is subtracted from gross precipitation to give net precipitation. Intercepted water does not infiltrate the soil or run off and the hydrologic importance of interception depends on climatic, soil physical, and vegetative characteristics. Harris-Pierce et al. [7] and Aguilar and Loftin [8, 9] evaluated the relationships between biosolids application rates, infiltration, and erosion in semiarid grasslands. Harris-Pierce et al. [7] reported that the quantity of runoff was unaffected by biosolids application rate. Aguilar and Loftin [8, 9] evaluated the effects of biosolids on the quantity of runoff water under both natural and simulated conditions. They reported that only one of four natural storms yielded significantly different runoff quantities between slope gradients. Mean runoff, however, was significantly different between biosolids-treated plots and untreated plots for all but one storm. Aguilar and Loftin [9] attributed the reduction in runoff with biosolids application to increased surface roughness. Aguilar and Loftin [8, 9] conducted additional simulated rainfall experiments, and significant differences in runoff were due to differences in soil water content.

The above articles evaluated the relationships between surface applied biosolids and infiltration but do not address infiltration properties and characteristics. Infiltration and surface runoff are the two dominant methods of water loss from precipitation and irrigation. With the use of a rainfall simulator, the excess runoff becomes what is known as "Horton" flow after Horton [10]. This differs from interpedal water flow which occurs within a pedon. The infiltration and redistribution of water takes three stages [11]. These stages are as follows: Stage I in which the soil takes in water as fast as it is applied and is known as "flux-controlled" infiltration [11]. Stage II is known as "profile-controlled" and as the name implies is controlled by the soil profile characteristics. These profile characteristics are determined by soil texture, soil depth, bulk density, and pore size, distribution, and arrangement. The final stage, III, is controlled by the final soil hydraulic conductivity and is a constant-value soil parameter not related to surface conditions.

Infiltration can be modeled using the Green and Ampt [12] equation. This century-old equation relates infiltration rate to stage III infiltration, hydraulic conductivity, and cumulative infiltration using an arbitrary soil constant. The interactions between infiltration (infiltrability Hillel [11]) parameters can be characterized using the Green and Ampt [12] equation cited by Hillel as follows:

$$i = i_c + \frac{b}{I}, \tag{1}$$

where i is infiltrability (Hillel's term) or infiltration rate cm hr^{-1}, i_c is the final infiltration rate, cm hr^{-1}, b is an arbitrary constant of infiltration, $cm^2\,hr^{-1}$, and I is the cumulative infiltration, cm.

Using the computed Green and Ampt "b" constant, surface alterations caused by antecedent water content, surface vegetation, or lack of vegetation, and gravel as well as biosolids can be used to compare and contrast the effects of surface application of biosolids on semiarid rangelands.

Extensive studies have evaluated biosolids application and soil physical properties at the Sierra Blanca Ranch [13–16]. All of these studies utilized biosolids transported by rail from New York, NY, to improve rangeland conditions in West Texas.

The overall objective of the research described below was to document the beneficial use of biosolids to improve soil physical properties by enhancing infiltration and, thereby, decrease surface runoff and erosion. Specific objectives were to evaluate the effects of: (1) antecedent soil-water content, (2) vegetative cover, and (3) gravel cover on infiltration and erosion as a function of biosolids application rate.

2. Materials and Methods

The study site was located within the northern Chihuahuan Desert in Hudspeth County, Texas. The experiments were conducted on the Sierra Blanca Ranch located 140 km southeast of El Paso, Texas, immediately north of Interstate Highway 10 [16]. The site is a typical northern Chihuahuan desert with grassland vegetation on basin floors and desert scrub on fan piedmonts. The climate of the area is subtropical semiarid with 308 mm annual precipitation, two thirds of which falls during the months of July through September [13]. The regional precipitation exhibits large interannual variations that range from 110 to 430 mm and average 308 mm [13]. Sixty-six percent of the annual precipitation comes as intense, short-duration, convective storms during the summer months. Average air temperatures are 4.9°C in January and 25.3°C in July.

The dominant soil evaluated was the Stellar very fine sandy loam, 1% to 3% slope (fine, mixed, superactive, thermic Ustic Calciargids). Soil particle size distribution differences between the bare and vegetated Stellar sites are significant in the crust layer and A horizon [13]. Crust layer texture for bare Stellar is loam, but it is within 1% sand of being very fine sandy loam. The particle size class for the crust layer in vegetated treatments and A horizon for both cover condition treatments is very fine sandy loam. Cover treatments differ in distribution of the separates in the crust and A horizon [13]. Erosion and deposition processes likely cause surface sealing that form the crust. The A

horizon below the crust is not as influenced by erosion and deposition. A second soil evaluated was the Chilicotal very fine sandy loam 1 to 3% slope (loamy-skeletal, mixed, superactive thermic Ustic Haplocalcids). The Chilicotal soil horizons differed little with clay content gradually increasing with depth [13]. While the mean particle size increased monotonically from 168 mm in the crust to 125 mm in the Bw1 horizon, all horizons of the Chilicotal soil are very fine sandy loam. The dominate vegetation was a typical desert grassland and was classified as a Loamy Range site with 5% basal cover, 69% bare ground and only 3% gravel cover.

Surface cover includes litter, gravel, plant base, dead plant base, and microphyte. Biosolids application contributes to cover. Surface cover parameters for the Chilicotal site differed from the Stellar site regardless of cover treatment. In the control bare Stellar treatments, 80% of the ground surface was unprotected (data not presented), but in the vegetated Stellar treatments, only 29% of the ground surface was unprotected. The Chilicotal site had little vegetation with ~50% of the ground surface unprotected and with gravel accounting for 48% of the ground cover. The bare Stellar soil had approximately 9% gravel as ground cover compared to the vegetated Stellar soil with 1% gravel surface cover. Litter provided cover for 2% of the ground surface in Chilicotal and 6% in bare Stellar. This level of cover was provided by 142 kg litter ha^{-1} in the bare Chilicotal and 100 kg ha^{-1} in the bare Stellar. The litter on the Chilicotal soil was generally creosote bush (*Larrea tridentata*) twigs that have a higher density than the grass- and forb-derived litter on the Stellar site. Sixty-three percent of the ground surface was protected by 1560 kg litter ha^{-1} invegetated Stellar treatments and 5% of ground surface cover was from plant bases.

Differences between vegetated and bare treatments may be due also to a physical interaction between biosolids and vegetation. Biosolids may have been suspended by vegetation above the ground surface and held together by the supporting force of adjacent plant stems and leaves. Bare Stellar plots had an average standing crop biomass of 56 kg ha^{-1}, while the vegetated plots had 4200 kg ha^{-1} standing crop biomass. Chilicotal plots had an average standing crop biomass of 52 kg ha^{-1}. Standing crop for bare Stellar and Chilicotal treatments resulted from unwelcome plants at the plot boundaries.

Litter also differed between soils although biosolids application rate had a significant effect on litter and this is likely an artifact of the technique. The vegetated plots without biosolids had 1560 kg litter ha^{-1}, whereas the 90 Mg biosolids ha^{-1} plots had only 1020 kg litter ha^{-1}. The difficulty in separating litter from biosolids may account for this difference. Most of the litter was beneath biosolids in the vegetated treatments. In bare treatments, the control treatments had very minor litter (100 kg litter ha^{-1}). For the 90 Mg biosolids ha^{-1} plots, the biosolids provided additional wind resistance which facilitated the accumulation of windblown litter. The total quantity of windblown litter, however, was small; it accumulated on top of biosolids, and it was not difficult to separate. Vegetated treatments had an average 59% canopy cover, whereas bare treatments had approximately 1% canopy cover. The canopy cover for the 90 Mg biosolids ha^{-1} rate was reduced from 62% to 49% for the control treatments. This was due to partial burial of the plant canopy at this high application rate.

The study plots were not clipped prior to surface application of biosolids or rainfall simulation. Biosolids were applied only once, and the growing-season rainfall during the study was below average. Wet biosolids were surface applied at rates of 0, 7, 18, 34, or 90 Mg biosolids ha^{-1} on an equivalent dry-weight basis. Biosolids depth and percent surface area covered by biosolids depended on application rate and increased when the application rate was increased. On average, 90 Mg biosolids ha^{-1} provided 69% surface cover at a depth of 20 mm. Specific depth and surface area coverage for this site were presented in Moffet et al. [13].

The simulated rainfall was applied as described by Moffet et al. [13]. Briefly, a portable single-nozzle rainfall simulator was placed 2 m above the soil surface. A tarp was placed around the simulator to protect the simulated rainfall from disturbance by the wind. A nozzle pressure of 20.7 kPa (3 psi) was maintained during simulation to produce a mean rainfall intensity of 16.4 cm hr^{-1} on a 0.5 m^2 plot. Median drop size was between 1.2 and 2 mm with the largest drops concentrated in the center of the plot. The water was moderately alkaline with a pH of approximately 8.2, an electrical conductivity of 0.7 dS m^{-1}, total dissolved solids of 444 mg L^{-1}, and a sodium absorption ratio of 6.7 [13].

A square 0.5-m^2 steel frame was positioned on each plot and driven into the ground, which bounded the plot edges on three sides. The fourth side of the frame, set on the lowest edge of the plot, was flush with the soil surface and was fitted with a runoff collection pan. The runoff collection pan was sheltered from the rain but allowed the plot runoff water to flow to the lowest corner of the pan.

The rainfall simulator was positioned on each plot, and a thirty-minute rainfall was simulated. The simulated rainfall was first applied to ("dry") plots at rates approximating 16 cm hr^{-1} and runoff was collected to determine the infiltration rate. The next day, simulated rainfall was again applied to the same plots, and these observations were labeled "wet." For both the dry and wet conditions, surface runoff was collected as a function of time. Runoff water that collected in the lowest corner of the collection pan was transferred through a nylon hose and suction pumped to a graduated cylinder. The volume was then recorded for each period. Runoff was measured and recorded every 2.5 minutes for the first ten minutes and every five minutes for the remaining 20 minutes. The difference in runoff and applied rainfall for each period was the infiltration for that period with infiltration divided by time being the infiltration flux.

The applied rainfall volume was estimated for each plot. At the end of the 30-minute simulation period, a calibration pan with the exact dimensions of the runoff plot was placed over the plot for a period of five minutes. At the end of the 5-minute period, the simulator was shut off, and the volume of water collected in the calibration pan was measured. The values for each plot rained on in a day were averaged, and the average was used as the estimate for all plots measured that day.

The simulated rainfall experiments required between 47 and 66 days to complete for each soil. Both wet and dry (antecedent soil water) conditions were run on 100 Stellar plots for a total of 200 observations. Only 47 days were needed to complete the 60 Chilicotal plots, since there were no antecedent soil water treatments.

Data that address the effects of antecedent soil water contents on infiltration flux, infiltration, and erosion were collected from the Stellar site. The alpha-level degree of certainty for conclusions drawn from F-tests in an analysis of variance depended on how well the assumptions were met. The assumptions made in an ANOVA generally deal with the shape of the treatment observation distribution about the mean, the dispersion homogeneity among treatments or treatment differences, and, lastly, that the treatment population is representative of the whole population. Tests may be used to determine how well these assumptions are met.

One of the most basic assumptions of parametric F-tests in an ANOVA is that experimental errors are normally distributed. Although F-tests are robust with respect to violations of normality, the normality was tested. For serious violations of normality, a log transformation was performed to normalize the data, and normality was tested using the Shapiro and Wilk [17] test.

3. Results

3.1. Infiltration Flux as a Function of Soil Wetness and Surface Cover. Two of the main factors that influenced infiltration and runoff from surface-applied biosolids sites were antecedent water content and soil surface cover. The following series of figures show the effects of antecedent water contents under bare and vegetated soil conditions on the Stellar very fine sandy loam soil. In the following figures, infiltration flux is plotted versus rainfall simulator run time for plots with different antecedent water contents and vegetation (i.e., bare dry, bare wet, vegetated dry, and vegetated wet). In the figures, applied biosolids range from 0 Mg biosolids ha^{-1} to 90 Mg ha^{-1}. The first figure (Figure 1) is for a Stellar soil that was bare/vegetated or dry/wet without biosolids. Water was applied using the rainfall simulator described previously.

All plots had application rates and initial infiltration fluxes of approximately 16 cm hr^{-1}. This is stage I infiltration, which is the initially high infiltration rate that is controlled by the water application rate. After several minutes, the infiltration flux decreases and stage II infiltration begins. Stage II infiltration is controlled by the soil profile and is the transition from stage I to the lower-flux, stage III infiltration. Stage III infiltration is the steady-state, soil hydraulic conductivity-controlled infiltration rate. The bare-wet infiltration flux decreased less rapidly, because the hydraulic gradient is less than that for the bare-dry soil. Both the wet and dry vegetated soils had longer stage I infiltration times than dry or wet bare soil. The stage I infiltration treatment flux time for vegetated soil was greater than bare soil due to a better pore size distribution. The 30-minute flux rates for bare soils were approximately 2 cm hr^{-1} compared to 9 cm hr^{-1} for the vegetated soils. The bare soils probably reached stage III infiltration flux, whereas the vegetated soils had not yet reached the stage III infiltration flux. Vegetation enhanced 30-minute infiltration flux by a factor of four or more when no biosolids were applied and delayed the time to stage III infiltration.

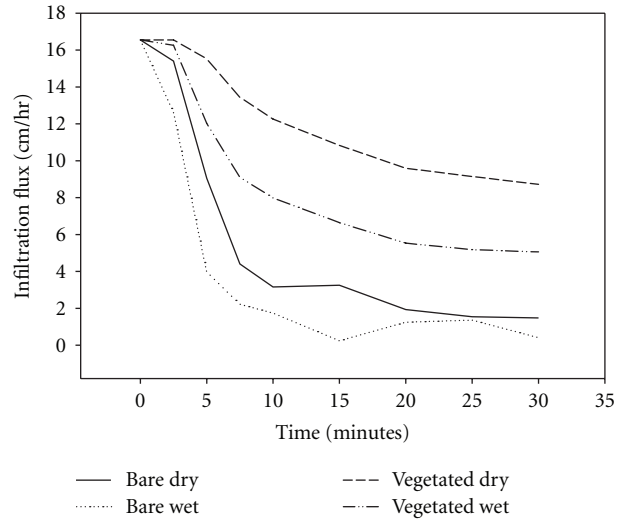

Figure 1: Infiltration flux for the Stellar soil under bare dry, bare wet, vegetated dry, or vegetated wet conditions on the Sierra Blanca Ranch.

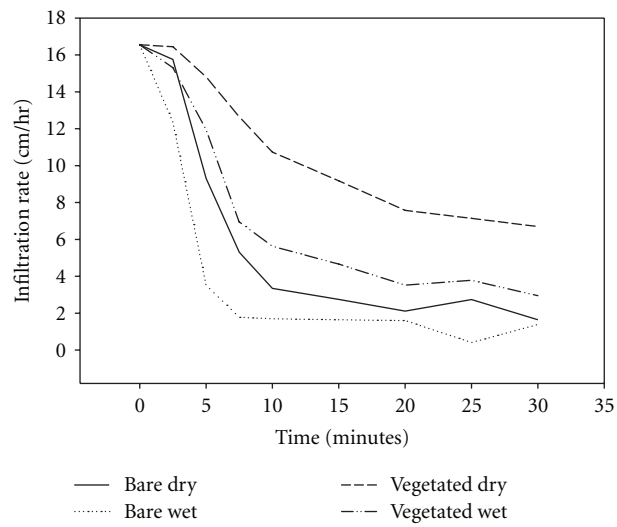

Figure 2: Infiltration flux for Stellar soil with 7 Mg biosolids ha^{-1} under bare dry, bare wet, vegetated dry, or vegetated wet conditions on the Sierra Blanca Ranch.

The infiltration flux was measured for the Stellar soil with 7 Mg biosolids ha^{-1} under bare dry, bare wet, vegetated dry, and vegetated wet conditions and stage I infiltration was approximately 16 cm hr^{-1} and the dry-soil stage I infiltration flux time exceeded that of the wet soil (Figure 2). The infiltration fluxes for the 7 Mg biosolids ha^{-1} treatments were similar to the soil without biosolids. The initial values were both approximately 16 cm hr^{-1} and decreased to 10 cm hr^{-1} for the dry soils and 8 cm hr^{-1} for the wet soils. The results

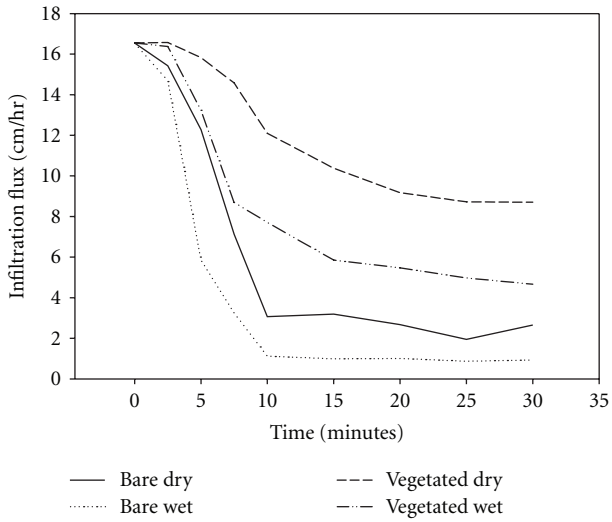

Figure 3: Infiltration flux for Stellar soil with 18 Mg biosolids ha^{-1} under bare dry, bare wet, vegetated dry, or vegetated wet conditions on the Sierra Blanca Ranch.

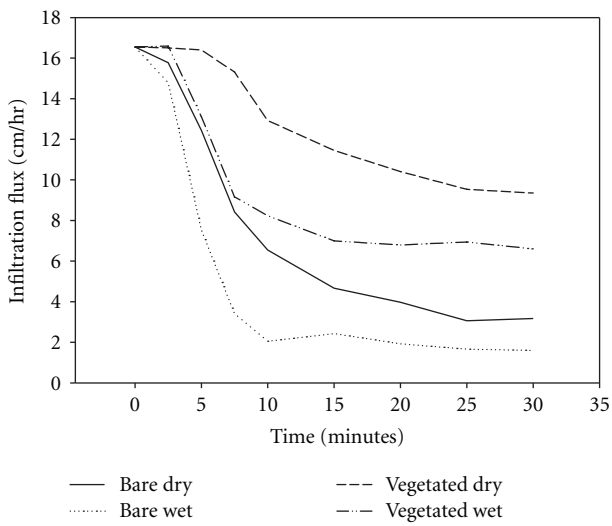

Figure 4: Infiltration flux for Stellar soil with 34 Mg biosolids ha^{-1} under bare dry, bare wet, vegetated dry, or vegetated wet conditions on the Sierra Blanca Ranch.

were similar for the Stellar soil with 18 Mg biosolids ha^{-1} (Figure 3) and 34 Mg biosolids ha^{-1} (Figure 4). At greater biosolids application rates, stage I infiltration times increased and the slope of stage II infiltration flux decreased. These differences occurred as added biosolids were increased from 7 Mg ha^{-1} through 18 and 34 Mg ha^{-1}. Adding 90 Mg biosolids ha^{-1} (Figure 5) lengthened the infiltration flux time for the Stellar soil. With the addition of 90 Mg biosolids ha^{-1}, only the wet-bare soil achieved a stage III, steady-state infiltration rate of approximately 2 cm hr^{-1}. The 90 Mg biosolids ha^{-1} bare-treatment stage-I infiltration fluxes were (Figure 5) similar to the vegetated treatments in which no biosolids were applied (Figure 1). Biosolids changed the soil surface properties enough that stage I infiltration flux was enhanced.

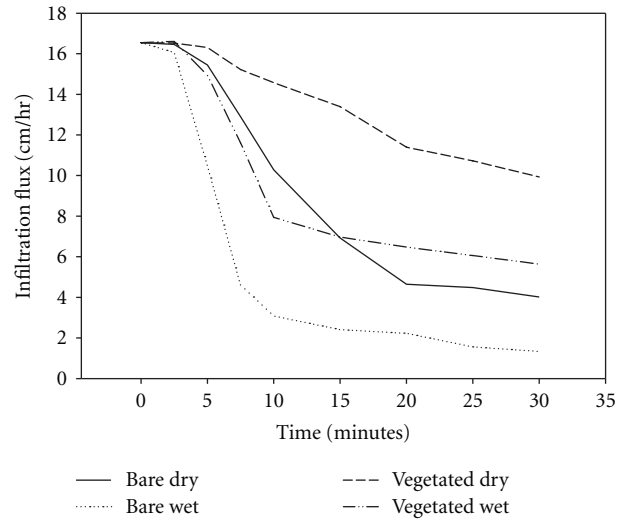

Figure 5: Infiltration flux for Stellar soil with 90 Mg biosolids ha^{-1} under bare dry, bare wet, vegetated dry, or vegetated wet conditions on the Sierra Blanca Ranch.

Infiltration flux was affected by interactions between biosolids rate, cover, and time (Figures 1–5). The time zero infiltration flux, for comparison, was the mean simulated rainfall intensity for the experiment. In general, wet, bare treatments reached a stage III, steady-state infiltration in less time than vegetated treatments regardless of biosolids rate. Stage III infiltration occurs when the infiltration flux reaches a plateau and does not change appreciably with time. Typically, an infiltration flux plateau formed between five and ten minutes after the initiation of simulated rainfall for the wet, bare treatments. Wet runs reached stage III infiltration within 30 minutes, whereas the comparable dry treatments did not.

Hydraulic conductivity in layered soils changes as the wetted region moves deeper into the soil profile. All soils had a surface crust. In the bare soil treatment, soil hydraulic conductivity was lowest in the crust, increased in the A horizon, and then decreased in the B horizon. Bare treatments had more contrasts in texture between the crust layer and A horizon than vegetated treatments and, therefore, had a short period of transient ponded infiltration (stage II).

