Moisture Effects on Nitrogen Availability in Municipal Biosolids from End-of-Life Municipal Lagoons

Nicholson N. Jeke, Francis Zvomuya,* and Lisette Ross

Abstract

Nitrogen (N) availability affects plant biomass yield and, hence, phytoextraction of contaminants during phytoremediation of end-of-life municipal lagoons. End-of-life lagoons are characterized by fluctuating moisture conditions, but the effects on biosolid N dynamics have not been adequately characterized. This 130-d laboratory incubation investigated effects of three moisture levels (30, 60, and 90% water-filled pore space [WFPS]) on N mineralization (Nmin) in biosolids from a primary (PB) and a secondary (SB) municipal lagoon cell. Results showed a net increase in Nmin with time at 60% WFPS and a net decrease at 90% WFPS in PB, while Nmin at 30% WFPS did not change significantly. Moisture level and incubation time had no significant effect on N0 in SB. Nitrogen mineralization rate in PB followed three-half-order kinetics. Potentially mineralizable N (N0) in PB was significantly greater at 60% WFPS (222 mg kg−1) than at 30% WFPS (30 mg kg−1), but rate constants did not differ significantly between the moisture levels. Nitrogen mineralization in SB followed first-order kinetics, with Nmin significantly greater at 60% WFPS (68.4 mg kg−1) and 90% WFPS (94.1 mg kg−1) than at 30% WFPS (32 mg kg−1). Low Nmin in SB suggests high-N-demanding plants may eventually have limited effectiveness to remediate the biosolids. Nitrogen mineralization rate in PB followed three-half-order kinetics. Potentially mineralizable N (N0) in PB was significantly greater at 60% WFPS (222 mg kg−1) than at 30% WFPS (30 mg kg−1), but rate constants did not differ significantly between the moisture levels. Nitrogen mineralization in SB followed first-order kinetics, with Nmin significantly greater at 60% WFPS (68.4 mg kg−1) and 90% WFPS (94.1 mg kg−1) than at 30% WFPS (32 mg kg−1). Low Nmin in SB suggests high-N-demanding plants may eventually have limited effectiveness to remediate biosolids in the secondary cell. While high Nmin in PB would provide sufficient N to support high biomass yield, phytoextraction potential is reduced under dry and near-saturated conditions. These results have important implications on the management of moisture during phytoextraction of contaminants in end-of-life municipal lagoons.

Core Ideas

• Moisture content affects N mineralization in primary municipal biosolids.
• Moisture effects on N mineralization are minimal in secondary biosolids.
• Nitrogen mineralization in primary cell biosolids greatest at 60% water-filled pore space.
management option. For example, in 2010, stringent nutrient regulations under the Manitoba, Canada, Water Protection Act forced the City of Winnipeg to reduce biosolids loading rates on receiving land during summer months and cease land application altogether during winter, which forced the City to adopt landfilling as an alternative (City of Winnipeg, 2013). However, this is a temporary solution for the City because the Canadian government is calling for a ban on landfilling and incineration of biosolids by 2020 to fight climate change (Ouimet et al., 2015). Clearly, there is a need to explore alternative approaches to manage biosolids, and in situ phytoremediation may be a cost-effective and environmentally friendly strategy for in situ treatment of biosolids in end-of-life lagoons.

Nitrogen mineralization is a microbial-mediated process and is, therefore, controlled by factors that affect microbial processes, such as temperature and moisture. Most published studies on \( N\text{min} \) in biosolids—or sewage-sludge-amended soils—were conducted at moisture levels near field capacity, which is considered optimum for mineralization (Gilmour et al., 2003; Er et al., 2005; Gil et al., 2011; Corrêa et al., 2012). Results from such studies may not reflect \( N\text{min} \) in end-of-life municipal lagoons, where optimal conditions are rarely achieved. Municipal lagoons are constructed at sites underlain by clay material, in the absence of which a compacted layer of clay is used to control seepage to groundwater (Spellman and Drinan, 2014). As a result, end-of-life lagoons are poorly drained, leading to ponding or saturation during spring snowmelt or periods of heavy rain. Lagoons can also become drier in periods of drought. Therefore, anaerobic and aerobic conditions are common in end-of-life lagoon environments.