The Chilicotal is a very fine, sandy loam soil that is similar in surface texture and slope to the Stellar soil, but is covered with gravel. The Chilicotal soil will be compared to the Stellar soil to evaluate surface gravel content and biosolids addition effects on infiltration flux. Infiltration flux for the Chilicotal soil is plotted versus rainfall simulator run time in Figure 6. The greater surface gravel content slightly reduced the initial infiltration rate to <16 cm hr^{-1} for this soil. The Chilicotal has more shrub vegetation and less total surface cover than the more vegetated Stellar soil. This lack of vegetation cover is made up in some way by the increased surface gravel. Infiltration rates for the Chilicotal soil (Figure 6) with added biosolids were similar to the Stellar (Figures 1–5). Stage I infiltration flux time increased as the biosolids application rate was increased. Additionally, the slope of stage II infiltration flux decreased with an increase

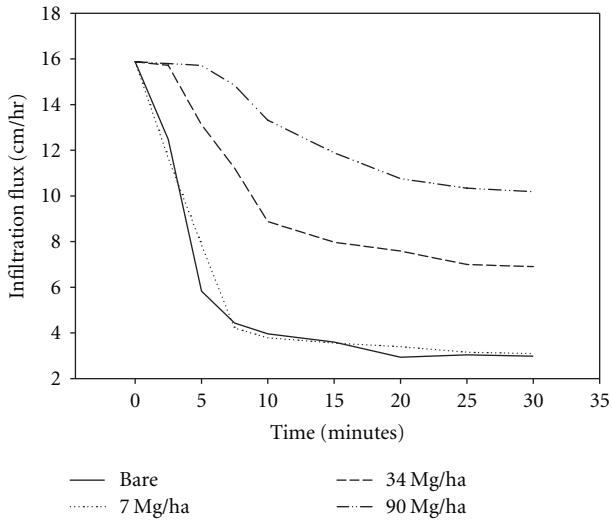

Figure 6: Infiltration flux as a function of time and rate of biosolids applied on a Chilicotal soil with a gravelly surface on the Sierra Blanca Ranch.

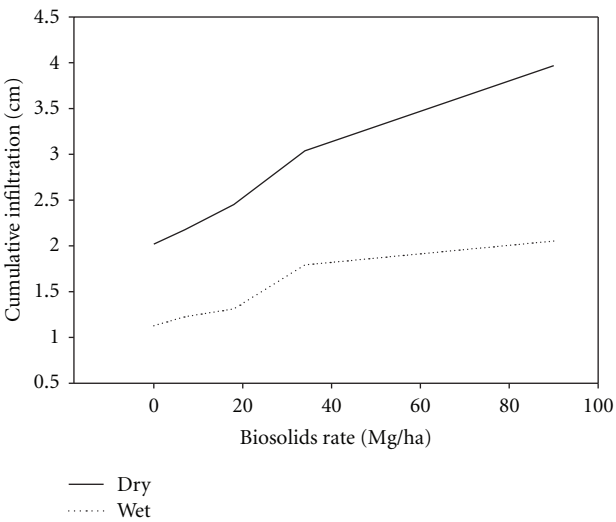

Figure 7: Cumulative infiltration for wet or dry conditions in bare treatments for the Stellar soil as a function of biosolids rate on the Sierra Blanca Ranch.

in biosolids application rate. For this gravelly soil, the 0 and 7 Mg biosolids ha^{-1} treatments had stage III infiltration fluxes of approximately 3 cm hr^{-1}, which are similar to the stage III infiltration of the Stellar soil.

Integrating infiltration flux curves yields cumulative infiltration. Effects of the wet/dry treatments on cumulative infiltration depended on cover and biosolids rate, not on the interaction of cover and rate (Figures 7 and 8). Cumulative infiltration was lower in the wet runs than in the dry runs. Additionally, cumulative infiltration was lower in the bare Stellar soil than in the vegetated Stellar soil.

In the Stellar soil, bulk density and percent clay increased with depth (data not presented). Hydraulic conductivity decreased as density and clay content increased and as structured soils with high clay content swell and close macropores.

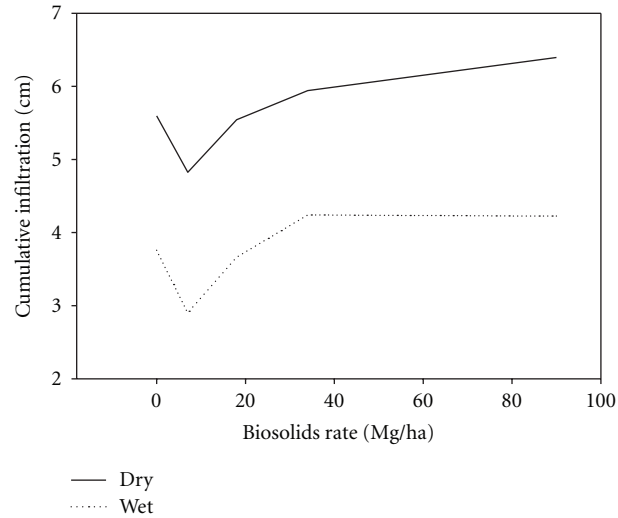

Figure 8: Cumulative infiltration for wet or dry conditions in vegetated treatments for the Stellar soil as a function of biosolids rate on the Sierra Blanca Ranch.

In crusted soils, the greatest limitation to infiltration may be the crust layer. After infiltration, soil water is redistributed. In a soil with little water infiltration, the matric potential (attraction of soil for water due to adhesion and cohesion) of the wetted region following a day of redistribution will be more negative than for a similar soil with more water infiltration. The vegetated soil treatment had significant infiltration the first day (dry run), and infiltration for wet run treatments was, thus, significantly reduced. Infiltration was limited in the bare soil treatments by the crust layer, and thus, redistribution had a greater effect on matric potential in vegetated soils. The matric potential in the wet region of bare soils may be more negative than in vegetated soils, because of the effect of redistribution. Therefore, in the bare soil, infiltration in the dry run did not cause as much of a reduction in infiltration as in the wet run.

There was an interaction between biosolids rate and water condition, which suggests absorption of water by the biosolids. Wet treatments with biosolids had 1.37 cm less infiltration than dry treatments without biosolids. Furthermore, wet soils with 90 Mg ha^{-1} biosolids applications had 2.05 cm less infiltration than dry treatments. From regression of wet and dry treatment infiltration differences and biosolids rate, it was determined that biosolids do absorb water at a rate of 0.74 m^3 water Mg^{-1} biosolids. This equals 6.7 mm at the 90 Mg biosolids ha^{-1} rate and is equivalent to biosolids with 73.5 percent water on a dry weight basis.

Summing the cumulative infiltration for the dry and wet rainfall simulator runs yields the two-day total infiltration. Vegetated soils treated with 90 Mg biosolids ha^{-1} infiltrated a total of 10.62 cm (less interception losses). The vegetated control (0 Mg biosolids ha^{-1}) and vegetated soils treated with 34 Mg biosolids ha^{-1} infiltrated 9.36 and 7.72 cm, respectively. Bare soil treated with 90 Mg biosolids ha^{-1} infiltrated 6.02 cm and control treatments infiltrated 3.25 cm. The difference between these quantities suggests other mechanisms

in addition to interception losses, since care was taken to minimize evaporation losses between runs. Fresh biosolids with 300% water (dry weight basis) contains 2.7 cm of water (90 Mg ha^{-1}) [8]. Using their [8] conservative estimate of 100% instead of the measured 50% to 60%, the amount of absorbed water is 0.9 cm.

3.2. Runoff. Runoff is influenced by plot characteristics, such as slope, surface roughness, and depression storage volume. The Chilicotal had 0.56 mm of depression storage (data not presented). Although the Stellar vegetated treatments had an average storage volume of 2.2 mm, most of this volume was an artifact of the treatment technique. In the Stellar vegetated treatment, water was trapped between the plot boundary and a region of higher ground at the plot center, but under normal conditions, the water would flow freely away from the plot center. The Stellar bare treatment had 0.90 mm of storage. On the basis of particle size distribution, the Stellar vegetated treatment appeared to be an area of very fine and fine sand accumulation.

3.3. Hydrologic Response to Biosolids. Surface-applied biosolids affected hydrologic response. The biosolids provided a protective cover on the soil that reduced erosion by absorbing raindrop energy. Biosolids may also intercept and absorb rainfall, reducing the net precipitation for an event. For small events, this may be an important loss on a percentage basis, but for large events like the simulation events, the loss was insignificant.

Infiltration flux was affected by the interaction of biosolids rate, cover, time, and antecedent soil moisture (Figures 1–5). The time zero infiltration flux, for comparison, is the mean simulated rainfall intensity for the experiment. In general, bare treatments reached stage III infiltration in a shorter time than vegetated treatments regardless of biosolids rate. Wet runs reached Stage III infiltration before companion dry treatments and terminated at a lower infiltration flux.

Hydraulic conductivity in layered soils changes as the wetted region deepens. In the bare soil treatment, soil hydraulic conductivity was lowest in the crust, increased in the A horizon then decreased in the B horizon. Bare treatments were more contrasting in texture between the crust layer and A horizon than vegetated treatments and, therefore, had a short period of transient ponded infiltration (stage II).

Integrating infiltration flux curves yields cumulative infiltration. For the Stellar soil, the effect of wetness condition treatment on cumulative infiltration depended on cover and rate but not on the combination of cover and rate. Cumulative infiltration was lower in the wet run than in the dry; this trend was more evident in vegetated treatments than in bare (Figures 7 and 8). Cumulative infiltration for the Chilicotal soil as a function of biosolids rate is presented in Figure 9.

As a wetting front moves into the soil, the hydraulic gradient term approaches unity. In the Stellar soil, bulk density and percent clay increase with depth. Hydraulic

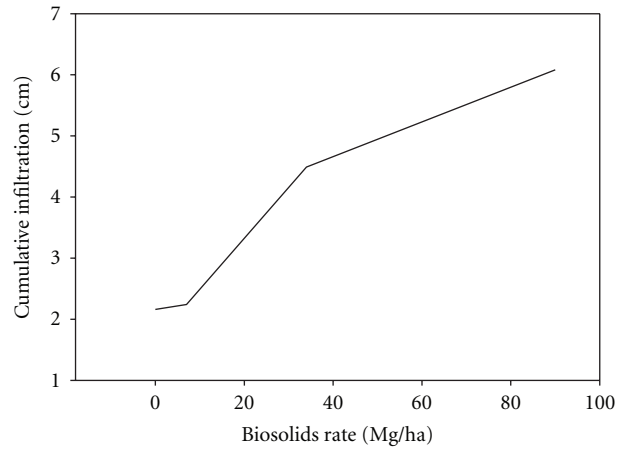

Figure 9: Cumulative infiltration for the Chilicotal soil as a function of biosolids rate on the Sierra Blanca Ranch.

conductivity decreases as density and clay content increase and as structured soils with high clay content swell and close macropores. In crusted soils, the greatest limitation to infiltration may be the crust layer. After infiltration, soil water is redistributed. In a soil that has infiltrated little water, the matric potential of the wetted region following a day of redistribution will be more negative than for a similar soil that infiltrated more water. The vegetated soil treatment had significant infiltration the first day (dry run) and infiltration for wet-run treatments was thus significantly reduced. Infiltration was limited in the bare soil treatment by the crust layer, and thus redistribution had more effect on matric potential in vegetated soils. The matric potential in the wet region of bare soils may be more negative than in vegetated soils because of the effect of redistribution. Therefore, in the bare soil, infiltration in the dry run did not cause as much of a reduction in infiltration in the wet run.

The Green and Ampt [12] "b" constants of infiltration were computed for the Stellar and Chilicotal soils using their respective infiltration rates, final infiltration rates and cumulative infiltration values. In these calculations, the final infiltration rates were assumed to be indicators of the stage III infiltration rates (Table 1). The final infiltration rate for the Stellar soil was assumed to be 0.4 cm hr^{-1} which is the stage III infiltration rate for the bare wet treatment. The final infiltration rate for the Chilicotal soil was assumed to be 3 cm hr^{-1} which is the stage III infiltration rate for this soil.

Conceptually, the b value is small for infiltration rate rapidly reaching the final infiltration rate, i_c. For large b values, the final infiltration rate is reached over a longer period of time. At the zero biosolids application rate, the bare, wet Stellar b values and the Chilicotal soil were both zero. The zero value for b indicates that the final infiltration was also the infiltration rate used to determine b value. A soil without applied biosolids would be expected to have a smaller b value than a soil with large quantities of applied biosolids. This proved to be true for the Stellar bare soils and the Chilicotal soil with low b values on soils not receiving biosolids and high b values for plots receiving high application rates of biosolids. Similarly, a wet soil would be

Table 1: Cumulative infiltration and Green Ampt "b" value for the Stellar soil under two wetness conditions and the Chilicotal soil on the Sierra Blanca Ranch.

Soil conditions	Cumulative infiltration, cm	"b" value
Stellar soil bare wet biosolids Mg ha^{-1}		
0	1.1	0
7	1.2	1.2
34	1.8	2.2
90	2.1	1.9
Stellar soil bare dry biosolids Mg ha^{-1}		
0	2	2.2
7	2.2	2.6
34	3	8.4
90	4	14
Stellar soil vegetated wet biosolids Mg ha^{-1}		
0	3.8	18
7	2.9	7.3
34	4.2	26
90	4.2	22
Stellar soil vegetated dry biosolids Mg ha^{-1}		
0	5.6	46
7	4.8	30
34	5.9	53
90	6.4	61
Chilicotal soil biosolids Mg ha^{-1}		
0	2.2	0
7	2.2	0.22
34	4.5	18
90	6.1	44

anticipated to have a smaller b value than a dry soil. This was shown in Table 1 for both the Stellar bare and vegetated soils. In general, for all of the soil treatments listed in Table 1, the b value increased as the biosolids amounts increased. The b values represent soil surface parameters that are altered by the addition of biosolids. The b values were larger in the vegetated plots compared to the bare plots, because the vegetation "caught" more of the biosolids and water than did the bare plots.

4. Summary and Conclusions

Biosolids, an end product of publicly owned treatment works, have been used as a soil amendment for years. Biosolids are not only a source of plant macro- and micronutrients, but also physically affect the ecosystem. In this study, biosolids surface-applied to soils were evaluated to reveal their influence on infiltration stage. While the magnitude of stage I infiltration is flux controlled, the length of time that water infiltrates at the application rate is controlled by surface conditions. Stage II infiltration is altered also by biosolids surface applications in that the water flux slope from stage I to stage III infiltration is more gradual for long stage I infiltration times. In general, soils with long stage I infiltration periods received the highest biosolids surface application rates. Vegetated Stellar soil plots had longer stage I infiltration times than bare Stellar soils and wetted Stellar soils had longer stage I infiltration times than dry Stellar soils. Biosolids lengthened stage I infiltration times most in dry or gravelly soils, which initially had short stage I infiltration times.

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Research Article

Impact of Indian Mustard (*Brassica juncea*) and Flax (*Linum usitatissimum*) Seed Meal Applications on Soil Carbon, Nitrogen, and Microbial Dynamics

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There is a critical need to investigate how land application of dedicated biofuel oilseed meals affects soil ecosystems. In this study, mustard (*Brassica juncea*) and flax (*Linum usitatissimum*) seed meals and sorghum-sudangrass (*Sorghum bicolor*) were added to soil at levels of 0, 1, 2.5, and 5% (w/w). Both the type of amendment and application rate affected soil organic C, total C & N, and C & N mineralization. Mustard meal amendment initially inhibited C mineralization as compared to flax, but >50% of mustard and flax organic C was mineralized within 51 d. Nitrogen mineralization was similar for flax and mustard, except for the 2.5% rate for which a lower proportion of mustard N was converted to nitrate. The mustard meal greatly impacted microbial community composition, appearing to select for specific fungal populations. The potential varying impacts of different oilseed meals on soil ecosystems should be considered when developing recommendations for land application.

1. Introduction

There is currently great interest in the use of various biofuels to supplement fossil fuel supplies. One potential source of biofuels is the production of biodiesel from oilseed crops. Oilseeds, such as soybeans, have been cultivated for hundreds of years with much of the oilseed meal, or press-cake, remaining after oil extraction being used for food, animal feed, or other industrial purposes. However, the cultivation of additional oilseed varieties, at the scale necessary to provide a significant supplement to worldwide demands for fossil fuels, may saturate existing markets for these oilseed meal coproducts [1]. Additionally, in order to avoid competition between food and fuel supplies, there are growing efforts to focus upon and/or develop nonfood oilseed crops that are dedicated to the production of biofuels and other industrial products [2]. However, many of the seed meals from these non-food, dedicated biofuel crops, such as castor, contain compounds or toxins which limit their use as food or animal feed [3–5].

One alternative use for these oilseed meals is as a soil amendment. Oilseed meals contain substantial amounts of N and varying levels of other nutrients needed for plant growth. Additionally, land application of the oilseed meals may increase levels of soil C and contribute positively to the net C effect of biofuels. Although there has been a substantial amount of research on the use of meals from some oil-producing crops as organic fertilizers, there has been a relatively limited amount of research for many of the dedicated oilseed crops [6–8].

The *Brassicaceae* family includes several oilseed plants, including Indian mustard (*Brassica juncea*), which have potential for use as dedicated oilseed crops. One unique property of many plants in the *Brassicaceae* family is that they contain glucosinolates, which when hydrolyzed are converted into biocidal chemicals such as isothiocyanates, nitriles, and

Table 1: Selected characteristics of Weswood loam soil.

Soil nutrient concentrations													Soil texture			
Total N	Total C	Organic C	P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu	Sand	Silt	Clay	pH
	$g\,kg^{-1}$						$mg\,kg^{-1}$							$g\,kg^{-1}$		
0.5	12.8	5.4	34	225	5440	236	15	210	0.95	0.15	0.61	0.26	420	380	200	7.9

ionic thiocyanates [9, 10]. These compounds have been documented to have broad biocidal effects and suppress a number of soil pathogens, insects, and weeds [11–14]. Although numerous studies have documented the beneficial usage of brassicaceous plants or green tissue as biofumigants, only a limited number of studies have been conducted on use of brassicaceous oilseed meals for this purpose [11, 15–17].

Additionally, although the impacts of isothiocyanates and related compounds on microbial pathogens have been well documented, the impacts of nonpathogenic, soil microorganisms are largely unknown. In the handful of studies that have been conducted on this, it has been reported that isothiocyanates and related compounds may affect bacterial and eukaryotic community structure [18] and inhibit populations and activity of nitrifying bacteria [19]. Since alteration of soil microbial communities may directly impact C mineralization, nutrient cycling, and numerous other aspects of soil quality, additional research is needed to specifically investigate the effects of brassicaceous oilseed meals on soil microbial ecosystems.

In this study, we used seed meals of mustard and flax along with sorghum-sudangrass biomass to represent oilseed meals from the *Brassica* and non-*Brassica* families and a "common" non-oilseed residue, respectively. The major objectives of this study were to compare the effects of application of a brassicaceous oilseed meal, nonbrassicaceous oilseed meal, and lignocellulosic biomass on (1) soil C and N dynamics, including organic, mineralizable, and total fractions of C and N and (2) soil microbial communities including populations of nitrifying bacteria.

2. Materials and Methods

2.1. Soil Collection and Characterization. Weswood loam (fine-silty, mixed, superactive, thermic, Udifluventic Haplustept) was used in this study (Table 1). It is an alluvial soil in the flood plain of the Brazos River in South Central Texas. Weswood soils are well-drained loamy soils and are used as irrigated cropland [20]. Bulk soil samples were collected from 0–15 cm depth and composited. Soils were kept at field moisture and then homogenized and passed through a 2-mm sieve. Soils were tested for total C, organic C, and total N by a combustion method using an Elementar Vario Max CN analyzer (Elementar Analysensysteme, Hanau, Germany) [21–23]. Organic C was determined at 650°C while total C was determined at 950°C. Soil phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), and sodium (Na) were extracted with Mehlich III solution and analyzed by inductively coupled plasma (ICP) spectrometry [24, 25]. Soil micronutrients including copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) were extracted using a 0.005 M DTPA, 0.01 M $CaCl_2$, and 0.1 M triethanolamine solution mixture and determined by ICP [26]. Soil particle size distribution was determined using the hydrometer method [27]. Soil pH was measured using a pH meter in a soil water suspension (10 g soil to 20 mL deionized water) after shaking 30 min at 180 rev min^{-1} on a reciprocal shaker followed by standing for 30 min [28].

2.2. Oilseed Meal and Sorghum-Sudangrass Analysis. Mustard (*Brassica juncea*) seed meal was obtained from the *Brassica* Breeding and Research group at the University of Idaho, USA. Flax (*Linum usitatissimum*) seed meal was made by processing seeds, collected from the Texas AgriLife Research Farm in Burleson County, Tex, USA with a Komet Oil Press (Model CA59, IBG Monforts Oekotec, Mönchengladbach, Germany). Sorghum-sudangrass (*Sorghum bicolor*) biomass was collected from the Texas AgriLife Research Farm in Burleson County, Tex, USA. Seed meals were ground with a mortar and pestle and passed through a 1-mm sieve. Sorghum-sudangrass was ground with a Wiley Mill no. 4 (20 mesh screen). Organic C, total C, and total N in the biomass amendments were determined by a high temperature combustion process using an Elementar Vario Max CN analyzer (Elementar Analysensysteme) [21, 29–31]. Organic C was determined at 650°C while total C was determined at 950°C. Plant B, Ca, Cu, Fe, K, Mg, Na, P, S, and Zn were determined using a nitric acid digestion and ICP analysis [32, 33]. Residual oil content in seed meals was determined by a Minispec mq-one nuclear magnetic resonance unit (Bruker Optics Inc., Billerica, Mass, USA) fitted with a 40-mm fuse with hydrogen probe.

Glucosinolate concentrations in seed meals and biomass were determined following the International Organization for Standardization methods [34] but with a few modifications as detailed below. Samples were first defatted with one extraction and two rinses of petroleum ether by vacuum filtration. Defatted material was weighed (300 mg) into 50 mL centrifuge tubes to which 500 mg of 5-mm glass beads [35] were added and then immediately vortexed. A hot (70°C) 70% methanol : H_2O solution (10 mL) was added to the samples that were then placed in a hot water bath for 20 minutes and vortexed intermittently. After which, the samples were centrifuged, and the supernatant was collected. An additional extraction was performed similar to above but with 5 mL of hot methanol rather than 10 mL. The extracts were combined, and 2 mL were added to a 0.6 mL plug of DEAE Sephadex A-25 anion exchanger and allowed to drain freely. The poly-prep chromatography columns (BioRad, Hercules, Calif, USA) were then rinsed with 1 mL deionized water and finally with two aliquots of 1 mL 0.02 M sodium acetate buffer (pH 4.5). Sulfatase solution (100 μL) was

added to the columns and allowed to sit overnight (16 hrs). Desulfoglucosinolates were eluted with 3 consecutive 1 mL volumes of deionized water. Samples were immediately separated and quantified by HPLC using a Waters 600s System Controller, 717 autosampler, and 996 photodiode array detector. The system was equipped with a Waters 3.5 μm Symmetry Shied RP8 column (2.1 × 150 mm), in which mobile phases flowed at 0.3 mL/min, and compounds were separated using an acetonitrile gradient starting at 2.0% and increasing to 95.0%. Expected retention behavior, such as time and sequence, and mass spectra were used to identify glucosinolate peaks. A calibration curve was constructed using sinigrin monohydrate (Science Lab, Houston, Tex, USA) as an external standard.

2.3. Experimental Design. This was a laboratory study using a completely randomized design (CRD) with factorial treatment structure. The two treatment factors were application rate and type of oilseed meals and residue. Application rates were 0.5, 1, 2.5, and 5% (w/w) (field equivalent of ∼9, 18, 45, and 90 Mg ha^{-1}, resp.), and added materials included mustard seed meal, flax seed meal, and sorghum-sudangrass biomass. The sorghum-sudangrass treatment was included as a lignocellulosic biomass for comparison with the oilseed meals. Each treatment had 3 replications. Blanks without meal applications were also used. In total, there were $4 \times 3 \times 3 + 3 = 39$ experimental units, which were randomly assigned in a 25°C incubator. Two separate incubations were conducted. A microcosm study (using 400 g of soil per microcosm) was used to determine changes in organic C, total C, total N, and microbial communities over time. A second, smaller scale (50 g of soil per microcosm) was used to determine C and N mineralization rates and effects on populations of nitrifying bacteria.

2.4. Organic C, Total C, and Total N Dynamics. Each microcosm (a 1-liter glass jar) contained 400 g of soil adjusted to 13% (w/w) water content (∼40% field capacity). The various seed meal and biomass amendments were added to each microcosm and thoroughly mixed. Jars were incubated with covers loosely sealed to maintain aerobic conditions at 25°C in a controlled environment incubator. Soil moisture content was adjusted weekly by adding deionized water to the desired level according to weight. Soil was sampled from each microcosm at intervals of 3, 14, 28, and 133 d. A portion of each sample was tested for pH, and another was air-dried, ground to pass a 150-μm sieve, and analyzed for organic C, total C, and total N as described above. An additional portion of the sample was immediately frozen and stored at −80°C for subsequent microbial analyses.

2.5. Soil C and N Mineralization. Each microcosm (a 1-liter glass jar) contained 50 g of soil adjusted to 13% water content (w/w) along with a beaker containing 10 mL of 1.0 N NaOH. Jars were then tightly sealed and were incubated for a total duration of 51 d. Carbon mineralization rates were determined at intervals of 2, 5, 10, 16, 23, 37, 44, and 51 d using acid titration to quantify CO_2 evolution. After each sampling, a new beaker with fresh 1 N NaOH solution was placed into the jar. At the completion of the incubation (51 d), a portion of the sample was immediately frozen and stored at −80°C for subsequent microbial analyses. Another portion of the soil was air-dried, processed, and analyzed for organic C, total C, and total N as described above. In addition, samples were extracted with 1 M KCl (1 : 2) and analyzed for NH_4-N colorimetrically by spectrophotometer [36] and with 1 M KCl (1 : 10) for NO_3-N by reduction to nitrite using a cadmium column followed by determination of nitrite concentrations with a spectrophotometer [37].

2.6. Microbial Community Composition and Dynamics. The impacts of biomass additions on soil microbial communities were determined by total lipid-fatty acid methyl ester analysis (TL-FAME) using a hybrid lipid extraction protocol as described by Ushio et al. [38]. Briefly, samples collected 3 and 28 d after soil amendment in the C and N dynamics experiment were freeze-dried and shipped to the Ecosystem Microbiology Laboratory at the University of Wisconsin Wis, USA for lipid extraction and identification using the Microbial ID method for FAME (MIDI, Inc., Newark, Del, USA). Detected lipids containing between 10 and 20 carbons in length were used for subsequent multivariate analysis. The indicator lipids used for quantification of specific microbial groups were (1) fungi: 18 : 1ω9c, 18 : 2ω6c {MIDI Sum in Feature 19}, 18 : 3ω6c, (2) Gram+ bacteria: 15 : 0, *a*15 : 0, *i*15 : 0, *i*16 : 0, 17 : 0, *a*17 : 0, *i*17 : 0, and (3) Gram− bacteria: 16 : 1ω7c, *cy*17 : 0, *cy*19 : 0, 16 : 0 2OH, 16 : 1 2OH, 18 : 1ω7c {MIDI Sum in Feature 8} [38–41].

In addition, effects of biomass additions on populations of nitrifying bacteria were determined in the C and N mineralization experiment using a quantitative-PCR- (qPCR-) based approach. Approximately 1 g of soil from each sample was extracted using a PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, Calif, USA) according to the manufacturer's instructions. The DNA extracts were purified using illustra MicroSpin S-400 HR columns (GE Healthcare Bio-Sciences Corp, Piscataway, NJ, USA) and were quantified using a Quant-iT PicoGreen dsDNA assay kit (Invitrogen Corp, Carlsbad, Calif, USA).

Population levels of nitrifying bacteria were determined using primers targeting the *amoA* gene [42]. The assays were performed in a 10-μL reaction mix containing 4.5 μL SYBR green real master mix (5Prime, Inc., Gaithersburg, Md, USA), 0.5 μL of each primer (200 nM), 1 μL template (2.5 ng), 1 μL bovine serum albumin (10 mg mL^{-1}), and 2.5 μL molecular-grade water (DNase-free). The qPCR conditions included an initial 10 min at 95°C followed by 40 amplification cycles of 94°C for 60 s, 60°C for 60 s, and 72°C for 60 s. Each run in the analysis included a set of standards, positive and negative controls, and samples (all including three analytical replicates) on a 96-well plate. Melting curve analysis of the qPCR products was performed after each assay to confirm qPCR amplification quality. The qPCR was performed using an Eppendorf Mastercycler ep realplex thermal cycler (Eppendorf, Hamburg, Germany).

Standards for qPCR were generated by PCR amplifying the *amoA* gene from the genomic DNA of *Nitrosomonas*

Table 2: Nutrient concentrations of flax and mustard oilseed meals and sorghum-sudangrass biomass.

Biomass type	C	N	C : N	Oil[a]	P	K	Ca	Mg	Na	S	Zn	Fe	Cu	Mn	B
	$g\,kg^{-1}$						$g\,kg^{-1}$						$mg\,kg^{-1}$		
Flax	493	59	8.4	157	6.6	19	3.1	5.4	1.3	3.3	44	32	15	46	30
Mustard	478	61	7.8	162	11.0	19	4.9	5.6	1.3	16.0	62	44	12	47	16
Sorghum	430	18	23.9	nd[b]	1.2	20	7.1	1.1	1.3	1.9	47	303	9	56	<9

[a]Residual oil content in seed meals following oil extraction.
[b]nd: not determined.

europaea [43]. The PCR products were confirmed on an agarose gel and then cloned into a pGEM-T Easy vector following the manufacturer's instructions (Promega, Madison, Wis, USA). Positive clones were isolated and extracted for plasmid DNA using a Wizard SV Miniprep kit (Promega). Plasmid DNA concentrations were quantified using the Quant-iT PicoGreen dsDNA assay kit (Invitrogen) and were used for preparing appropriate dilution standards for the qPCR assays. The plasmid DNA concentrations ranging from 5.0×10^{-3} to 5.0×10^{-7} ng μL^{-1} DNA were used to generate the qPCR standard curves and to quantify the absolute *amoA* gene copy numbers per gram of soil.

2.7. Statistical Analyses. Statistical analyses were conducted using the R statistical programming language v2.10.1 [44] and the Rcmdr package v1.6.0 [45]. Analysis of variance (ANOVA) was used to evaluate the effects of biomass amendment, rate, and/or time on soil and microbial community characteristics. Two-way and repeated measures ANOVA were used, where appropriate, and in cases where interactive effects were significant, the data sets were broken down into simpler subsets and analyzed using one-way ANOVA. Where possible, post hoc evaluations of means were conducted using Tukey tests. Data that did not meet assumptions of normality or homogeneity of variance were log-transformed prior to analysis. All results are presented as nontransformed values, and results were considered to be statistically significant when $P \leq 0.05$. Additionally, nonmetric multidimensional scaling of the soil microbial communities, based upon FAME composition, was carried out using the Bray-Curtis similarity metric in the PAST software package, version 2.03 [46].

3. Results

3.1. Soil Characteristics. Weswood soils were slightly alkaline with a pH of 7.9 (Table 1). Soil total C was 12.8 g kg^{-1}, of which 5.4 g kg^{-1} was organic C. Soils also contained various concentrations of extractable macro- and micronutrients. Phosphorus was in the moderate category, while K, Ca, Mg, and S were rated as high in availability according to Texas AgriLife Extension Guidelines. Iron, Zn, Mn, and Cu were low.

3.2. Composition of Seed Meals and Sorghum-Sudangrass Biomass. A major difference between oilseed meals and sorghum-sudangrass biomass was their N concentrations and hence C : N ratios (Table 2). The mustard seed meal used in this experiment contained 478 g kg^{-1} C and 61 g kg^{-1} N with a C/N ratio of 7.8. Similarly, flax seed meals had 493 g kg^{-1} C and 59 g kg^{-1} N with a C/N ratio of 8.4. Sorghum-sudangrass had a much lower N concentration (18 g kg^{-1}) and a much higher C/N ratio (24). Mustard seed meal contained about 5 times as much S as flax seed meal and more than 8 times that of sorghum-sudangrass. Both seed meals also had much higher concentrations of P, Mg, and B, while sorghum-sudangrass had substantially more Fe. Concentrations of other elements, such as K, Na, Zn, and Mn were comparable in all three amendments. Residual oil contents were similar in mustard and flax seed meals, both being about 160 g kg^{-1}. The mustard seed meal contained several glucosinolate-related compounds with the dominant being 2-propenyl glucosinolate (sinigrin) at a concentration of 157.9 ($\pm$15.1) μmol g^{-1}. Neither the flax seed meal nor the sorghum-sudangrass residue contained detectable levels of glucosinolates.

3.3. Organic C, Total C, and Total N Dynamics. A significant portion of the added oilseed meal organic C was degraded rapidly following addition to soil, with organic C losses slowing after few weeks (Figure 1). For example, about 21, 21, 33, and 37% of organic C in the 0.5, 1, 2.5, and 5% flax treatments, respectively, were lost within the first two weeks. After 4 months of incubation, approximately 48, 57, 67, and 72% of added organic C had been removed in the 0.5, 1, 2.5, and 5% flax treatments resulting in soil organic C values of 6.7, 7.5, 9.5, and 12.3 g kg^{-1}, respectively. Degradation of organic C was similar in the mustard-amended treatments with approximately 30, 61, 70, and 79% of added organic C removed after 4 months in the 0.5, 1, 2.5, and 5% mustard treatments, respectively. The loss of organic C in sorghum-sudangrass treatments was smaller relative to the seed meal treatments. About 4, 18, 14, and 17% of added organic C in the 0.5, 1, 2.5, and 5% sorghum-sudangrass treatments, respectively, was lost within two weeks. By the end of the 4-month incubation, only 21 to 44% of added organic C had been degraded resulting in soil organic C levels of 6.9, 8.8, 11.6, and 17.5 g kg^{-1} in the 0.5, 1, 2.5, and 5% sorghum-sudangrass treatments, respectively, which were greater than their oilseed treatment counterparts. There were no significant changes among the control samples throughout the incubation. Soil total C dynamics, which included organic and carbonate C, followed a pattern similar to organic C (Figure 2).

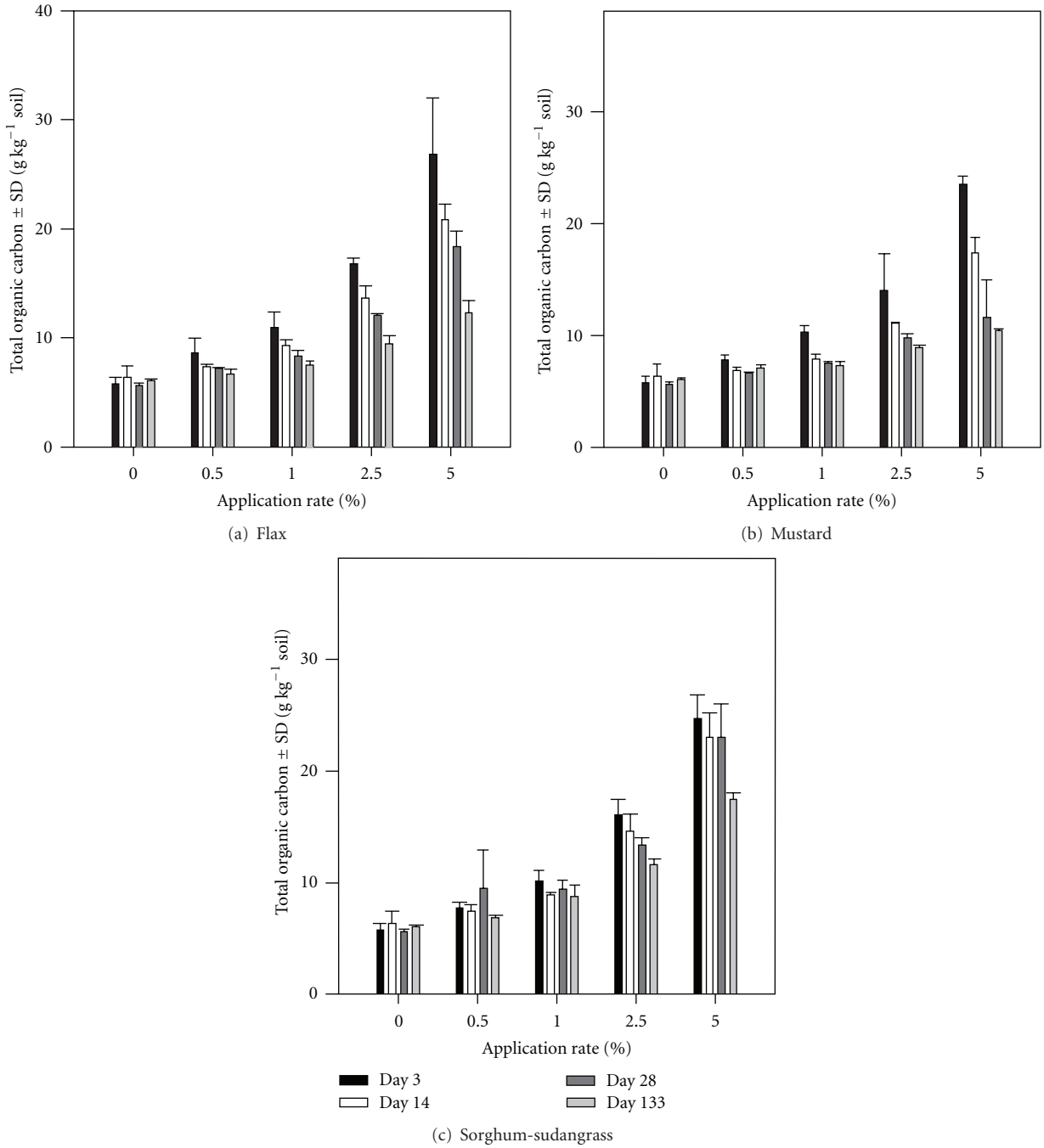

Figure 1: Soil organic C dynamics as affected by added oilseed meals and sorghum-sudangrass biomass after 3, 14, 28, and 133 d of addition. Means are based on 3 replications. Bars represent ± standard deviation of the mean.

Biomass addition significantly enhanced soil total N levels with flax and mustard seed meals having larger effects than the sorghum-sudangrass biomass (Figure 3). In contrast to the soil C dynamics, soil total N did not exhibit significant changes over the incubation period.

Application of oilseed meals and sorghum-sudangrass had different impacts on soil pH. The initial soil pH was 7.9. After 4 months of incubation, soil pH decreased to 6.8 and 6.9 with 5% mustard and flax seed meal additions, respectively. The pH reduction was less in treatments receiving 0.5 and 1% amendments, usually within 0.5 unit of the original soil pH. There was no change in pH change among sorghum-sudangrass treatments.

3.4. Carbon Mineralization. Both the type of biomass amendment and application rate had significant effects on soil C mineralization rates (Figure 4). Regardless of amendment type, C mineralization increased with increasing application.

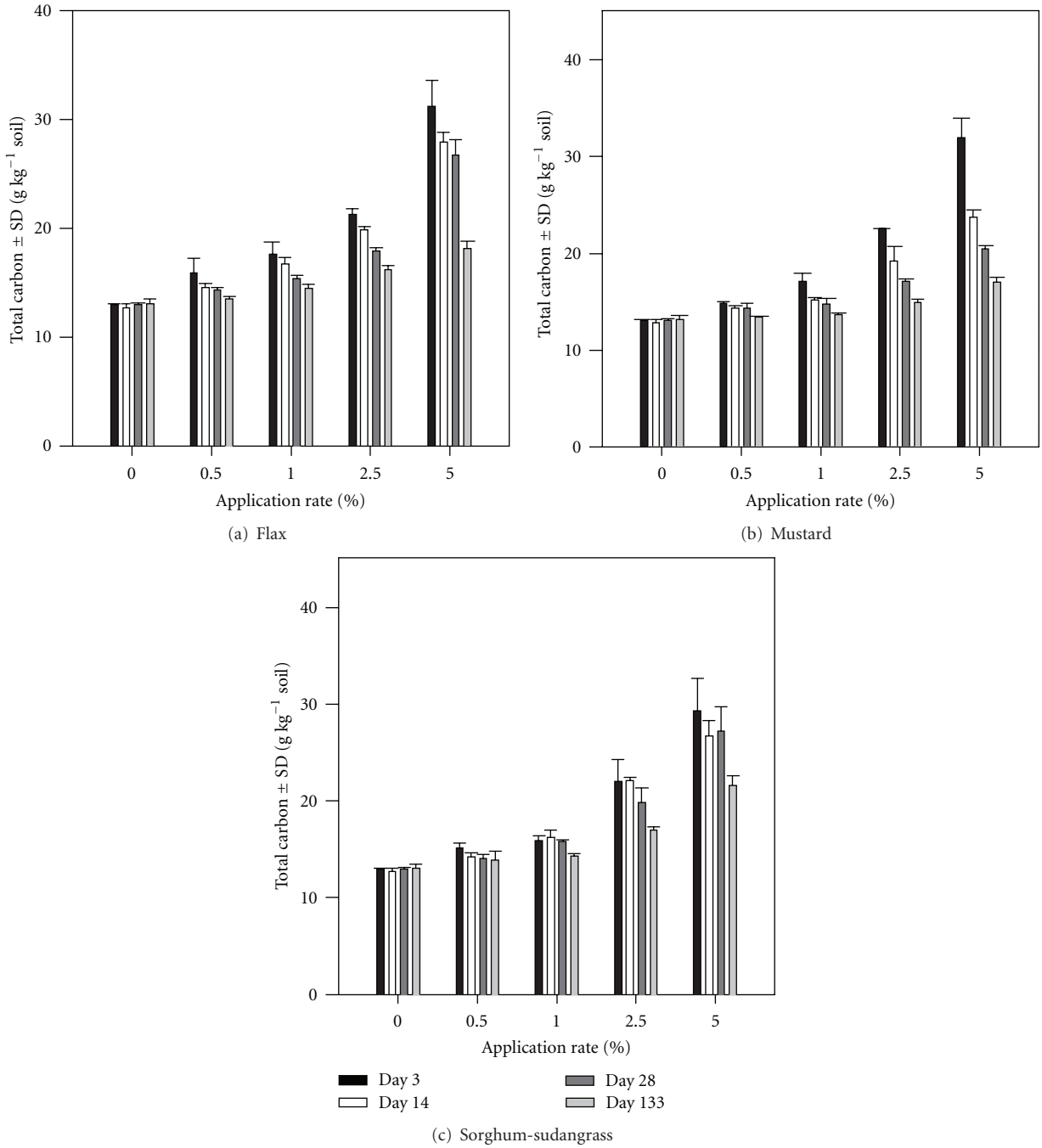

Figure 2: Soil total C dynamics as affected by added oilseed meals and sorghum-sudangrass biomass after 3, 14, 28, and 133 d of addition. Means are based on 3 replications. Bars represent ± standard deviation of the mean.