An understanding of moisture effects on \( N\text{min} \) can help predict \( N\text{min} \) under fluctuating moisture conditions typical of end-of-life lagoons. The removal of contaminants from lagoon biosolids by harvesting plants depends on the plant biomass yield and concentrations of contaminants in the biomass. Nitrogen available for plant uptake in biosolids affects plant biomass yield and hence phytoremediation performance. Moisture content of biosolids in the lagoon affects microbial processes involved in N transformation. Therefore, the availability of N at different moisture levels in lagoons provides insight into the potential impacts of N on biomass yield and potential phytoextraction of N and other nutrients and contaminants in the harvested biomass.

Nitrogen mineralization in biosolids is influenced by biosolids type and properties, the wastewater treatment method used to generate the biosolids, and the type and length of the stabilization process used (Coggins et al., 2006; Corrêa et al., 2012). Generally, \( N\text{min} \) has been found to be greater in aerobically digested than in anaerobically digested biosolids (Hseu and Huang, 2005). Wang et al. (2003) reported mineralization rates of 32% of organic N in aerobically digested and 15% of organic N in anaerobically digested municipal biosolids incorporated into soil and incubated for 26 wk. Biosolids composting or storage in lagoons can result in a decrease in \( N\text{min} \) potential (Gilmour et al., 2003). Corrêa et al. (2012) reported the absence of mineralization in composted sewage sludge during a 23-wk incubation experiment. Biosolids stored in a lagoon for more than 15 yr did not mineralize in a laboratory incubation at 25°C and moisture content near field capacity, and this was attributed to stabilization by long-term lagoon storage (Gilmour et al., 2003).

High-N biosolids, such as digested, lime-stabilized, and heat-treated biosolids, which are typically stabilized over short periods, are more reactive than low-N biosolids that are treated under long residence times in lagoons (Brown and Henry, 2002). Nitrogen mineralization is likely to be less sensitive to water content as substrates become less decomposable (Paul et al., 2003). Wastewater treatment lagoons in small communities have average lifespans of 30 to 35 yr (Ross et al., 2003), and, therefore, biosolids generated under long residence times in such lagoons may respond differently to changes in water content compared with biosolids stabilized over short periods. Therefore, characterization of potential N supply for plant growth may provide insight into the potential success of phytoremediation of the biosolids, which relies on optimum plant growth and, hence, the supply of N and other nutrients.

There is currently a dearth of published information on the effects of moisture on \( N\text{min} \) in biosolids that are not mixed with soil. Many of the studies on sewage- or biosolids-amended soils have been conducted at moisture levels near field capacity, which is appropriate for agricultural crop production. However, results from such studies are not directly applicable to end-of-life lagoons, which are characterized by widely fluctuating moisture conditions. The overall objective of this study was, therefore, to determine the effects of moisture level (30, 60, and 90% WFPS) on \( N\text{min} \) in biosolids from an end-of-life municipal lagoon destined for in situ phytoremediation.

Materials and Methods

**Biosolids**

Biosolids samples were collected from an end-of-life municipal lagoon in Niverville, Manitoba, Canada (49°35'42.7" N, 97°02'50.3" W). The lagoon, which operated for 37 yr (1971–2008), consisted of a primary cell (4.6 ha) and a secondary cell (8.8 ha), which operated in a series flow. The primary cell was the treatment cell, which received raw wastewater, while the secondary cell was the holding cell, which received effluent from the primary cell for further treatment and storage. The lagoon treated primarily domestic water and served a population of 2500 people (Statistics Canada, 2007). Sewage sludge settled at the bottom of the lagoon and was stabilized during long-term storage in the lagoon without any pretreatment. When the lagoon ceased operation, the volume of biosolids in the primary and secondary cells were approximately 20,000 and 28,000 m³, respectively. Biosolids samples were collected from several points to give a broad spatial coverage in each of the cells in the summer of 2011. The biosolids depth in the two cells averaged 20 cm at the time of sampling. Biosolids were sampled using shovels across the entire depth and placed into 20-L plastic pails. At the time of sampling, the biosolids had dewatered by evaporation.