Interestingly, flax and mustard seed meals and sorghum-sudangrass biomass each had distinct C mineralization patterns over the incubation period. For flax, the CO_2 flux peaked shortly (~2–5 d) after flax seed meal addition and was followed by a rapid decrease. By the end of the third week, the CO_2 flux had generally stabilized, except that addition treatments of 2.5 and 5% were greater than controls, 0.5, and 1% rates. Similarly for sorghum-sudangrass, soil CO_2 flux showed a very sharp initial increase following the addition of biomass, decreased precipitously within 2 weeks, and thereafter maintained a relatively stable rate which was still higher than the controls, especially at higher application rates. In contrast, the soil CO_2 peak flux following addition of mustard did not occur until around 10 d of incubation for most addition rates and was followed by a more gradual decrease to levels approaching the unamended soil. Data for the 5% amendment rate for all biomass types is not shown due to several early (2–5 d) CO_2 flux values that exceeded

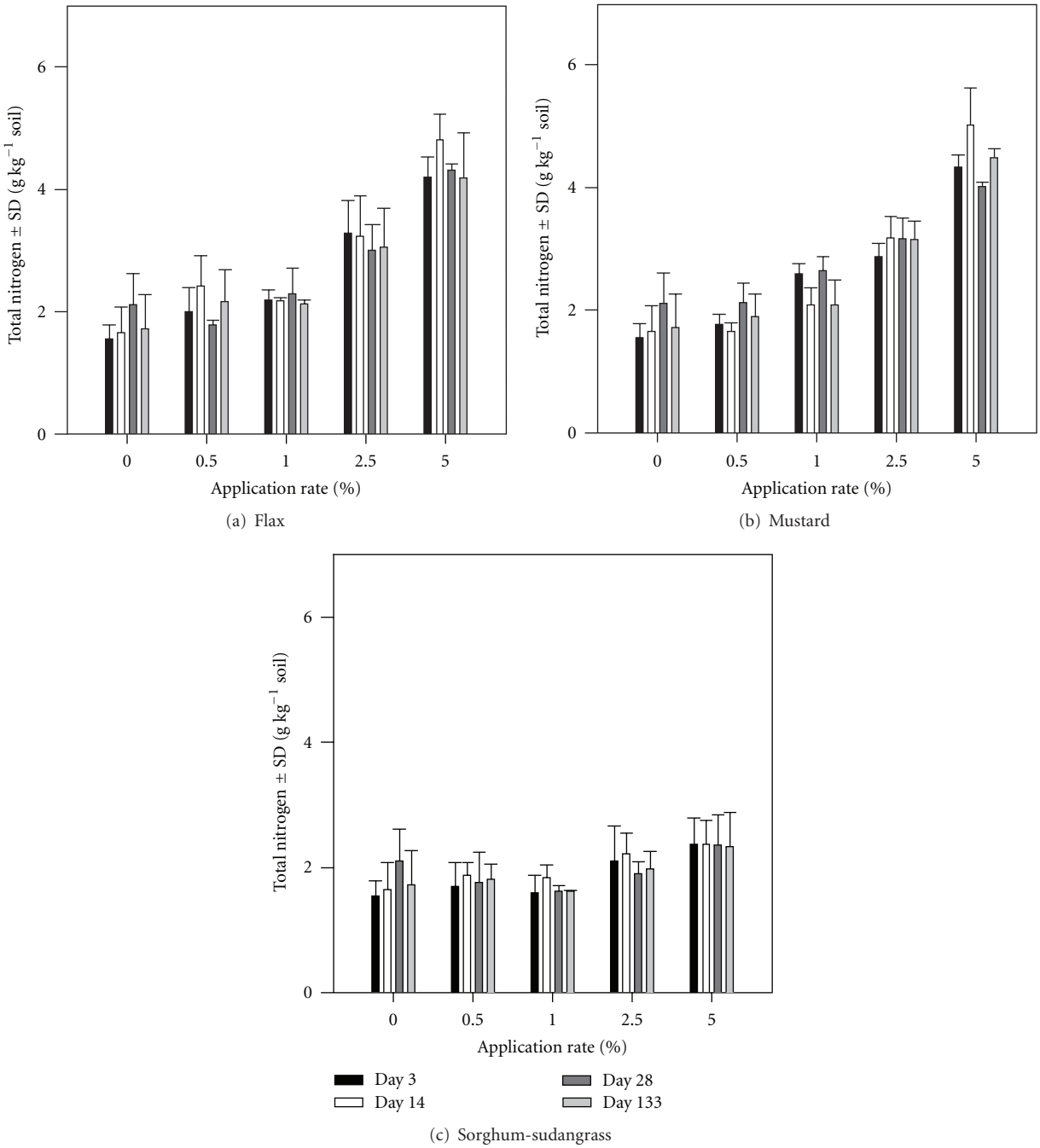

Figure 3: Soil total N dynamics as affected by added oilseed meals and sorghum-sudangrass biomass after 3, 14, 28, and 133 d of addition. Means are based on 3 replications. Bars represent ± standard deviation of the mean.

detection limits; however, the 5% amendment results generally trended with those for the 2.5% amendment rate.

Cumulative C mineralization and removal of organic C during the 51 d of incubation followed the order of mustard ≈ flax > sorghum-sudangrass (Table 3). After 51 d, approximately 25 to 53% of added organic C remained in the mustard- and flax-amended soil in contrast to the sorghum-sudangrass-amended soil for which 60 to 71% or the added organic C remained.

3.5. Nitrogen Mineralization. Similar to the C mineralization results, treatments receiving flax and mustard meals resulted in much higher amounts of mineralized N than treatments receiving sorghum-sudangrass biomass (Table 3). After application of oilseed meals, the levels of soil inorganic N increased dramatically in proportion to application rate, reaching maximum levels of 705 and 857 mg kg^{-1} at the 5% amendment rate. In contrast, soil inorganic N levels were much lower in the sorghum-sudangrass-amended soils

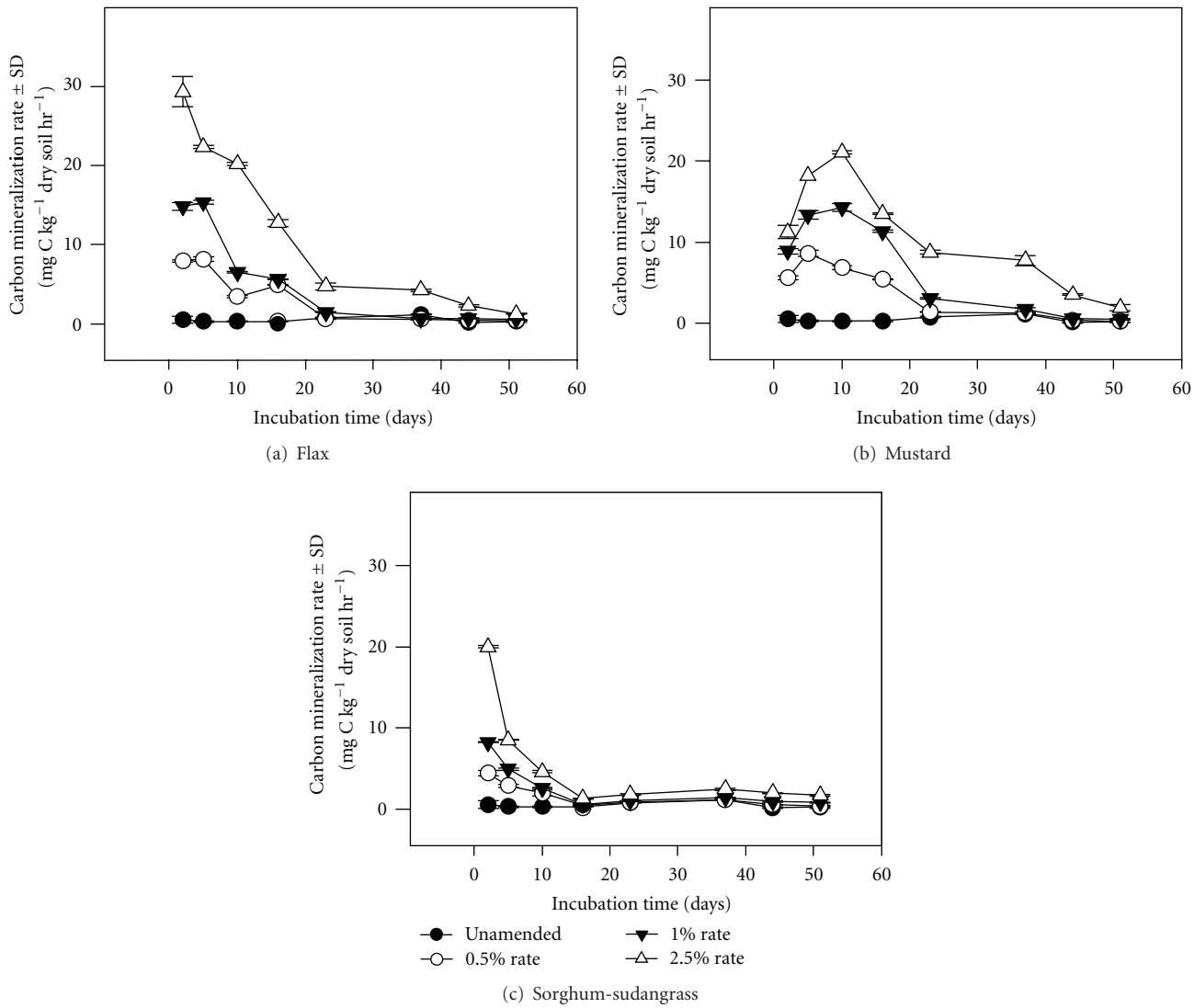

Figure 4: Soil C mineralization dynamics as affected by added oilseed meals and sorghum-sudangrass biomass with time. (•: unamended; ◯: 0.5%; ▼: 1%; △: 2.5% application rate). Means are based on 3 replications. Bars represent ± standard deviation of the mean. Error bars are hidden when smaller than the symbols.

(7.8 to 26.6 mg kg^{-1}) and tended to decrease at higher application rates. Approximately 14–30% of total N in the mustard- and flax-amended soils was inorganic N after 51 d in comparison to only 4.4% in the unamended soil and 0.5 to 3.2% in the sorghum-sudangrass-amended soil.

The amount of the soil inorganic N existing as NH_4-N or NO_3-N varied depending upon both the rate and type of biomass amendment. For the sorghum-sudangrass treatments, the majority of mineralized N at all amendment rates was NH_4-N (Table 3). In contrast, for flax and mustard treatments, the primary form (>90%) of inorganic N at the end of incubation for the lower rates of amendment (0.5 and 1%) was NO_3-N. At the highest amendment rate (5%), essentially all (>99%) of the inorganic soil N was NH_4-N. For the mustard and flax oilseed meals, the levels of soil NH_4-N and NO_3-N were similar for most application rates except for the 2.5% application rate for which mustard resulted in much higher levels of NH_4-N (297 v. 24 mg kg^{-1}) and lower levels NO_3-N (243 v. 601 mg kg^{-1}) than did the flax treatments.

3.6. Microbial Community Composition and Dynamics. Both the type and rate of biomass addition affected the abundance of specific microbial groups and the overall composition of the soil microbial community. Within 3 d, levels of soil microbial indicator lipids had increased approximately 2- to 10-fold in amended soils (Table 4). At each application rate, the increase was greatest in the mustard-amended soils. As illustrated by the fungal : bacterial ratios, this was largely a result of increased fungal biomass in the mustard-amended soils in contrast to the flax and sorghum-sudangrass where there was less of a fungal impact. Generally, after 28 d,

Table 3: Soil carbon and nitrogen concentrations 51 d after amendment with various rates of flax or mustard oilseed meals or sorghum-sudangrass biomass.

Amendment rate (%)	Biomass type	Soil nutrient concentrations					
		Total C ($g\,kg^{-1}$ soil)	Organic C ($g\,kg^{-1}$ soil)	Added organic C remaining (%)	Total N ($g\,kg^{-1}$ soil)	NH_4-N ($mg\,kg^{-1}$ soil)	NO_3-N ($mg\,kg^{-1}$ soil)
0.5	Unamended	12.95^{a}	5.77^{a}	—	0.90^{a}	2.33^{a}	37.67^{a}
	Flax	13.84^{b}	6.57^{b}	45.68^{a}	1.06^{b}	6.93^{ab}	147.20^{b}
	Mustard	13.33^{ab}	6.70^{b}	53.36^{a}	1.13^{b}	10.82^{ab}	161.83^{b}
	Sorghum-sudangrass	13.60^{ab}	6.96^{b}	71.13^{a}	0.82^{a}	16.82^{b}	9.73^{c}
1.0	Unamended	12.95^{a}	5.77^{a}	—	0.90^{a}	2.33^{a}	37.67^{a}
	Flax	14.42^{ab}	7.76^{bc}	47.12^{ab}	1.33^{b}	11.26^{b}	239.17^{b}
	Mustard	13.68^{a}	7.04^{b}	33.71^{a}	1.36^{b}	13.68^{b}	278.27^{b}
	Sorghum-sudangrass	16.52^{b}	8.00^{c}	60.20^{b}	1.15^{ab}	23.02^{c}	3.50^{c}
2.5	Unamended	12.95^{a}	5.77^{a}	—	0.90^{a}	2.33^{a}	37.67^{a}
	Flax	17.22^{b}	9.76^{b}	35.18^{a}	2.07^{b}	23.66^{b}	601.17^{b}
	Mustard	15.34^{c}	8.42^{c}	25.26^{a}	1.90^{b}	296.58^{c}	243.30^{c}
	Sorghum-sudangrass	19.24^{d}	21.60^{d}	67.44^{b}	1.16^{c}	5.52^{a}	3.03^{d}
5.0	Unamended	12.95^{a}	5.77^{a}	—	0.90^{a}	2.33^{a}	37.67^{a}
	Flax	23.30^{b}	16.57^{bc}	45.36^{a}	2.72^{b}	701.66^{b}	2.97^{b}
	Mustard	22.25^{b}	14.99^{b}	40.52^{a}	2.98^{b}	853.64^{b}	3.73^{b}
	Sorghum-sudangrass	25.18^{b}	19.34^{c}	65.45^{b}	1.46^{a}	4.93^{c}	2.83^{b}

Means within a column, for a given amendment rate (including the unamended control), followed by the same letter(s) are not significantly different at $P < 0.05$.

total levels of microbial indicator lipids remained higher in amended soils, but the fungal : bacterial ratios were not significantly different from the unamended soil.

All of the biomass types impacted the overall soil microbial community composition within 3 d of amendment (Figure 5). However, the mustard and sorghum-sudangrass had the greatest impacts. Interestingly, the microbial community in the mustard-amended soil was much more similar to the sorghum-sudangrass-amended soil than that of the flax-amended soil. After 28 d, all of the amended treatments remained different than the unamended soil; however, they were generally more similar to the unamended soil than they were after 3 d.

Likewise, both the rate and type of biomass amendment impacted levels of nitrifying bacteria in the C & N mineralization experiment. After 51 d, application of oilseed meals increased the levels of nitrifying bacteria between 7- and 74-fold, at the 0.5, 1, and 2.5% amendment rates, as compared to the unamended soil (Figure 6). However, neither flax nor mustard oilseed meal increased populations of nitrifying bacteria at the 5% amendment rate. Levels of nitrifying bacteria for sorghum-sudangrass-amended soil, at all application rates, were not different from the unamended control.

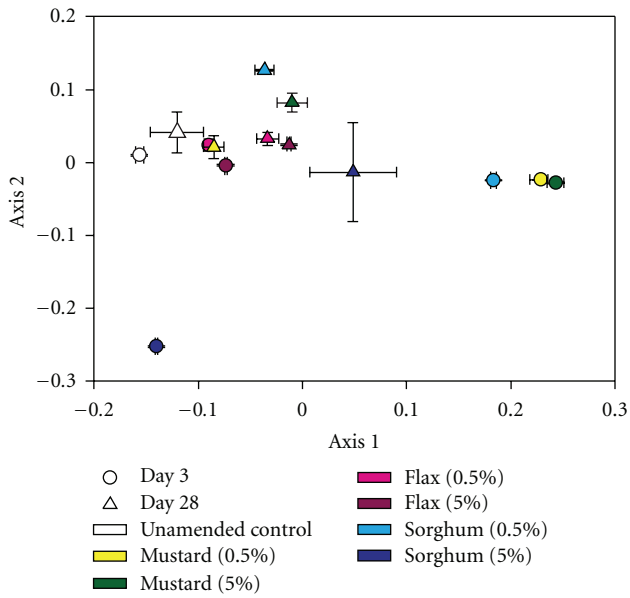

Figure 5: Nonmetric multidimensional scaling analysis of soil microbial communities, based upon total lipid-fatty acid methyl ester profiles, 3 and 28 d after amendment of soil with 0.5 or 5% rates of flax or mustard oilseed meals or sorghum-sudangrass biomass. Means are based on 3 replications. Bars represent ± standard error of the mean.

4. Discussion

4.1. Soil C Dynamics. Carbon added with the oilseed meals and sorghum-sudangrass exhibited different mineralization patterns in soil. Organic matter in the oilseed meals showed extensive mineralization, particularly shortly after application. Carbon mineralization, however, was greatly reduced

Table 4: Microbial lipid biomarkers in soil 3 and 28 days after amendment with various rates of flax or mustard seed meal or sorghum-sudangrass biomass.

Time (days)	Amendment rate (%)	Biomass type	TL-FAME[1] markers (nmol g^{-1} soil)					
			Total bacteria	Gram + bacteria	Gram − bacteria	Fungi	Total[2]	Fungal : bacterial ratio
3	0	Unamended	15.86^{a}	7.64^{a}	8.22^{a}	9.30^{a}	25.16^{a}	0.59^{a}
	0.5	Flax	30.21^{bc}	15.54^{b}	14.68^{a}	23.19^{b}	53.40^{b}	0.77^{a}
	0.5	Mustard	25.83^{b}	18.29^{b}	7.54^{b}	113.36^{c}	139.20^{c}	4.44^{b}
	0.5	Sorghum-sudangrass	33.69^{c}	25.02^{c}	8.67^{a}	86.84^{d}	120.53^{d}	2.57^{c}
3	0	Unamended	15.86^{a}	7.64^{a}	8.22^{a}	9.30^{a}	25.16^{a}	0.59^{a}
	5.0	Flax	57.80^{b}	35.54^{b}	22.25^{b}	40.95^{b}	98.74^{b}	0.71^{b}
	5.0	Mustard	42.82^{c}	24.00^{c}	18.82^{bc}	206.10^{c}	248.91^{c}	4.80^{c}
	5.0	Sorghum-sudangrass	56.37^{b}	42.79^{b}	13.58^{ac}	2.31^{d}	58.69^{d}	0.04^{d}
28	0	Unamended	16.66^{a}	8.21^{a}	8.44^{a}	16.09^{a}	32.75^{a}	0.97^{a}
	0.5	Flax	34.45^{b}	18.05^{b}	16.40^{b}	41.92^{ab}	76.38^{ab}	1.21^{ab}
	0.5	Mustard	45.19^{b}	25.96^{c}	19.23^{b}	34.42^{ab}	79.61^{ab}	0.76^{a}
	0.5	Sorghum-sudangrass	33.74^{b}	22.42^{bc}	11.32^{a}	73.04^{b}	106.78^{b}	2.51^{b}
28	0	Unamended	16.66^{a}	8.21^{a}	8.44^{a}	16.09^{a}	32.75^{a}	0.97^{a}
	5.0	Flax	83.66^{bc}	49.14^{b}	34.52^{b}	99.58^{a}	183.24^{b}	1.19^{a}
	5.0	Mustard	120.34^{b}	97.62^{c}	22.72^{ab}	202.59^{b}	322.92^{c}	1.69^{a}
	5.0	Sorghum-sudangrass	81.67^{c}	59.58^{b}	22.10^{ab}	103.26^{a}	184.93^{b}	1.34^{a}

Means within a column, for a specific amendment rate (including the unamended control) and day, followed by the same letter(s) are not significantly different at $P < 0.05$.
[1]TL-FAME: total lipid-fatty acid methyl ester analysis.
[2]Total TL-FAME marker represents the sum of Gram-positive bacteria, Gram-negative bacteria, and fungi for a given community.

after 2 to 3 wks. The C compounds were apparently more labile in the oilseed meals than the sorghum-sudangrass since the application of the oilseed meals resulted in more rapid and greater cumulative C mineralization. This is not surprising since typical crop residues, such as sorghum-sudangrass, contain large amounts of lignin and lower amounts of protein [47], while oilseed meals usually contain relatively large amounts of protein and fatty acids but less than 20% fiber [48]. The lower C : N ratios in oilseed meals also likely helped to maintain higher C mineralization rates relative to the sorghum-sudangrass.

In addition to differences in the rate and extent of C mineralization for the oilseed meals as compared to the sorghum-sudangrass biomass, there were also differences between the mustard and flax oilseed meals. For flax, the C mineralization peaked very quickly (within 2 d) while it was delayed until around 10 d in the mustard-amended soil. In addition, the initial CO_2 flux from 5% mustard seed meal addition was smaller than that from 1 and 2.5% additions. These results are consistent with those of Snyder et al. [8] who found that a 2% amendment of mustard seed meal (*B. juncea*) delayed the peak and magnitude of C mineralization as compared to the same amount of rapeseed (*B. napus*) containing a much lower glucosinolate content. Thus, in both studies, this initial reduction in C mineralization was likely due to the high level of glucosinolate compounds in the mustard [10, 49]. However, these secondary chemicals are susceptible to microbial degradation and have

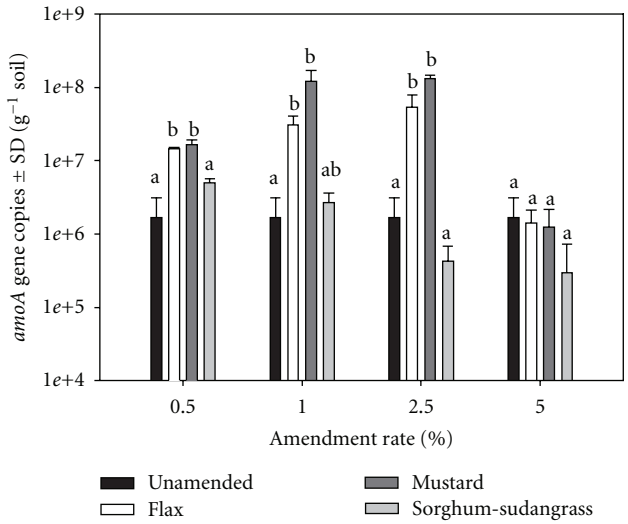

Figure 6: Populations of nitrifying bacteria (expressed as copies of *amoA*) 51 d after amendment of soil with flax or mustard oilseed meals or sorghum-sudangrass biomass. Means are based on 3 replications. Bars represent ± standard deviation of the mean. Means with different letters for the same application rate are significantly different ($P < 0.05$).

a relatively short half-life once applied to soils, often being degraded within two weeks [18, 50, 51]. This is consistent with our results in that the CO_2 flux peaked after the likely

degradation of the biocidal compounds in mustard treatments. It is also possible that after the initial effect, the soil microbial communities adapted to the chemicals and selected for microorganisms that were more resistant to the biocidal compounds which then flourished to produce the peak in CO_2 flux. Although C mineralization in the mustard-amended soil was delayed at the beginning of the experiment, by the end of the experiment the cumulative C mineralization was equal to that in the flax-amended soil.

At the end of the 4-month incubation, soil total C increased from 12.8 to 14.5 g kg^{-1} in the 1% flax meal treatment, amounting to an addition of about 5×10^3 kg of C ha^{-1} on a field basis (assuming the same mineralization rate, soil bulk density of 1.2 g cm^{-3}, and biomass applied to a depth of 0–15 cm). This result has two implications. First, once land-applied, not all of the C contained in the meals can be counted as sequestered C. A large portion, over half in this study, is likely to be mineralized quickly and released into the atmosphere. Second, the remaining C, however, may have the potential for temporary or longer-term C sequestration thus enhancing the "C neutrality" of any resulting biofuels.

The reduction in soil pH for oilseed meal-amended treatments presumably occurred due to the production of organic and/or inorganic acids during organic matter degradation. Although this could be a concern if applying high rates of oilseed meals to acidic soils, application rates would likely be below 18 Mg ha^{-1} (1%) under typical field conditions and result in relatively small (<0.5 unit) decreases in pH, depending upon the soil. A reduction in pH could be beneficial for alkaline soils; however, this might result in the release of inorganic soil carbon and contribute negatively to the carbon budget of the produced biofuel. Although we did not see evidence of large losses of soil C due to pH reduction in this experiment, it should be considered in subsequent experiments on alkaline soils.

4.2. Soil N Dynamics and Nitrifying Bacteria. While considerable C was lost through respiration, total N remained in the soil under our experimental conditions, as was demonstrated by nearly constant values in all treatments throughout the incubation period. However, a very different scenario might occur under field conditions where there is greater potential for soil N losses through denitrification, runoff, and/or leaching.

Given that the oilseed meals had much higher N content and lower C : N ratios than the sorghum-sudangrass biomass, it was not surprising that they resulted in a greater amount of mineralized N. Under the same assumptions as previously stated for C, there would be about 400 kg mineralized N ha^{-1} for a 1% rate of flax or mustard meal addition in the field. This indicates that oilseed meals have great potential as a slow-release N fertilizer [7]. In contrast, adding sorghum-sudangrass actually resulted in significant N immobilization.

Generally, after 51 d of incubation, there was no difference in the amount of ammonification and nitrification in the mustard- and flax-amended soils, for a given level of amendment rate, with essentially all inorganic N being either NO_3-N (0.5 and 1% amendments) or NH_4-N (5% amendment) for both seed meal types. However, there were significant differences in nitrification at the 2.5% rate of amendment. After 51 d of incubation, essentially all inorganic N in the flax treatment was NO_3-N while approximately 55% of the inorganic N in the mustard treatment was NH_4-N. Others have demonstrated that isothiocyanates (ITC) can inhibit soil nitrifying bacteria through direct reductions in their populations and activities, resulting in nitrification being inhibited by 35–65% [19]; however, in that study, NH_4-N was added to the soil concurrent with the ITCs. It is possible that allelochemicals released from the mustard oilseed meal, added at high rates, may have inhibited nitrification in the current experiment; however, we found no significant differences in the population sizes of nitrifying bacteria in the mustard- and flax-amended soils, for a given application rate. Another possible explanation is a reduction in ammonification. For oilseed meals, the organic N would first have to be converted to NH_4-N before it could be nitrified. A recent study in which a 2% rate of mustard meal (*B. juncea*) was applied to soil found a reduction in mineralization of N and levels of NH_4-N and NO_3-N as compared to the same rate of rapeseed (*B. napus*) containing a much lower glucosinolate content [8]; however, levels of both NH_4-N and NO_3-N in the mustard-amended soil were essentially equivalent to the those in the rapeseed-amended soil by 30 to 45 d. Since we did not detect differences in the numbers of nitrifying bacteria in the 2.5% mustard and flax treatments, it is likely that other processes such as delayed or reduced ammonification contributed at least partially to the lower levels of NO_3-N after 51 d. It is possible that, although the biocidal chemicals in the mustard did not appear to impact population sizes of nitrifying bacteria, that they did impact their metabolic activity and thus decreased levels of nitrification; however, the abundance of NH_4-N and very low values of NO_3-N in both the 5% mustard and 5% flax treatments indicates that additional processes, such as high levels of ammonia or volatile sulfur compounds, were also likely contributing to low nitrification activities at high levels of seed meal amendment [52–54].

4.3. Soil Microbial Communities. Not surprisingly, each of the added biomass types impacted the soil microbial communities as compared to the unamended control. However, it was somewhat surprising that the mustard generally selected for a microbial community that was more similar to the sorghum-sudangrass than to the flax oilseed meal. This appeared to be largely due to greater fungal biomass in the mustard and sorghum-sudangrass treatments. For the mustard, this is especially interesting given the number of studies documenting its adverse effects on various soil fungi [11, 15, 16]. Our initial presumption was that the mustard would broadly inhibit soil fungal populations. The initial suppression of C mineralization in the mustard treatments tended to agree with this. However, it appears that the mustard selected for a population(s) of fungi that were resistant to the presence of any biocidal products produced from the glucosinolates in the mustard. Only a handful of studies have investigated the impacts of isothiocyanates and related compounds on soil microbial communities [18, 55, 56]. Even though

these studies have focused upon the impact of ITCs added as pure chemicals, in harvested green tissue, or in living plants instead of being from seed meals, they all have generally indicated that glucosinolate breakdown products (e.g., ITCs) can at least transiently impact microbial communities, especially fungi, at high concentrations. In the current study, the microbial communities in all amended treatments had recovered to compositions more resembling the unamended soil after 28 d, but it is unclear if these changes might have longer-term impacts on other soil processes. Ongoing, DNA-based analyses are being used to further characterize the impacts of these seed meals on the soil microbial communities.

5. Conclusion

Oilseed meals are different from traditional soil additives such as crop residues in that they contain greater amounts of N and easily decomposable C. Three aspects require special attention when considering land application of oilseed meals. First, a large proportion of C in oilseed meals will be rather quickly respired and released into the atmosphere as CO_2. Carbon dynamics in subsequent months and years following application will determine how much C is eventually incorporated into soil organic matter and more information regarding these long-term effects is needed in order to develop a more complete picture of C cycling with seed meals. Second, oilseed meals contain large amounts of N, which like C, will be mineralized once in soil. On the one hand, N mineralization from oilseed meals can provide N needed for crop growth and should reduce N fertilizer requirements. On the other hand, large amounts of available N may result in environmental degradation. Therefore, site-specific field experiments are needed to determine the release rate and fate of N from oilseed meals, which can then be used to develop best management practices regarding N. Third, many oilseed meals from dedicated biofuel crops, such as mustard, may release allelochemicals which can affect soil microorganisms. Mustard seed meals at higher rates demonstrated inhibitory effects on C mineralization at the beginning of incubation and also dramatically altered the soil microbial communities. However, this effect appeared to be transient, and soil biological activities soon recovered either due to the degradation of the allelochemicals and/or soil microbial adaptation. Further research is needed on the longer-term effects of these oilseed meals on soil C dynamics and soil ecosystems in order to develop specific recommendations for land application of these biofuel co-products.

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Research Article

Potential Use of Organic- and Hard-Rock Mine Wastes on Aided Phytostabilization of Large-Scale Mine Tailings under Semiarid Mediterranean Climatic Conditions: Short-Term Field Study

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The study evaluated the efficacy of organic- and hard-rock mine waste type materials on aided phytostabilization of Cu mine tailings under semiarid Mediterranean conditions in order to promote integrated waste management practices at local levels and to rehabilitate large-scale (from 300 to 3,000 ha) postoperative tailings storage facilities (TSFs). A field trial with 13 treatments was established on a TSF to test the efficacy of six waste-type locally available amendments (grape and olive residues, biosolids, goat manure, sediments from irrigation canals, and rubble from Cu-oxide lixiviation piles) during early phases of site rehabilitation. Results showed that, even though an interesting range of waste-type materials were tested, biosolids (100 t ha^{-1} dry weight, d.w.) and grape residues (200 t ha^{-1} d.w.), either alone or mixed, were the most suitable organic amendments when incorporated into tailings to a depth of 20 cm. Incorporation of both rubble from Cu-oxide lixiviation piles and goat manure into upper tailings also had effective results. All these treatments improved chemical and microbiological properties of tailings and lead to a significant increase in plant yield after three years from trial establishment. Longer-term evaluations are, however required to evaluate self sustainability of created systems without further incorporation of amendments.

1. Introduction

Copper mining operations may adversely affect the environment due to deposition of large volumes of a number of hard-rock waste materials in nearby areas, such as sterile rocks, smelter slags, smelter dust, and tailings, among others. When sulfide copper ores are concentrated by flotation, approximately 80% of total wastes are tailings, which still contain a concentration of metals (i.e., Cu, Zn, Mo, Ni, Pb, Cd) and metalloids (i.e., As) that may pose environmental risks after inadequate deposition and management [1–6].

Currently, tailings are deposited in artificial dumps, or tailings storage facilities (TSFs), where fine solid particles (tailing sands) are separated from water by gravity [7]. Abandonment of postoperative TSFs under semiarid Mediterranean climatic conditions, such as in north-central Chile, led to complete water evaporation from upper tailings [7]. Without proper closure management, this fine, homogenous, and noncohesive material is left exposed to physical and chemical environmental forces [1, 8], causing erosion by wind. Deposition of metal/metalloid-rich tailings into nearby soils and surface waters may pose risks to human health, agricultural activities, and wildlife [3, 9, 10]. Depending on tailings mineralogy (i.e., content of indigenous iron-/sulfur-oxidizing bacteria [11, 12]), and geographic location, acid mine drainage and lixiviation of metals may also occur with some secondary environmental impacts on surface and ground waters [7].

After a long period since abandonment, postoperative TSFs are generally devoid of vegetation or covered with a rather scarce vegetation cover [13–15]. Spontaneous plant colonization of TSFs is a very slow process as mine tailings are usually characterized by bad drainage, compactation, absence of organic matter and nitrogen, neutral to low pH [7, 14, 16], and limited soil-type microbiota to make energy and nutrients of litter available to plants [10]. Additionally, under semiarid climatic conditions, these problems are exacerbated by the builtup of salinity, as a high proportion of rainfall and the water contained in TSFs undergo evaporation rather than infiltration [7, 13]. Therefore, physical and chemical characteristics of mine tailings, among other factors, interact to almost completely suppress seed germination, plant growth, and microbial activity [10, 17].

Aided phytostabilization is recognized as a potentially cost-effective and ecologically sound approach to containment of metal-polluted soils and mine tailings [5, 15, 18–20]. Their primary objectives are to reduce the mobility, ecotoxicity, and dispersion of metals/metalloids through the environment [5, 15, 19, 20]. This technique requires both incorporation of adequate inorganic and/or organic amendments to topsoils and revegetation with adequate plant species (i.e., metal tolerant plants, likely with an excluder phenotype) for immobilizing metals/metalloids in the rhizospheric zone and providing erosion control and wildlife habitat [2–5, 5–22]. Use of native plants is a focus of this technology because they often demonstrate tolerance to local environmental conditions and provide a foundation for natural ecological succession. However, success of aided phytostabilization on mine tailings also depends on the rapid improvement of most, if not all, limiting physical and chemical factors of the substrate for proper plant establishment and growth, including the establishment of proper soil microbial activity [16, 23–25]. Microbial activity is fundamental for the biochemical cycling of carbon, nitrogen, and phosphorus and the processes of organic matter turnover [26], and thus for achieving self-sustainable ecosystems [27].

Establishment of a vegetation cover helps to control erosion and provide organic matter in the substrate, increasing substrate aggregation and sequestration of pollutants [5, 15]. Most of these functions can be induced through proper application of amendments into mine tailings, such as organic and/or inorganic materials. In general, inorganic amendments improve either physical characteristics of tailings, such as compactation and drainage, or some limited chemical characteristics, such as pH and excessive soluble metal levels [25, 28–30]. Organic amendments improve physical characteristics of tailings, mitigate their metal toxicity, inoculate them with soil microorganisms, and incorporate required macronutrients and organic matter [5, 15, 25, 27, 28, 31–33].

Even though aided phytostabilization has been effectively used on metal-enriched soils and hard-rock mine wasted, such as TSFs, particularly in temperate areas (i.e., [10, 23, 24, 34, 35]), its use on postoperative TSFs of north-central Chile represent a new challenge. On one hand, postoperative TSFs being generated by large-scale copper mine operations in Chile have larger surface areas than the ones generated in other mining countries (range of 300 to 3,000 ha); therefore, local availability of very large amounts of proper amendments is by far a more limiting aspect for application of the technology than in other copper mining countries. This represents an opportunity for generating alternative disposal areas for other massive wastes that cannot be largely applied on croplands (i.e., agrowastes, biosolids, hard-rock mine wastes); however, their efficacy to improve physical, chemical, and biological properties of tailings have to be evaluated. On the other hand, experiences of aided phytostabilization on TSFs mainly gained in temperate climates (i.e., Canada) may not be directly applied to TSFs located in semiarid regions, such as north-central Chile. For example, organic covers (approx. 50 cm depth) made with biosolids and/or other organic wastes generated by cellulose-producing plants or agricultural activities have been effectively used on aided phytostabilization programs on TSFs in temperate areas (i.e., [10, 23, 24, 34, 35]), but they are inadequate under Mediterranean semiarid climate type conditions as salinization of organic covers strongly limit plant establishment and growth (i.e., [5, 25, 36, 37]). Therefore, alternative management options, such as incorporation into upper tailings, have to be evaluated.

The main objective of the present study was to assess the efficacy of a range of locally available organic and hard-rock mine waste-type materials on aided phytostabilization of postoperative copper TSFs under semiarid Mediterranean climate type conditions, with emphasis on chemical and biological parameters. The use of locally available wastes as tailing amendments, through promotion of integrated waste management practices at local level, was prioritized in order to achieve the large volumes that will be required by the large-scale copper mine operations in north-central Chile. Plant yield, metal uptake and translocation to aerial tissues, and the evolution of a number of microbiological and chemical parameters of the substrate were assessed.

2. Materials and Methods

2.1. Study Site. The study was conducted at *La Cocinera* TSF (6.7 ha; 6.618.700 N-291.300 E) owned by the Ovalle copper mining plant of ENAMI. This postoperative and dried TSF is located in north-central Chile, Coquimbo Region, in an area under semiarid Mediterranean climate type conditions. Annual rainfall in the area averages 237 mm, concentrated during April to September (Autumn-Winter). The dry season extends from October to May. During this period the water balance is negative, resulting in a soil moisture deficit. Mean temperature of the warmest month (January) and the coldest month (July) are 23°C and 7°C, respectively [36].

Tailings of *La Cocinera* TSF are characterized by a clay loam texture, slightly alkaline pH (7.98), elevated electric conductivity (EC, 5.62 mS m^{-1}), very low organic matter (0.48%) and available N-P contents, low cation-exchange capacity (CEC, 6.28 meq 100 mg^{-1}), and very high concentrations of sulphate (2,912 mg L^{-1}), total copper (4,393 mg kg^{-1}, dry weight basis (d.w.)), total zinc

Table 1: Experimental treatments and description of amendments and doses used on the field trial established at *La Cocinera* tailings storage facility, north-central Chile.

Treatment	Amendment	Dose
Control, C	—	—
RM	Rubble from lixiviation piles	1000 t ha^{-1}
	Manure (goat)	108 t ha^{-1}
RMS	Rubble	1000 t ha^{-1}
	Manure	108 t ha^{-1}
	Sediment of irrigation canals	Layer of 5 cm
B1	Biosolids	100 t ha^{-1}
B2	Biosolids	200 t ha^{-1}
G1	Grape residues	89 t ha^{-1}
G2	Grape residues	200 t ha^{-1}
O1	Olive residues	91 t ha^{-1}
O2	Olive residues	200 t ha^{-1}
GB	Grape residues	131 t ha^{-1}
	Biosolids	20 t ha^{-1}
GM	Grape residues	91 t ha^{-1}
	Manure	70 t ha^{-1}
OB	Olive residues	135 t ha^{-1}
	Biosolids	19 t ha^{-1}
OM	Olive residues	96 t ha^{-1}
	Manure	67 t ha^{-1}

(1,619 mg kg^{-1} d.w.), total iron (87,094 mg kg^{-1} d.w.), and total calcium (35,504 mg kg^{-1} d.w.; [15]). The existing literature [38, 39] describes the local soils as clay loam in texture, from colluvial and alluvial origin, neither saline (EC of 2.29 mS m^{-1}) nor sodic, with organic matter content around 1.5–2.8%, pH of 7.42, and CEC of 13.27 meq 100 mg^{-1}. Even though, some physical and chemical characteristics of study tailings are similar to local soils, such as texture and pH, most of the others need to be improved in order to support a native plant cover. Therefore, the main emphasis of the present study was to evaluate the efficacy of selected amendments to improve limiting chemical and biological parameters of tailings.

2.2. Experimental Design. A 2,400 m^2 field trial (60 m long × 40 m wide) was conducted at *La Cocinera* TSF to evaluate some biological and chemical endpoints of mine tailings amended with several local available organic and inorganic residues for a total of 12 treatment plots and a control (no amended tailings) plot (Table 1); each treatment was replicated three times. Selected amendments were discarded rubble from Cu-oxide lixiviation piles (R), available at the same mine operation; goat manure (M) from nearby cattle yards; sediments from the cleaning process of local irrigation canals (S); air-dried biosolids (B) from a municipal water treatment plant; solid pressing grape residues (G) from a spirit (*pisco*) producing plant; solid olive mill residues (O) from an olive oil producing plant. General properties of selected amendments are given in Table 2, and details (amendment types and doses) of experimental treatments are shown in Table 1. Application rates of organic residues were either decided according to available information in the literature for hard-rock wastes or metal-polluted degraded soils, when added alone (i.e., [40]), or with a target of 5% OM and a C : N ratio of 30 in amended tailings when added in mixtures of C-rich (i.e., grape residues, olive residues) and N-rich organic residues (i.e., biosolids, manure (i.e., [24])). Application rate of discarded rubble from Cu-oxide lixiviation piles was decided with a preliminary laboratory evaluation to change tailings texture from loam, to sandy loam while sediments from irrigation canals were used according to their availability. Amendments were mixed on the upper layer of tailings (0–20 cm depth) with a gasoline-operated rototiller, to avoid salinization problems in amended tailings; the exception was sediment from irrigation canals which were applied on top of tailings, as we considered them equivalent to preserved topsoils used as top covers on mine rehabilitation (i.e., [1, 13]). The experimental layout was a complete randomized block design.

On March 2006, each experimental plot (5 × 5 m) was first amended and then seeded with 800 g of perennial ryegrass (*Lolium perenne* var *Nui*) and 38 g of a mixture of local grasses/herbs previously collected from either nearby wild areas and from a grass population (*Polypogon australis*) spontaneously established in a restricted area of the same TSF, following the methodology of Gold et al. [41]. This represents a seeding rate of 335 kg ha^{-1}, a value higher than the range of 20 to 120 kg ha^{-1} recommended for remediation of metal-contaminated soils of hard-rock wastes (i.e., [42–44]), but conservative considering that no information about metal tolerance of the species was available. The study area was wire-mesh fenced, and all grazing from domestic and wild herbivores was excluded; wind-breakers were built in two sides of the perimeter in order to reduce wind erosion. An irrigation system was established in the site with spray sprinklers located in the center of every experimental plot to assure plant establishment and development in the early stages. Irrigation was kept from March to November, during three consecutive years (2006 to 2008), to complement natural winter precipitations; irrigation water was obtained from a nearby stream.

2.3. Substrate Sampling and Analysis. Substrate samples were collected from all experimental plots at the time of addition of amendments (March 2006) and after two (March 2008) years, using a manual stainless steel soil auger. Each plot sample consisted of two bulked subsamples (25 cm^3 cores) randomly collected at 0–20 cm depth. A composite sample was made from mixing both subsamples which were placed in a hermetically sealed plastic bags, homogenized in the field at collection time, and stored at 4°C in the dark until their transportation to the laboratory. Roots were manually removed from all samples prior analyses and processing in the laboratory. Plot samples were divided into two aliquots; one was used for microbial analyses, and the other was used for chemical characterization as described below.

Table 2: General properties of selected organic and inorganic amendments for aided phytostabilization of Cu-sulfidic tailings under semiarid Mediterranean climate conditions.

Amendment	Bulk density (g/mL)	pH (water)	SOC (%)	EC (mS/cm)	Sulphate (mg/L)	Total metal (mg/kg) Cu	Zn	Fe	Total P (mg/kg)	Total K (mg/kg)	Total N (%)	Total C (%)	C : N
Biosolids (B)	0.36	6.20	81	7.04	600	483	525	5493	9780	6638	7.4	46.9	6.3
Grape residues (G)	0.45	5.64	94	1.94	201	31	21	937	2800	24500	2.9	56.8	19.6
Olive residues (O)	0.41	5.71	95	2.91	1.25	16	11	2076	800	10500	1.0	54.3	53.8
Goat Manure (M)	0.32	7.92	71	12.18	642	46	58	8517	4100	29700	2.5	40.1	16.2
Sediments (S)	1.12	6.91	0.36	4.20	1386	333	177	n.d.	700	3400	0.12	0.02	0.2
Rubble (R)	1.70	5.28	0.23	5.55	3454	7412	510	n.d.	1300	1500	0.01	0.32	32.0

2.3.1. Substrate Analysis (Bulk and Pore Water). Composite samples were made mixing replicated plots for each experimental treatment, as substrates underwent several analyses (bulk, pore water, field capacity at 100%, etc.). Substrate samples were air dried and sieved (<2 mm) before analytical determinations. The total percentage of the sample corresponding to the soil whose particle size was greater than 2 mm was registered (retained by the sieve), and the fraction less than 2 mm was determined by granulometry using the method of Bouyoucos [40]. The pH and electrical conductivity (EC) were measured in a 1 : 1 substrate to water solution using a glass electrode. Soil organic carbon (SOC) was determined by the Walkey and Black wet dichromate oxidation method [40]. Cation exchange capacity (CEC) and total N and Cu were determined according to protocols of the USDA [40] and US EPA [45], respectively. In the case of total Cu determinations in bulk substrate samples, every digestion batch included one blank sample, one standard reference material (SRM) sample (Loam-B, catalog CRM-LO-B; High-Purity Standard, Charleston, SC, USA), one duplicate sample, and one quality-control sample for the quality-assurance and quality-control criteria. Digested samples were analyzed for total Cu contents by flame atomic absorption spectrometry (FAAS, AAnalyst 300; Perkin-Elmer). Background nonatomic absorption was corrected with a deuterium continuous lamp. The atomic absorption analytical device was housed in a class 1000 clean-room laboratory, and the loading of the autosampler tray was done in a class 100 laminar flow cabinet. The calibration standard was prepared with high-purity (>18 MΩ cm^{-1}) deionized water and acidified with HNO_3 Suprapur (Merck) to 0.2%. For performance control of the atomic absorption spectrometer, a certified multielement standard was used (Spectrascant Certified, Teknolab). The quality-assurance and quality-control criteria were satisfied when the measured parameter of the standard reference material (Loam-B, catalog no. CRM-LO-B; High-Purity Standard, USA) and the quality-control sample (a previously characterized soil sample with a known concentration of metals) differed by no more than 5%. Available N, P, K contents were determined according to Sadzawka et al. [46].

To assess the chemical evolution of substrates under different treatments, 35 mL of pore-water samples were taken from each experimental substrate using Rhizon soil pore-water samplers (Rhizosphere Research Products, Wageningen, The Netherlands), following the method described in Vulkan et al. [47]. Substrate pore-water samples were kept in acid-washed polyethylene plastic vials (50 mL), and then a subsample was acidified with HNO_3 suprapur (Merck) and analyzed for total dissolved Cu (method SW-486 of US EPA [45]) by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS; Perkin Elmer ELAN6100 with auto sampler). The other subsample was acidified with H_3PO_3 suprapur (Merck) and analyzed for dissolved organic carbon (DOC; method 415.1 of US EPA [48]), using an Apollo 9000 TOC analyzer (Tekmar-Dohrmann, USA).

2.3.2. Microbial Analysis. Substrate aliquots for microbial analysis were kept in hermetically sealed plastic bags, as collected in the field, and stored at 4°C in the dark until their microbiological analysis. Basal respiration was determined by placing 50 g of each substrate sample at 70% of field capacity in a 0.5 L air-tight sealed jar along with 10 mL of 1 N NaOH, followed by incubation for 28 days in the dark at 28°C. The C-CO_2 evolution was periodically determined by titration [49]. Basal respiration rate was calculated based on cumulative CO_2 evolution over the 28 days period. Microbial biomass C (MBC) was determined by the chloroform fumigation extraction method [50]. This parameter results from the difference between fumigated and nonfumigated samples, corrected with the K_{EC} value of 0.45 [51]. The microbial metabolic quotient was calculated as basal respiration (μg C-CO_2 h^{-1}) per mg of microbial biomass C according to Anderson and Domsch [52].

2.4. Plant Sampling and Analysis. Aboveground plant biomass was determined at each experimental plot by clipping vegetation at ground level in three randomly placed quadrats (0.35 m × 0.35 m or 0.12 m^2) by the end of September 2006 and 2008, after seed production in grasses and at the mid growing season of herbs. Plant biomass of each experimental plot was harvested, placed in a preweighed paper bag, and transported to the laboratory where they were washed with tap water and deionized water in order to eliminate external contamination. Shoots were then dried in a forced air oven at 60°C and weighed after 3 d to obtain the aerial dry biomass. Plant tissues were ground to powder in an agate ball mill and digested with HNO_3–HF–H_2O_2 in a microwave oven (Milestone 1200; Milestone Microwave System, Monroe, Conn, USA). Copper content in shoots was determined by ICP-MS (Perkin Elmer ELAN 6100 with

Table 3: Texture of experimental substrates (bulk) at the time of establishment of the field assay (year 1, 2006), at a depth of 0–20 cm. Codes of treatments follow Table 1.

Treatment	Texture (%)			Texture type*	Particles >2,000 μm
	<2 μm	2 μm–50 μm	50 μm–2,000 μm		(%)
C	14	42	44	Loam	11
RM	13	33	54	Loam	39
RMS	12	25	63	Sandy loam	39
B1	12	43	45	Loam	21
B2	20	43	37	Loam	38
G1	16	44	40	Loam	15
G2	19	40	41	Loam	19
O1	9	37	54	Loam	8
O2	11	30	59	Sandy Loam	18
GB	21	42	37	Loam	15
GM	15	37	47	Loam	20
OB	11	50	39	Silt Loam	19
OM	18	40	42	Loam	17

* According to the soil textural classification chart of the US Department of Agriculture.

an auto sampler) according to methods SW-486 [45]. Every digestion batch included one blank sample, one SRM sample (1573a tomato leaves; National Institute of Standards and Technology, Gaithersburg, Md, USA), one duplicate sample, and one quality-control sample for the quality-assurance and quality-control criteria. The quality-assurance and quality-control criteria were satisfied when the measured parameter of the standard reference material (1573a tomato leaves) and the quality-control sample differed by no more than 5%.

2.5. Statistical Analysis. Significance of plant and microbial response variables due to experimental treatments were tested by one- (treatment) or two-way (treatment and time) analysis of variance followed by the LSD Fisher test when required. Normality and homogeneity of variances were checked with the Shapiro-Wilks and Levene tests, respectively; logarithmic transformations were used when required. Simple lineal regressions and Pearson's correlation analyses were used to determine correlations among variables (i.e., substrates). Statistical analyses were conducted using the software InfoStat [53].

3. Results

3.1. Substrate Properties. Tailings at the experimental site have loam texture, according to the soil textural classification of the USDA (Table 3). In general, addition of amendments, either alone or in mixtures, did not modify the percentage of particle size distribution below 2,000 μm, hence maintaining the loam texture. Exceptions were RMS, O2, and OB treatments (Table 3); RMS treatment had the same sandy loam texture of pure sediments, as they were applied at the soil surface without incorporation. Slight changes in soil texture detected in the other two treatments (O2 and OB) may be only explained by typical variations of tailings texture [7]. When the coarse fraction is considered (>2,000 μm), higher percentages (among 1.4 to 3.5 times higher) are, in general, found for amended tailings when compared to control tailings (Table 3). Increases were higher for B than for G and O, and they showed to be dose dependent, as most of the OM incorporated into experimental plots was retained on the sieve. As expected, the highest values of coarse particle fraction (>2,000 μm) were found on plots where rubble from Cu-lixiviation piles was incorporated (Table 3), as this material has 68% of particles >2,000 μm.

At the beginning of the assay (year 1 or 2006), addition of amendments improved most chemical (i.e., CEC, SOC, and DOC) and nutritional (i.e., N, P) properties of tailings (Tables 3 and 4). Biosolids and grape residues, either alone or mixed with other materials, produced the more marked changes in chemical and nutritional properties of tailings, particularly in terms of CEC, SOC, and available N-P-K concentrations, when compared to control (Table 4). Olive residues mainly improved SOC of tailings in a dose-dependent form (Table 4). However, all organic amendments contained soluble salts, and their addition into tailings increased EC with respect to control (5.3 mS cm^{-1}), up to 26.4 mS cm^{-1}, particularly when applied in high doses; this effect was more pronounced with biosolids (Table 4) but also occurred after addition of high dosages (200 t ha^{-1}) of grape and olive residues.

The carbon to nitrogen ratio largely varied among treatments and generally decreased with addition of organic amendments, particularly in the case of biosolids and manure as these are N-rich materials (Table 4). Incorporation of amendments into tailings did not change or slightly reduced total Cu concentrations in the substrate, thus having no or minor dilution effects. The exception was treatment G2 with addition of 200 t ha^{-1} of grape residues which reduced 1.8 times total Cu (Table 4). The pH of substrates only varied from slightly to moderately alkaline values, in the range of 7.40 to 8.20, and it almost did not vary with time (Table 4).

By the end of the experiment (year 3 or 2008), CEC, SOC, available N-P-K, and C : N ratio markedly decreased

Table 4: Evolution of chemical properties of experimental substrates (bulk) from the time of establishment of the field assay (year 1, 2006) and after 2 years (year 3, 2008), at a depth of 0–20 cm. Codes of treatments follow Table 1.

Treatment	pH		EC ($mS\,cm^{-1}$)		CEC ($meq\,100\,g^{-1}$)		SOC (%)		$N_{available}$ ($mg\,kg^{-1}$)		$P_{available}$ ($mg\,kg^{-1}$)		$K_{available}$ ($mg\,kg^{-1}$)		C : N ratio		Total Cu ($mg\,kg^{-1}$)	
	Y 1	Y3	Y 1	Y3	Y 1	Y3	Y 1	Y3	Y 1	Y3	Y 1	Y3	Y 1	Y3	Y 1	Y3	Y 1	Y3
C	8.0	8.0	5.3	7.4	3,7	5.8	2.0	0.7	9	Bdl	8	8	142	73	56.6	6.7	6248	6140
RM	7.6	7.9	9.1	11.9	18,1	7.8	3.3	1.5	14	13	139	53	2821	826	12.7	7.9	6445	6604
RMS	7.4	7.8	5.9	6.9	21,5	9.7	2.6	1.2	35	11	42	25	469	269	18.7	9.0	4910	5728
B1	8.1	8.0	13.5	15.0	9,3	4.9	2.0	1.1	480	425	287	47	616	232	10.8	4.3	4655	5876
B2	7.8	7.7	26.4	19.6	31,5	7.0	5.0	1.3	984	814	993	87	2127	404	6.4	4.9	4013	4839
G1	8.2	7.9	8.9	7.5	12,6	4.9	2.5	1.4	24	9	54	13	1264	155	48.3	9.1	6506	6879
G2	7.6	8.0	12.0	8.9	24,0	8.2	3.4	2.0	180	6	269	40	4727	422	8.5	7.3	3394	5208
O1	8.1	8.0	8.5	6.5	6,5	5.8	2.4	1.1	5	Bdl	15	8	713	81	28.3	14.4	8588	7369
O2	7.8	8.0	10.2	7.9	8,4	5.6	5.8	1.6	26	Bdl	16	8	1464	188	37.3	12.8	6739	5215
GB	8.0	8.1	12.0	9.3	15,5	4.5	3.3	1.3	444	32	269	41	1850	295	21.2	9.1	7163	5928
GM	8.2	8.0	13.1	8.7	23,6	6.3	4.8	1.7	207	22	175	33	3088	333	91.8	7.4	7391	7353
OB	7.8	8.0	9.7	6.6	7,3	10.0	4.4	1.5	178	Bdl	77	16	885	102	23.4	12.6	5856	6178
OM	8.0	8.2	14.5	10.8	36,5	8.0	5.1	1.1	24	Bdl	84	20	3055	512	19.9	6.4	5857	5317

Y1: year 1; Y3: year 3; EC: electrical conductivity; CEC: cation exchange capacity; SOC: soil organic carbon; Bld: below detection limit.

Table 5: Evolution of the concentration of dissolved organic carbon (DOC) and total dissolved Cu in the pore water of substrates from the time of establishment of the field assay (year 1 or 2006) and after 2 years (year 3 or 2008). Codes of treatments follow Table 1.

Treatment	DOC ($mg\,L^{-1}$)		Cu ($mg\,L^{-1}$)	
	Y1	Y3	Y1	Y3
C	11	29	0.04	0.17
RM	542	795	4.52	0.06
RMS	218	148	1.22	4.01
B1	2412	274	29.12	0.57
B2	3048	600	116.92	1.13
G1	202	72	2.81	0.11
G2	523	113	8.24	0.05
O1	310	57	0.69	0.65
O2	831	138	2.28	0.51
GB	516	253	5.63	1.24
GM	780	190	7.79	1.00
OB	813	243	28.04	0.40
OM	918	165	6.96	2.44

Y1: year 1; Y3: year 3.

in all treatments, but in all cases values were higher than control plots (Table 4). Even though available N tended to decrease with time, minor reductions were detected on biosolids-amended plots when compared to other organic amendments (Table 4). Total Cu concentrations in the substrate did not show relevant variations with time while EC values either slightly decreased or increased with time (Table 4).

Dissolved organic carbon in pore water was very low in tailings (control plots), but it increased by 1 to 2 orders of magnitude with incorporation of amendments (Table 5). Specifically, DOC reached highest values in those plots where only biosolids were added (B1 and B2). In general, DOC values decreased with time, but they were more marked in some treatments, such as B1 and B2 (Table 5). Total dissolved Cu in pore water of substrates increased from 17 to 2,923 times with addition of amendments when compared to control plots (Table 5). Biosolids amended plots showed the higher increases reaching concentrations of 29.1 and 116.9 $mg\,L^{-1}$ in treatments B1 and B2, respectively (Table 5). However, Cu concentrations in pore water markedly decreased with time, reaching values quite alike among treatments after three years (Table 5). During the first year of the study, a positive and significant relation between DOC and total dissolved Cu in pore water was found ($R^2 = 0.75$, $P < 0.05$), but by the third year this relation was not significant ($R^2 = 0.01$, $P < 0.73$).

3.2. Plant Responses. Figure 1 shows the variation of aerial plant biomass (dry weight basis, d.w.) among experimental treatments with time, while Table 6 shows variation of Cu content in shoots. A two-way ANOVA for aerial plant biomass indicated significant differences among experimental treatments ($F = 7.08$, $P < 0.05$), year since amendments addition ($F = 51.07$, $P < 0.05$), and the interaction among these factors ($F = 7.46$, $P < 0.05$). Control plots showed very low aerial biomass production (4.5 to 5 $g\,m^{-2}$ dry weight basis (d.w.)), irrespective of the year (Figure 1). During the first growing season, aerial plant biomass strongly varied among treatments, being higher on treatments RMS and G1 (19 and 11 $g\,m^{-2}$ d.w., resp.) and null or very limited on treatments with addition of biosolids (0 to 0.3 $g\,m^{-2}$ d.w.; Figure 1); all other treatments showed an aerial biomass production that ranged from 2.5 to 7.8 $g\,m^{-2}$ d.w. Plant biomass tended to increase with time on most treatments (Figure 1). However, after three years, the highest increase in aerial plant biomass production occurred on treatment

Table 6: Evolution of the concentration of copper in aerial plant biomass (mean ± standard deviation, $n = 3$) from the time of establishment of the field assay (year 1 or 2006) and after 2 years (year 3 or 2008). Codes of treatments follow Table 1. Values followed by the same letter are not significantly different at $P < 0.05$ according to a two-way ANOVA and LSD Fisher test.

Treatment	Cu in shoot (mg kg^{-1})	
	Y1	Y3
C	237 ± 120.3^{abc}	203 ± 19.6^{abc}
RM	310 ± 215.7^{bcd}	390 ±433.4^{cde}
RMS	90 ± 8.3^{a}	270 ± 217.0^{bcd}
B1	826 ± 480.6^{f}	207 ± 90.5^{abc}
B2	—	144 ± 40.9^{ab}
G1	189 ± 69.8^{abc}	345 ± 268.8^{bcde}
G2	231 ± 114.9^{abc}	187 ± 48.4^{abc}
O1	559 ± 428.9^{e}	—
O2	507 ± 493.2^{de}	280 ± 202.4^{bc}
GB	427 ± 274.4^{de}	154 ± 31.5^{ab}
GM	316 ± 293.3^{bcde}	180 ± 35.4^{abc}
OB	235 ± 104.9^{abc}	207 ± 94.2^{abc}
OM	275 ± 83.4^{bcd}	312 ± 162.8^{bcde}

Y1: year 1; Y3: year 3.

Table 7: Evolution of metabolic quotient (mean ± standard deviation, $n = 3$) in experimental substrates (0-20 cm) from the time of establishment of the field assay (year 1 or 2006) and after 2 years (year 3 or 2008). Codes of treatments follow Table 1. Values in the same column followed by the same letter are not significantly different at $P < 0.05$ according to ANOVA.

Treatment	Metabolic quotient (μg C-CO_2 mg Cbio^{-1} h^{-1})	
	Y1	Y3
C	5.0 ± 0.75^{a}	4.0 ± 0.83^{a}
RM	3.0 ± 0.50^{b}	2.0 ± 0.62^{b}
RMS	3.0 ± 0.35^{b}	1.6 ± 0.71^{b}
B1	6.9 ± 0.68^{c}	0.6 ± 0.51^{c}
B2	7.5 ± 0.71^{d}	0.9 ± 0.77^{c}
G1	3.2 ± 0.51^{b}	2.8 ± 0.68^{d}
G2	3.4 ± 0.65^{b}	1.2 ± 0.80^{bc}
O1	7.1 ± 0.92^{c}	4.7 ± 1.03^{a}
O2	7.5 ± 1.09^{d}	4.8 ± 0.92^{a}
GB	3.9 ± 0.71^{bc}	1.6 ± 0.73^{b}
GM	3.7 ± 0.78^{bc}	1.2 ± 0.50^{bc}
OB	3.6 ± 0.80^{b}	1.8 ± 0.62^{b}
OM	4.7 ± 0.85^{ab}	2.9 ± 0.75^{b}

Y1: year 1; Y3: year 3; Cbio: microbial biomass C.

B1 (480 g m^{-2}, d.w.), followed by treatments B2 (283 g m^{-2} d.w.) and RM (112 g m^{-2} d.w.). On the contrary, treatments O1 and O2, with addition of olive residues had marked reductions in aerial production, reaching values of only 0.1 to 0.2 g m^{-2} d.w. (Figure 1).

A two-way ANOVA for Cu concentration in shoots indicated significant differences among experimental treatments ($F = 2.33$, $P < 0.05$), year since amendments addition

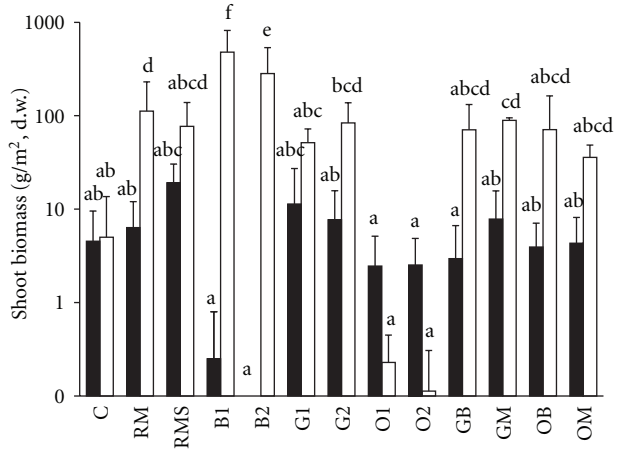

Figure 1: Evolution of plant aerial biomass (dry weight basis: d.w.; log 10 scale) in experimental treatments (mean ± standard deviation, $n = 3$) from the time of establishment of the field assay (year 1 or 2006 in black columns) and after 2 years (year 3 or 2008 in white columns). Codes of treatments follow Table 1. Same letters indicate no significant differences at $P < 0.05$ according to a two-way ANOVA and LSD Fisher test.

($F = 7.34$, $P < 0.05$), and the interaction among these factors ($F = 3.52$, $P < 0.05$). Plants in control plots reached mean values of 203–237 mg Cu kg^{-1} in aerial tissues with no significant variation among years (Table 6). During the first year, all other treatments did not show significant differences in Cu concentrations in shoots (i.e., RM, RMS, G1, G2, OB, OM) or showed significant increases (B1, O1, O2, GB) when compared to control plots. The maximum Cu concentrations in shoots occurred in treatment B1 (826 mg Cu kg^{-1}), followed by treatments O1, O2, and GB that showed values that ranged from 427 to 559 mg kg^{-1}. In most treatments, Cu contents in shoots remained the same or significantly decreased with time (Table 6). Specifically, Cu contents in shoots of plants in treatments B1, O2, and GB were reduced from 2 to 4 times with time (Table 6). With the exception of control and treatments with olive residues addition (O1 and O2), no visual metal toxicity symptoms (i.e., redness and chlorotic leaves, stunted plants) were, however, detected on field plants. Furthermore, biomass production increased with time in most treatments as shown above (Figure 1), with exception of control and treatments with olive residues, thus indicating no metal phytotoxicity of most amended substrates.

3.3. Microbiological Properties. Results of microbiological parameters are given in Figure 2 (accumulated basal respiration), Figure 3 (biomass C), and Table 7 (metabolic quotient). Values of both microbial basal respiration (MBR; Figure 2) and microbial biomass C (MBC; Figure 3) were low and constant in time in control plots, while the metabolic quotient (Table 7) was higher and constant in time in control plots when compared to experimental treatments.

Two-way ANOVAs for MBR and MBC indicated in both cases significant differences among experimental treatments,

Table 8: Tables of two-way analysis of variance for microbial accumulated basal respiration and microbial biomass C (mean ± standard deviation, $n = 3$) in experimental substrates (0–20 cm) from the time of establishment of the field assay (year 1 or 2006) and after 2 years (year 3 or 2008).

Parameter	Source of variation	Degrees of freedom	Mean squares	F	P
Basal respiration	Model	25	105727	152.2	<0.01
	Treatment	12	57253	82.4	<0.01
	Year	1	1352296	1947.2	<0.01
	Treatment × Year	12	50320	72.5	<0.01
	Error	52	695		
Biomass C	Model	25	55094	253.9	<0.01
	Treatment	12	23278	107.3	<0.01
	Year	1	819816	3779.4	<0.01
	Treatment × Year	12	23183	106.9	<0.01
	Error	52	217		

Table 9: Pearson's correlation coefficients between plant aerial biomass and several microbial and chemical (bulk and pore water) properties of experimental substrates ($n = 39$).

Variable	Plant aerial biomass
DOC	−0.20
SOC	0.54
EC	0.47
Microbial basal respiration	0.55
Metabolic quotient	−0.65
Cu concentration in pore water	0.51
Microbial biomass C	0.60
Cu concentration in shoots	−0.42

DOC: dissolved organic carbon; SOC: soil organic carbon; EC: electric conductivity.

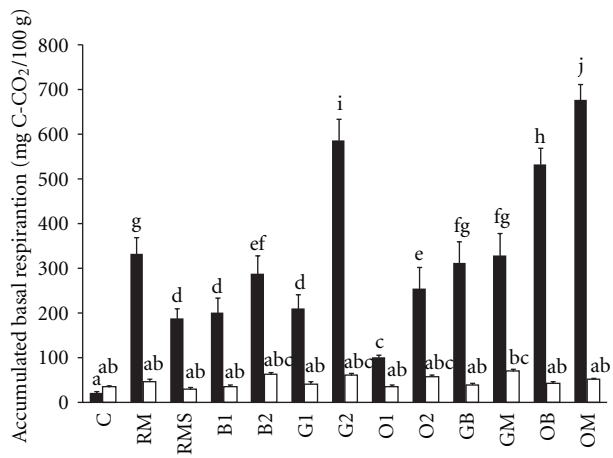

Figure 2: Evolution of accumulated basal respiration (28 days; mean ± standard deviation, $n = 3$) in experimental substrates from the time of establishment of the field assay (year 1 or 2006 in black columns) and after 2 years (year 3 or 2008 in white columns). Codes of treatments follow Table 1. Same letters indicate no significant differences at $P < 0.05$ according to a two-way ANOVA and LSD Fisher test.

year since amendments addition, and the interaction among these factors (Table 8). At the beginning of the assay (year 1 or 2006), both MBR and MBC significantly increased with addition of all amendments (Figures 2 and 3). Increase in MBR was higher in treatments RM, G2, OB, and OM (from 16 to 32 times) and lower in treatment O1 (5 times) with respect to control. In the case of MBC, increase was higher in treatments G2, OB, B2, and RMS (from 24 to 32 times) with respect to control; furthermore, for both parameters and all organic amendments used (B, G, O), the increase was dose dependent. MBR and MBC tended to decreased with time in all treatments, MBR showing more marked reductions than MBC (Figures 2 and 3). After three years, BMR values of all treatments reached values similar to control plots, with the exception of treatment GM that showed values 2 times higher than control plots (Figure 2). In the case of MBC, even though this parameter decreased in time to values similar to control plots, treatments B2, O1, and O2 remained significantly higher than control plots, reaching values up to 3.5 times higher than control (Figure 3). A significant simple lineal regression existed among MBR and MBC ($R^2 = 0.37$, $F = 21.7$, $P < 0.05$) during the first year of the assay, but this disappeared after three years ($R2 = 0.09$, $F = 3.8$, $P = 0.0577$) of experimentation. Finally, the metabolic quotient tended to decrease with time (Table 7). The highest values were recorded in treatments O1 and O2 for both years of evaluation. Treatments B1 and B2 showed high values in year 1, similar to treatments O1 and O2, but markedly decreased on the following years, reaching the lowest values in year 3 (Table 7).

3.4. Correlation between Biological and Chemical Parameters of Substrates. Pearson's correlations between all soil properties (bulk and pore water) and plant-related parameters were calculated. The most relevant are shown in Table 9. The strength of the associations was interpreted according to the Hopkins' correlation classification [54]: insubstantial (0.0–0.1), low (0.1–0.3), moderate (0.3–0.5), high (0.5–0.7), very high (0.7–0.9), and nearly perfect (0.9–1.0). Correlation analysis of plant aerial biomass showed high positive relationship with SOC, MBR, and Cu concentration in pore

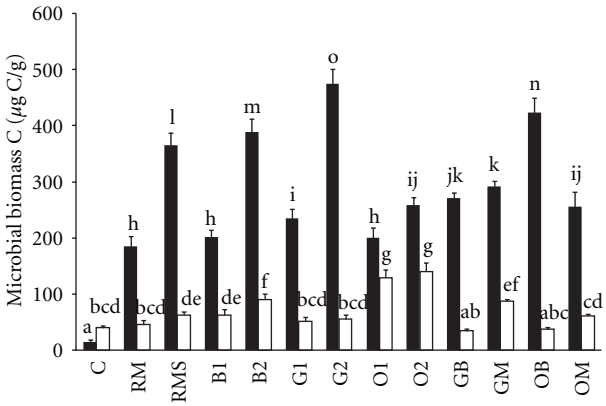

Figure 3: Evolution of microbial biomass carbon (MBC; mean ± standard deviation, $n = 3$) in experimental substrates from the time of establishment of the field assay (year 1 or 2006 in black columns) and after 2 years (year 3 or 2008 in white columns). Codes of treatments follow Table 1. Same letters indicate no significant differences at $P < 0.05$ according to a two-way ANOVA and LSD Fisher test.

water; a very high negative relationship with metabolic quotient; a very high positive relationship with MBC; a moderate negative relationship with Cu concentration in shoots.

4. Discussion

4.1. Substrates Properties. Soils are generally classified as saline when they have EC values higher than $4\,\mathrm{mS\,cm^{-1}}$ [55]. Under this guideline, all treatments were moderately to strongly saline, even after three years since establishment, and even though organic amendments were incorporated into tailings (mixed). Secondary salinisation of substrates, due to application of organic residues, can be a major constraint in aided phytostabilization strategies, particularly under semiarid environments where evapotranspiration exceeds infiltration of water due to scarce rain events (e.g., [56]). For example, Lombard et al. [37] found that a composted biosolid-treated calcareous soil increased EC by 21 and 41% above the control soil when applied at rates of only $14\,\mathrm{t\,ha^{-1}}$ and $44.5\,\mathrm{t\,ha^{-1}}$, respectively, in an area with semiarid climate. The increase in EC was expected because biosolids are high in complex mixtures of soluble salts [37]. Salinisation of tailings (increased EC) by amendments may be limiting for plant establishment and growth, but it would be compensated by high CEC of organic amendments (with the exception of olive residues) and discarded rubble from lixiviation piles. In semiarid climates, salinity is usually combined with high soil pH (alkalinity), because of $CaCO_3$ enrichment in the uppermost soil layers; hence calcareous soils are common; in the case of the experimental area, pH of substrates only ranged from slightly to moderately alkaline, but Ca content of tailings is high (1.5%). Although the effect of pH on the activity of soil microorganisms has been reported [57, 58], variations in soil pH in the present study do not seem to be sufficiently great to establish differences in the biological activity since no correlation between MBC and pH was found.

The average SOC in mine tailings of semiarid areas of north-central Chile is only about 0.23% [39]. This might predict low or lack of microbiological activity. Indeed, indigenous microbial communities associated with mine tailings often show limited density and diversity, relative to undisturbed sites [11, 59], and mainly correspond to iron-/sulfur-oxidizing bacteria [12]. However, SOC reached a value of 2% in *La Cocinera* TSF. Most of the SOC in tailings probably comes from flotation reagents used for the copper concentration process in the flotation plant. The level of SOC of all organically amended tailings increased noticeably with respect to control during the first year, in agreement with literature (i.e., [31]), but it decreased along the third year of the experiment, probably due to mineralization of easily degradable materials added [60]; however, in all cases, it remained above that of control plots and thus assuring microbial activity and plant growth. This may be also attributed to the presence of the plant cover developed after amendments application, with organic inputs such as root exudates and plant remains compensating C losses through mineralization, and due to the exhaustion of the labile organic C and the increase of the recalcitrant fraction of C, which is less prone to mineralization [61, 62]. For example, Müller da Silva et al. [27] found beneficial changes in ecosystem functioning, such as enhanced biological cycling of nutrients, in *Eucalyptus* plantations where biosolids were incorporated into degraded soils; as a result of increased plant biomass production, due to improved OM and nutrient soil levels, higher transfers of these components to the litter and subsequently to the soil were measured; if litter degradation is adequate, then self-sustainability of the system may be reached with no need of further additions of amendments, particularly for plant formations adapted to nutrient-poor soils like in semiarid areas. Furthermore, the slow release of nutrients contained within the biosolids and other organic amendments, when compared to chemical fertilizers [63], makes it possible to restore soil nutrient stocks throughout the development cycle of revegetated areas. Longer-term evaluations of the experimental plots established in the present study, in terms of OM and nutrients cycling among other parameters, are thus required for better conclusions.

Addition of organic amendments decreased C : N ratio of tailings, hence improving fertility of substrate; however, a large variation in this parameter existed among treatments, as locally available organic amendments have different N concentrations. For example, biosolids have large N concentrations (7.4%), while grape and olive residues have much lower concentrations (2.9% and 1%, resp.). Variations in C : N ratio determine different effects on microbiological processes and therefore in N availability to plants, not only in the short term but also in the long term (i.e., [24, 64]). For example, the decrease of this ratio to recommended values of 20 to 30 means that N is mineralized and thus available for soil microorganisms and plant roots [65]. However, very low (<20) C : N ratios may generate excessive N mineralization with consequent lixiviation of nitrates to groundwater and

release of ammonia [27, 66] and CO_2 to the atmosphere [67], thus restricting seed germination and early stages of plant development [27, 66]. This effect may have occurred on biosolids-amended tailings, particularly when elevate doses were applied (treatment B2), as no seed germination was observed during the first year (data not given). On the other hand, elevated values of this ratio (>30) indicate low N concentrations, which is a less favorable condition for soil microbial activity and plant development; this last situation is further limiting if the main C forms of the material are recalcitrant (i.e., lignin), which is resistant to microbial degradation [68]. In this case, N is sequestered into soil microorganisms and not available for plant root uptake [69]. For example, addition of olive residues resulted in elevated C : N rates (>28), and it remained high after three years. A shortage of N, due to the low content of this element in olive residues, may have occurred after three years thus explaining the marked reduction in aerial biomass production under these treatments (O1 and O2); mixture of olive residues with other N-rich materials, such as biosolids and goat manure, allowed longer-term N availability, and thus sustained aerial plant biomass production in time should be possible without further additions to maintain adequate fertility.

The organic amendments increased the initial level of DOC, an effect which was still present at the third year but with much reduced concentrations. The high content of low molecular weight organic acids in biosolids and olive residues at dressing time, such as fulvic acids, may pose a metal toxicity risk for plants due to their capability for increasing metal solubility and bioavailability in the substrate solution (e.g., [31, 32, 70, 71]). Metal cations, such as Cu^{2+}, are complexed by DOC, and these soluble organo-metallic complexes can be readily absorbed by plant roots [31, 32, 72–75] and/or leached into deep substrate layers [76]. Indeed, higher copper contents have been detected in shoots of grasses and some trees growing on biosolids-mixed tailings [25] and soils [31, 32, 77]. This phenomenon was detected in the present study in both biosolids and olive residues amended tailings, particularly during the first year. A decrease in the concentration of soluble metals would be expected on the longer term, as detected in the present study, once the mineralization of labile organic matter in biosolids or other organic materials leads to stabilized organic matter, as it has been shown by Al-Wabel et al. [78].

4.2. Biological Properties. The establishment of a vegetation cover on mine tailings located in semiarid Mediterranean climates is fundamental to protect these sites against erosive processes and for in situ immobilization of metals in the substrate; at the same time, it contributes to increase the soil organic matter content. In addition, a plant cover and particularly, the builtup of plant rhizosphere will influence the biological quality of the substrate by favoring the soil microbial activity and phytostabilization of these sites. On the other hand, soil microbiota is a key component to assure nutrient cycling for plant availability and long-term sustainability.

Application of amendments on mine tailings under semiarid Mediterranean climatic conditions improved the biological properties of original substrate. Organic amendments also favored plant growth which protect substrate from natural forces (i.e., wind and rain) and contribute to phytostabilization. The species used in the present study were able to survive in the tailings, but growth measured as dry aerial biomass largely varied among treatments. Biosolids inhibited seed germination (data not shown) and showed to be limiting for plant establishment and growth at the beginning of the assay, irrespective of application dose (100 and 200 t ha^{-1}). This may be result of the high salinity of this material, but also due to the large concentration of DOC which mobilized Cu into soil pore water thus posing Cu toxicity risks to plants. However, at the end of the assay, these treatments showed the highest plant yields, maybe as a result of salt and DOC leaching through the profile due to irrigation and natural precipitation, as it has been shown in other field studies where biosolids were used as organic amendments for mine tailings (e.g., [25, 56]). Furthermore, temporal changes in C : N ratio of organic-amended tailings may also affect plant establishment and productivity as demonstrated by Brown et al. [64]. They demonstrated that increasing the C : N ratio of organic amendments added to mine tailings to $\geq 20:1$ increased plant species richness and growth, thus affecting native plant restoration.

Olive residues allowed plant establishment but they were inadequate for sustained plant growth, showing poor yield of biomass after three years; however, they improved microbial properties (MSR and MBC) of tailings even after three years since plot establishment. One explanation of this result may be based on the presence of phenolic compounds, which are toxic [79, 80]. The organic matter of olive residues mainly consists of oil, polysaccharides, sugars, polyphenols, polyalcohols, proteins, organic acids (i.e., acetic and formic acid), phenols, lipids, and tannins, some of them known to be toxic to plants [70] or biorecalcitrant [79, 81]. However, inorganic constituents at the concentration levels found in olive residues are not toxic. In fact, it has been proved that they may potentially act as a good source of plant nutrients [82–84]. According to this, the use of composted olive residues is suggested as when olive residues undergo through a proper biodegradation process, like composting, the toxic organic compounds are broken down, and remaining components of these residues are suitable as good source of plant and microbial nutrients [84]. Other explanation may be the low N fertility of this material as discussed above, as when olive residues were mixed with biosolids and goat manure (N-rich sources), plant biomass increased 59% with respect to control. Thus it is not advisable to apply directly olive residues to mine tailings for phytostabilization. Several treatments showed to be adequate for plant establishment and growth in the short term, besides of improving microbiological properties of tailings (e.g., RM, B1, B2, OB). These treatments showed the highest biomass yield, MSR, and MBC; however, plots have to be evaluated in the longer term for better conclusions.

The negative correlation between qCO_2 and plant aerial biomass found in the present study reflects environmental

stress probably as a result of phytotoxicity effects from some organic amendments mentioned above, like olive residues and biosolids. Treatments which had the higher plant biomass yield showed the lower qCO_2 values. On the other hand, a positive correlation was found between MBC and plant aerial biomass, confirming the usefulness of MBC as an indicator of changes in vegetation. These results suggest that qCO_2 and MBC constitute sensitive indicators of plant growth and thus for phytostabilization progress [85]. The present study showed that herbaceous/grass species can grow in a wide range of metal concentrations (Cu, Zn, and Fe) in mine tailings. Zinc and Cu are essential for normal plant growth and development at low concentrations [86] and play important roles in several metabolic processes in plants. However, excess Cu and Zn in soil may retard plant growth [87, 88]. Kabata-Pendias and Pendias [89] reported that total fractions in soil equal to 70 to 400 $mg\,kg^{-1}$ of Zn and 60 to 125 $mg\,kg^{-1}$ of Cu are toxic to plants. The metal contents in the substrates studied greatly exceeded these ranges.

With regard to metal accumulation in shoots, it has been stated in the literature that organic amendments that contain a high proportion of humified OM (i.e., compost) can decrease the mobility of some metals due to the formation of stable chelates [90, 91]. However, the residues used in the present study were not stabilized at the beginning of the assay, and the humified OM could have been low, as shown by elevated DOC levels in pore water. Instead, they might facilitate metal transport in substrate by acting as carriers through formation of metal-organic complexes [92], at least in the short term. As a result, Cu contents in shoots were alike or slightly higher than in control plots. However, the concentration of metals in shoots tended to decrease with time in most treatments, suggesting that an immobilization process is taking place, probably because of organic matter (OM) stabilization. Shoot copper concentrations found in all treatments were well above critical concentrations described in plants and the maximum tolerable level in animals [88, 89, 93]. Nevertheless, it is important to state some relevant aspects. First, critical concentrations of copper have been defined for sensitive plants, normally represented by crops and vegetables (i.e., [88, 89, 93]); in the present study, local adapted plants were used, which may be more tolerant to elevated copper levels in aerial tissues. Second, some researchers have reported that part of the metals found in shoots might be adhered onto stem and leaves surface and are not absorbed into the internal structures of the plant, suggesting that total metal content in shoots is overestimated (e.g. [87, 94, 95]). This external contamination may exist even after standard washing protocols, particularly in xerophytic plants of semiarid environments as trichomes and glands are common morphological structures on leaves adapted to drought (i.e., [96]). Finally, the maximum tolerable level of copper in animals assumes that animal has no other feed or forage source, which is not the case, because these sites are not destined for grazing. Thus, the high concentration of copper in shoots reported in the present study does not necessarily imply risk for food webs.

5. Conclusions

Aided phytostabilization based on the use of local amendments and plant sources is a feasible field-scale technology for large-scale postoperative Cu-sulfidic mine TSF under semiarid Mediterranean climate conditions. A broad range of organic- and hard-rock mine wastes showed to be adequate for improving chemical and biological properties of tailings. However, selection of adequate local available amendments and management options for long-term release of limiting conditions of tailings for microbial and plant establishment and development are key aspects.

Biosolids, grape residues, and goat manure, either alone or in mixtures, are adequate organic materials for improving chemical and biological properties of tailings; however, high doses should be avoided ($\geq$200 ton ha^{-1} d.w.), particularly in the case of biosolids, as salinization, ammonia volatilization, and Cu mobilization into pore water may secondary occur with consequent limiting conditions for plant establishment. Even though biosolids are useful organic amendments to speed up the builtup of plant biomass and restore microbial properties of Cu-sulfidic mine tailings, lixiviation of excess salts and DOC during early stages of aided phytostabilization is required in order to get proper plant establishment and growth.

Olive residues are adequate materials for restoring microbial properties of tailings, but their low N fertility and high content of phytotoxic compounds made it inadequate to sustain plant development in the long term. Their mixture with N-rich materials, such as biosolids and manure, would be a better management option for these applications; furthermore, preliminary composting may be another alternative, particularly to eliminate the compounds responsible of phytotoxicity, but this needs to be further evaluated.

Discarded rubble from copper-oxide lixiviation piles can be a useful amendment for tailings, but it needs to be mixed with organic amendments (i.e., manure) to assure microbial inoculation of tailings and improvement of nutritional properties that may limit plant establishment and grow. Furthermore, general chemical characteristics of rubble and tailings where it will be incorporated should be first evaluated in order to determine its efficacy and define specific management options (i.e., pH management).

Local grass/herb species are appropriate for aided phytostabilization of abandoned and postoperative TSF under semiarid Mediterranean climate conditions, as they rapidly build up a continuous plant cover, but high Cu concentrations found in shoots may increase the potential risk of metal transfer to the food chain, an aspect that should be further evaluated, considering background metal contents in wild plants.

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Research Article

Soil Nutrient Availability, Plant Nutrient Uptake, and Wild Blueberry (*Vaccinium angustifolium* Ait.) Yield in Response to N-Viro Biosolids and Irrigation Applications

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We compared the impact of surface broadcasted N-Viro biosolids and inorganic fertilizer (16.5% Ammonium sulphate, 34.5% Diammonium phosphate, 4.5% Potash, and 44.5% s and/or clay filler) applications on soil properties and nutrients, leaf nutrient concentration, and the fruit yield of lowbush blueberry under irrigated and nonirrigated conditions during 2008-2009 at Debert, NS, Canada. Application rates of N-Viro biosolids were more than double of inorganic fertilizer applied at a recommended N rate of 32 kg ha^{-1}. The experimental treatments NI: N-Viro with irrigation, FI: inorganic fertilizer with irrigation, N: N-Viro without irrigation, and F: inorganic fertilizer without irrigation (control) were replicated four times under a randomized complete block design. The NI treatment had the highest OM (6.68%) followed by FI (6.32%), N (6.18%), and F (4.43%) treatments during the year 2008. Similar trends were observed during 2009 with the highest soil OM values (5.50%) for NI treatment. Supplemental irrigation resulted in a 21% increase in the ripe fruit yield. Nonsignificant effect of fertilizer treatments on most of the nutrient concentrations in soil and plant leaves, and on ripe fruits yield reflects that the performance of N-Viro was comparable with that of the inorganic fertilizer used in this study.

1. Introduction

Local authorities both overseas and in Canada are continually searching for environmentally acceptable and economically viable means of sewage sludge disposal. The Halifax Regional Municipality in Nova Scotia, Canada, has entered into a partnership with the N-Viro company to treat its biosolids for producing N-Viro biosolids that have been registered as a fertilizer under the Canada Fertilizer Act. The United States Environmental Protection Agency promotes the recycling of sludge material on some crop lands since it is an excellent source of several plant nutrients. The use of N-Viro biosolids as a fertilizer on agricultural lands provides essential plant nutrients while improving the physical and chemical soil properties and enhancing agricultural yield [1, 2].

Nutrients contained in the processed biosolids are slowly released, as those from organic manures, and are stored for a longer time in the soil, thereby ensuring a positive residual effect on plant root development and growth leading to higher crop yields [3, 4]. Literature reports that the release of nitrogen (N) from impregnated waste paper (biosolids) was found to be slow and steady [5]. The N-enriched cow dung used in maize production gave yield comparative inorganic fertilizer and increased the soil nutrient contents [6]. The application of commercially processed vermicomposts produced from food wastes, paper wastes, and cattle manure to soils increased their microbial biomass and dehydrogenase activity, in addition to increasing the yields of pepper growth and nutrient availability [7]. Application of biosolids to the dryland winter wheat is a feasible method of recycling plant nutrients [8].

Northeastern North America is the World's leading producer of wild blueberry with over 86,000 ha under management, producing 112 million kg of fruit valued at $470 million annually [9]. Blueberry fields are developed from native stands on deforested farmland by removing competing vegetation [10]. The crop is unique, as it is native to North America and has never been cultivated. Wild blueberries follow a two-year production cycle where one year produces vegetative growth, followed by a year in which bloom, pollination, and fruit growth and development occur. The majority of fields are situated in naturally acidic soils (pH range 4.5 to 5.5) that are low in nutrients and have high proportions of bare spots, weed patches, and gentle to severe topography [11]. Most of the soils in lowbush blueberry production in Canada are prone to wind, have very low nutrient and water storage capacities, and therefore, require constant application of nutrients and irrigation water.

Despite environmental management threats, in cases of less care, the application of nitrogenous manure with irrigation management can help minimize nutrient losses in vadose zone and improve crop production [12, 13]. The effect of N-Viro application on the soil properties, soil nutrient availability, plant nutrient uptake, and the fruit yield of the lowbush blueberry under irrigated and nonirrigated Nova Scotian conditions is unknown. This study was conducted to fill this knowledge gap in order to develop economically viable and environmentally accepted nutrient management strategies for the use of N-Viro biosolids in lowbush blueberry production in the Atlantic Canada.

2. Materials and Methods

2.1. The Study Site. This study was conducted at the field station of Nova Scotia Wild Blueberry Research Institute, Debert (45° 26′ N, 63° 28′ W), NS, Canada from the vegetative sprout year of 2008 (May 18) to the crop year of 2009 (August 15). The selected field had been under commercial management over the past decade and received biennial pruning by mowing for the past several years along with conventional fertilizer, weed, and disease management practices. The soil at the experimental site is classified as sandy loam (Orthic Humo-Ferric Podzols), which is a well-drained acidic soil. These strongly acidic soils, known as "Truro 52", are of the Hebert association and are mostly found in the Colchester County of Nova Scotia [14].

2.2. Experimental Design and Treatments. A lowbush blueberry field of 0.12 ha was divided into 16 plots of equal size (9 m × 3 m). There was a 3 m buffer strip around and between the 8 irrigated and the 8 nonirrigated plots. The irrigated plots were separated from the nonirrigated plots in order to minimize moisture movement from one treatment to the other. The following 4 experimental treatments were replicated 4 times under randomized complete block design (RCBD):

(i) NI; N-Viro with irrigation,

(ii) FI; inorganic fertilizer with irrigation,

(iii) N; N-Viro without irrigation, and

(iv) F; inorganic fertilizer without irrigation (control).

Table 1: Chemical composition of N-Viro biosolids on dry weight basis.

Components	Value ($\times 10^3$), mg kg^{-1}
Total nitrogen (N)	8
Water insoluble nitrogen (WIN)	5
Available phosphoric acid (P_2O_5)	10
Soluble potash (K_2O)	5
Calcium (Ca)	100
Organic matter	200
Neutralizing value ($CaCO_3$ equivalency)	250
Maximum moisture content	38%
Fineness passing # 10 mesh Tyler screen	65%
Fineness passing # 100 mesh Tyler screen	40%

2.3. Application of N-Viro Biosolids and Inorganic Fertilizer. The N-Viro biosolids used in this study were a product of sludge prepared through chemical and thermal treatments and contained 0.8% of available N and a range of macro- and micronutrients (Table 1). These biosolids were the residual solids left over after the treatment of municipal wastewater. They include organic matter and inorganic compounds from the sewage itself and those produced in the treatment process. The biosolids can be in either liquid or solid form, depending on the treatment process. In this case, the mixing, drying, and pasteurizing of municipal sewage sludge produced a solid granular material, that is, N-Viro biosolids for use as liming material nutrient sources in agricultural soils. Detailed procedure of N-Viro preparation is available at http://www.n-viro.ca/nviro/process-overview.

The N-Viro biosolids were applied to the plots by surface broadcast method at beginning of every year. Initial soil nutrient contents were determined from the soil samples collected prior to the fertilizer application. Representative N-Viro samples were sent for chemical analysis to the Quality Evaluation Division Laboratory, Nova Scotia Department of Agriculture, Truro, NS, which uses the combustion method [15], for total N and the Mehlich-3 soil extraction method [16], to extract the rest of the reported nutrients from soil solutions. Nutrient extraction from N-Viro samples is achieved by using the wet digestion procedure [17] and concentrated nitric acid (HNO_3). Nutrient concentrations of the samples were assessed by inductively coupled plasma emission spectroscopy (ICP OES) using the Atom Scan 16 (Thermo-Jarrell Ash, Franklin, Mass, USA) with appropriate standard curves.

The inorganic fertilizer comprised Ammonium sulphate (16.5%), Diammonium phosphate (34.5%), Potash (4.5%), and sand and/or clay filler (44.5%). The inorganic fertilizer was also surface broadcasted at the recommended N rate of 32 kg ha^{-1} at beginning of every year. Application rate of N-Viro biosolids was more than double of inorganic fertilizer. The inorganic fertilizer and the N-Viro biosolids

used in this study were supplied by the Truro Agromart Ltd. (547 Onslow Road, Truro NS B2N 5G7 Canada; http://www.truroagromart.ca/).

2.4. Soil Sampling and Analysis. Bulk and intact soil core samples were collected from the top 20 cm soil layer prior to the start of experiment and during both years of the study. The bulk soil samples were used to determine soil textural class using hydrometer method [18], for sand, silt, and clay content. These soil samples were also analyzed for macro- and micronutrients by the Quality Evaluation Division Laboratory, Nova Scotia Department of Agriculture, Truro, NS. The macronutrients included N, Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Sodium (Na), and Sulphur (S). The micronutrients included Boron (B), Iron (Fe), Manganese (Mn), Copper (Cu), and Zinc (Zn). The Laboratory follows protocols of the combustion method [15], for total N and the Mehlich-3 soil extraction method [16], to extract the above macro- and micronutrients from soil solutions using the wet digestion procedure [17], and concentrated nitric acid (HNO_3). Nutrient concentrations of the samples are assessed by ICP OES using the Atom Scan 16 (Thermo-Jarrell Ash, Franklin, Mass, USA) with appropriate standard curves.

The soil intact core samples collected with a soil sampler (Soilmoisture Equipment Corp., Santa Barbara, Calif) were used to determine soil bulk density (ρ_b) using the standard procedure [19], that involves oven-dry (at 110°C for 24–48 h) soil mass (M) and volume (V) of the intact cores as

$$\rho_b = \frac{M}{V}. \tag{1}$$

In the third week of July 2008 and 2009, the bulk soil samples were again collected and analyzed to determine the availability of soil macro- and micronutrients for plant uptake. These samples were different from those used for oven-drying.

2.5. Plant Leaf Sampling and Analysis. Concurrent with soil sampling, plant leaves were also randomly sampled [20], from each treatment plot in order to determine the plant leaf nutrient uptake. The plots were divided into sampling areas avoiding low spots, trouble spots, and areas with obvious differences in the plant health. The leaves were collected by hand from 20 random blueberry stems at four to six locations zigzagged within each grid. The dusty leaves or those having soil on them were rinsed with distilled water and dried. To complete the drying process the leaves were placed in the oven at 65°C for 8–10 h [11]. The leaf samples were analyzed by the Quality Evaluation Division Laboratory, Nova Scotia Department of Agriculture, Truro, NS, for macro- and micronutrients listed in the soil analysis section using standard methods [15–17].

2.6. Supplemental Irrigation and Weather Information. Initial baseline soil water characteristics were established prior to the supplemental irrigation application. The values of wilting point (WP) and field capacity (FC) were determined for the soil of the study site by constructing a soil water release curve. A line-source sprinkler irrigation system was used for supplemental irrigation to the plots in irrigation treatment as the soil moisture reached WP conditions. For first year, irrigation was applied on July 11 and August 1, 2008. For second year, irrigation was applied on June 5, June 18, July 20, and August 6, 2009. The soil water contents were monitored at 20 cm soil depth in each plot with a time domain reflectometry (TDR) probes. The plots were irrigated to FC conditions. Quantity of supplemental irrigation was precisely recorded with flow meters. The Environment Canada website, that is, http://www.weatheroffice.gc.ca/canada_e.html was accessed to download daily rainfall and temperature data for Debert, NS.

The crop year 2009 (1,147 mm year^{-1}) was drier than the sprout year 2008 (1,414 mm year^{-1}). The mean minimum and maximum temperatures in the year 2009 were −23.8 and 26.2°C on January 26, 2009 and August 08, 2009, respectively. Because of more precipitation received during the year 2008, comparatively less extreme temperatures were recorded during this year. The mean, minimum and maximum temperatures in the year 2008 were −19.3 and 22.8°C on January 21, 2008 and July 24, 2008, respectively.

Comparatively wetter conditions during sprout year (2008) did not require substantial supplemental irrigation. A total of 189 L of supplemental irrigation was provided to the irrigated plots on July 11, 2008 (115 L) and on August 01, 2008 (74 L), respectively, that amounted to 0.87 mm irrigation per plot during the whole sprout year. On the other hand, the overall lower precipitation and comparatively warmer summer during 2009 demanded frequent supplemental irrigations. Four supplemental irrigations were applied on June 05, June 18, July 20, and August 06 in 2009 that resulted in the application of 149 mm of the supplemental irrigation per plot during the whole fruit year (2009). The supplemental irrigation increased the soil water contents in the irrigated plots.

2.7. Fruit Yield. Fruit yield was harvested between August 10 and 25, 2009. The berries were collected from a randomly selected 1 m^2 area in each experimental plot using hand rakes. The berries were then separated and weighed into unripe and ripe fruit. It is not usual to report the yield of unripe berries. However, we calculated and have reported the yield for both ripe and unripe berries.

3. Statistical Analysis

Analysis of variance (ANOVA) for macro- and micronutrients in soil and plant leaves and fruit yield was performed using statistical software package [21]. Factorial RCBD was considered for constructing ANOVA. Irrigation and fertilizer were the two factors. The macro- and micronutrients in soil, plant leaves and the fruit yield were the dependent variables. When ANOVA indicated a significant effect of any factor or their interaction(s), the treatment means were separated using Tukey's mean separation test. Tukey's mean separation was shown by homogenous group letters, that is, the mean value with letter "a" is significantly different (and large) from

Table 2: Initial physical, hydrological, and chemical properties of the soil at the field station of Nova Scotia Wild Blueberry Research Institute, Debert, NS, Canada.

Parameter	Value
Physical properties	
Sand	72%
Silt	22%
Clay	6%
Bulk density	1.27 g cm^{-3}
Hydrological properties	
Field capacity	0.22 cm^3 cm^{-3}
Wilting point	0.09 cm^3 cm^{-3}
Chemical properties	
pH	5.0
Cation exchange capacity	8.5 meq (100 g)$^{-1}$
N	0.0 mg kg^{-1}
P_2O_5	27 mg kg^{-1}
K_2O	44 mg kg^{-1}
Ca	98×10^3 mg kg^{-1}
Mg	22×10^3 mg kg^{-1}
Na	7×10^3 mg kg^{-1}
S	25 mg kg^{-1}
B	0.11 mg kg^{-1}
Fe	186 mg kg^{-1}
Mn	19.5 mg kg^{-1}
Cu	0.22 mg kg^{-1}
Zn	2.4 mg kg^{-1}

those represented by letters other than "a" and not different from those represented with same letter or its combination with any other letter(s), for example, "ab or ac".

4. Results and Discussion

4.1. Soil Properties. According to the soil analyses results, the soil of the experimental site is sandy loam comprising 70% sand and 6% clay. The soil is neutral with average CEC and low concentrations of macro- and micronutrients (Table 2).

Irrigation treatment effected CEC during 2008 period. The studied soil properties including organic matter (OM) and pH remained unaffected during 2008, and OM, CEC, and pH were unaffected during 2009. Mean values of the soil OM were 5.90 and 4.76% during 2008 and 2009, respectively. This, approximately 20%, decrease in soil OM over the period of two years resulted in the release of nutrients from N-Viro biosolids due to their slow breakdown. However, there was an increasing trend in the soil OM content with irrigation and N-Viro treatments. The NI treatment had the highest OM (6.68%) followed by FI (6.32%), N (6.18%), and F (4.43%) treatments during the year 2008. Similar trends were observed during the year 2009 with the highest soil OM values (5.50%) for the NI treatment (Table 3).

The irrigation treatment improved ($P < .05$) CEC of the soil. In general, the soil CEC values increased from 8.31 in 2008 to 9.51 meq (100 g)$^{-1}$ in 2009. There was a trend for greater CEC to occur in plots treated with irrigation and N-Viro, compared to that in soils managed without irrigation and/or with inorganic fertilizer (Table 3). The NI treatment resulted in the highest CEC during the two years that is, 9.50 meq (100 g)$^{-1}$ in 2008 and 10.7 meq (100 g)$^{-1}$ in 2009, among all the treatments. Comparatively higher CEC values for the irrigated N-Viro plots indicate a considerable improvement in nutrient exchange capacity of the soils in presence of larger soil water contents and organic biosolids.

Table 3: Means separation for the values of soil organic matter (OM), cation exchange capacity (CEC), and pH.

Nutrients	Year	NI[(a)]	FI[(b)]	N[(c)]	F[(d)]
OM (%)		6.68a	6.32a	6.18a	4.43a
CEC (meq/100 g)	2008	9.50a	7.75a	8.75a	7.25a
pH		4.98a	5.05a	4.95a	4.93a
OM (%)		5.50a	4.30a	4.40a	4.83a
CEC (meq/100 g)	2009	10.7a	8.83a	9.50a	9.35a
pH		4.95a	4.85a	4.83a	4.75a

[(a)] N-Viro with irrigation; [(b)] Commercial fertilizer with irrigation; [(c)] N-Viro without irrigation; [(d)] Commercial fertilizer without irrigation. [(d)] Tukey's LSD letters mean differences in mean values within a row.

A decreased soil pH was recorded in 2009 from 2008 for all treatments and their interactions. The wild blueberries are distinct among fruit crops in their soil and fertility requirements as they require an acidic (low pH) soil. Iron chlorosis results if soil pH is appreciably higher than 5.5, whereas, when soil pH drops below 4.8, the possibility of Mn toxicity arises. In either case, plants do not perform well [22]. In case of iron chlorosis the plants cannot uptake enough iron through their roots. Overall, there was a decreasing trend in the soil pH from 2008 to 2009. These results are in agreement with the findings of Eaton et al. [20] who collected soil and leaf samples from 44 lowbush blueberry fields throughout Nova Scotia in 1989-90 and in 1997-98 and reported that the soil pH was decreased by repeated fertilizer applications between the two sample periods.

4.2. Macro- and Micronutrients in Soil. There was no effect of irrigation or fertilizer treatments on any of the macronutrients in the soil samples collected during 2008. In the same year, however, Cu was affected ($P < .05$) by irrigation. During 2009, Ca availability in soil was also ($P < .01$) affected by irrigation and fertilizer treatments and by their interaction. The interaction of irrigation and fertilizer was also significant ($P < .05$) on P and Mg presence in soil during 2009. The soil N did not differ significantly among the treatments.

The Tukey's mean separation test showed that the mean values of N, P, K, Ca, Na, and S during 2008, and all the macronutrients except Ca during 2009, for the all treatments were not different from one another. During 2009, irrigation (393 mg kg^{-1}) and N-Viro (390 mg kg^{-1}) resulted in greater presence of Ca in soil than in nonirrigated (120 mg kg^{-1}) and inorganic fertilizer (123 mg kg^{-1}) plots. Among the individual treatments the mean values of Ca in soil were the

greatest in NI (648 mg kg^{-1}) followed by FI (138 mg kg^{-1}), N (132 mg kg^{-1}), and F (108 mg kg^{-1}) treatments.

4.3. Leaf Nutrient Concentration. Analysis of variance for leaf nutrient concentration of macronutrients, during 2008 season, showed that there was an effect ($P < .01$) of irrigation treatments on Mg concentration in plant leaves. Fertilizer treatment and the interaction between irrigation and fertilizer had a highly significant ($P < .01$) effect on P concentration in plant leaves, while during 2009 there was an effect of the irrigation treatment on N ($P < .01$) and Ca ($P < .05$) concentrations; however, there was no effect for fertilizer treatment and the interaction between the two factors. For concentration of micronutrients in plant leaves during 2008, there was an effect of irrigation on Mn and Zn ($P < .01$) and on Cu ($P < .05$) concentrations. Fertilizer treatment also affected Cu ($P < .01$) and B concentrations in plant leaves. During 2009, the fertilizer treatment affected ($P < .01$) Fe and Zn concentrations in plant leaves.

Results of Tukey's mean separation test for macronutrient show that, during 2008, the Mg concentration in plant leaves was higher under irrigation (0.180 mg kg^{-1}) compared to no irrigation treatment (0.146 mg kg^{-1}). Higher Mg concentration was possibly due to the plant method of nutrient uptake, that is, water uptake from soil [23]. Phosphorus concentration was higher under N-Viro treatments (0.131 mg kg^{-1}) than in inorganic fertilizer treatments (0.118 mg kg^{-1}). Phosphorus concentration under NI treatment appears to have had the highest mean value (0.138 mg kg^{-1}) compared to other interactions. Similarly, NI treatment had the highest mean values for Mg (193 mg kg^{-1}) concentration in plant leaves. During 2009, N and Ca concentrations in plant leaves were higher in irrigated plots compared to nonirrigated plots; mean values were 1655 and 1568 mg kg^{-1} for N and 0.628 and 0.567 mg kg^{-1} for Ca, respectively. Phosphorus was higher under N-Viro treatment compared to inorganic fertilizer treatment; mean values were 0.113 and 0.108 mg kg^{-1}, respectively.

For micronutrients, during 2008, the mean values of Mn, and Zn were higher with irrigation compared to without irrigation treatments; their mean values were 2598 and 1990 mg kg^{-1}, and 16.8 and 14.6 mg kg^{-1}, respectively. Boron under N-Viro treatment (26.3 mg kg^{-1}) was higher than fertilizer treatment (24.3 mg kg^{-1}). The NI gave the highest mean values for plant leaf Mn and it was statistically different from the other three treatments. The N-Viro with irrigation treatment gave highest mean for plant leaf Zn, and it was statistically different and larger from FI and F treatments and not different from N treatment. The mean values of Zn uptake were higher in irrigated plots (16.8 mg kg^{-1}) than in nonirrigated plots (14.6 mg kg^{-1}). The NI treatment resulted in higher and larger uptakes of Zn (17.4 mg kg^{-1}) than the rest of the treatments.

During 2009, the Fe uptake under irrigation treatment was higher (47.8 mg kg^{-1}) than that without irrigation (40.2 mg kg^{-1}). A similar trend was shown by Zn uptake as it was higher under irrigation treatments (14.7 mg kg^{-1}) compared to without irrigation treatments (13.2 mg kg^{-1}). That might be related to the fact that most plant nutrient uptake occurs with water uptake through the roots in soil [23]. Interactions between irrigation and fertilizer treatments on micronutrient concentration in plant leaves were significant for all the micronutrients except for Cu. The highest mean values were under NI, 17.4, 21.5, 1572, and 15.4 mg kg^{-1} for B, Fe, Mn, and Zn, respectively. Our results agreed with the findings of previous studies [20, 24–27].

4.4. Fruit Yield. Supplemental irrigation resulted in a 21% increase in the ripe fruit yield from nonirrigated (2620 kg ha^{-1}) and irrigated (3320 kg ha^{-1}). Our results are in agreement with the findings of Seymour et al. [12] who studied the effect of irrigation on lowbush blueberry crop yield and quality near the coast of Maine; they reported that the irrigated plots averaged 43% higher yield than rain fed plots for the 2000-2001 production cycle. There was an effect ($P < .05$) of irrigation on the yield of unripe fruits also; however, the Tukey's mean separation test did not result in separation of mean values of either ripe or unripe berries' yield for different treatment. These results show that the application of N-Viro biosolids resulted in fruit yield comparable to that from inorganic fertilizer application.

Based on the quotation from the nutrient sources supplier for this experiment, Truro Agromart Ltd. (547 Onslow Road, Truro NS B2N 5G7 Canada; http://www.truroagromart.ca/), the N-Viro and the inorganic fertilizer cost $116 and $280 CAD, respectively, per hectare. This results in approximately 2.5 times economical availability of N-Viro than the commercial inorganic fertilizer. We concluded that the increasing prices of inorganic fertilizers would compel farmers to use economically available N-Viro that, coupled with supplemental irrigation, performed better than the inorganic fertilizer regarding improved soil properties, soil nutrient availability, plant nutrient uptake, and the lowbush blueberry yield during this study.

5. Summary and Conclusions

Since the processed biosolids (N-Viro) are rich in organic matter and plant nutrients, they should supply the required nutrient to plants as do the commercially available inorganic fertilizers. This study investigated the impact of N-Viro biosolids application on soil properties, soil nutrient availability, leaf nutrient concentration, and the fruit yield of lowbush blueberry (*Vaccinium angustifolium* Ait.) grown under irrigated and nonirrigated conditions during 2008 and 2009 at the Wild Blueberry Research Institute, Debert, NS, Canada. Four treatments, that is, N-Viro with irrigation (NI), inorganic fertilizer with irrigation (FI), N-Viro without irrigation (N), and inorganic fertilizer without irrigation (F) were replicated four times under randomized complete block design. Soil samples, collected from the top 20 cm soil layer, and the plant leaf samples were analyzed for concentration of macro- and micronutrients in soil and plant leaves, respectively.

We have discussed our findings on the impact of (1) N-Viro biosolids and (2) supplemental irrigation on the availability of (A) macro- and (B) micronutrients, plant nutrient concentration, and the fruit yield of lowbush blueberry.

Results showed that the soil pH decreased over time in all the treatments in the range of 4.93 (2008) and 4.79 (2009). Largest amount of soil organic matter was recorded for NI treatment (6.68%) followed by FI (6.32%), N (6.18%), and F (4.43%) treatments during the year 2008. During the subsequent year (2009), a similar trend was observed when soil organic matter was highest for NI treatments (5.50%).

Presence of soil moisture coupled with organic decomposition of N-Viro resulted in more availability of soil macronutrients for plant uptake. Higher plant leaf nutrient uptake was recorded in irrigated plots than in nonirrigated plots. Irrigation or fertilizer did not significantly ($P > .05$) affect the ripe fruit yield; however, irrigation had significant ($P < .05$) effect on the yield of unripe fruits. A 21% increase in the ripe fruit yield was recorded for irrigated treatments ($3320\,\mathrm{kg\,ha^{-1}}$) in comparison with yield from nonirrigated treatments ($2620\,\mathrm{kg\,ha^{-1}}$). Best nutrient management practices may include the use of economically viable organic N-Viro to substitute the expensive and potentially hazardous commercial fertilizers.

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