**Microcosm Setup**

Biosolids samples from each cell were composited and thoroughly mixed. Subsamples (90 g dry wt.) from the composites were packed into plastic containers (6-cm diam. by 7-cm height) to bulk densities of 0.66 Mg m⁻³ for PB and 0.77 Mg m⁻³ for SB. A total of 180 microcosms (i.e., two biosolids × three water contents × three replicates × 10 sampling dates) were prepared to accommodate the ten sampling dates. Reverse osmosis water was
added to bring the biosolids to 30, 60, and 90% WFPS, which corresponded to 0.22, 0.44, and 0.67 kg H₂O kg⁻¹ for PB and 0.12, 0.32, and 0.48 kg H₂O kg⁻¹ for SB. These moisture levels correspond to relatively dry, field-capacity, and near-saturation moisture conditions, respectively, in the biosolids. After watering, the plastic containers were capped with lids, which had four 2-mm diam. holes to allow gaseous exchange.

The experiment was laid out as a completely randomized design with a factorial combination of biosolids (PB and SB) and moisture level (30, 60, and 90% WFPS). The microcosms were placed in random order in the dark in an incubator set at 25°C. A humidifier (Sunbeam Humidifier Model SUL 496-CN) was placed in the incubator to reduce evaporation from the biosolids. The microcosms were weighed every 5 d and reverse osmosis water was added to replace any moisture lost via evaporation. Triplicate units from each treatment were removed from the incubator on Days 0, 3, 6, 13, 20, 30, 50, 70, 100, and 130 and moisture conditions, respectively, in the biosolids. After watering, the plastic containers were capped with lids, which had four 2-mm diam. holes to allow gaseous exchange.

Laboratory Analysis

Samples were extracted for inorganic N concentrations and filtrates analyzed within 5 d of retrieval from the incubator. Total Kjeldahl N was determined using a flow injection analyzer (FIAlab 2500, FIAlab Instruments) following digestion with sulfuric acid (Bremner and Mulvaney, 1982). Total C was determined by dry combustion using a CNS2000 analyzer (Leco Corp.) on samples that had been air-dried (~20°C) and finely ground (<0.1 mm). Inorganic N (nitrate N [NO₃⁻-N] + nitrite N [NO₂⁻-N] + ammonium N [NH₄⁺-N]) concentration was determined by dry combustion using a Model AA3 autoanalyzer (Bran+Luebbe) following extraction of a 5-g subsample of moist biosolids with 25 mL of 2 M KCl (Mulvaney, 1996). A subsample from each unit was oven-dried for determination of gravimetric moisture content so that concentrations could be expressed on a dry-weight basis.

Calculations

Water-filled pore space (percentage) was calculated as follows:

\[ \text{WFPS} = 100 \times \frac{\theta_v}{\rho_v} \]

where \( \theta_v \) is the volumetric water content (\( \theta_v = \theta_t \times \rho_v \)), \( \rho_v \) is total porosity (\( 1 - \rho_b/\rho_s \)), \( \rho_b \) is the gravimetric water content (g g⁻¹), \( \rho_s \) is the bulk density (Mg m⁻³), and \( \rho_s \) is particle density (assumed to be 1.3 Mg m⁻³ for the biosolids [Hillel, 1998]).

Net mineralized N (\( N_{\text{min}} \)) concentration (mg kg⁻¹) was calculated as follows:

\[ N_{\text{min}} = (N_i - (N_i)_0) \]

where \( (N_i) \) is inorganic N concentration (mg kg⁻¹) at time \( t \) (d) and \( (N_i)_0 \) is inorganic N concentration (mg kg⁻¹) at time 0.

Statistical Analysis

Analysis of Variance

All data were analyzed with the GLIMMIX procedure for repeated measures in SAS version 9.3 (SAS Institute, 2011), with biosolids and moisture level as fixed effects and time as the repeated measure. Various covariance structures were compared using the Akaike information criterion (AIC), and the compound symmetry structure, which had the lowest AIC, was chosen as the best fit for the repeated measurements analysis. Treatment differences were deemed significant if \( P < 0.05 \) using the Tukey–Kramer adjustment for multiple comparisons.

Kinetic Model Fitting

Five kinetic models (first-order, combined first- and zero-order, three-half-order [linear and exponential], and a first-order double compartment model) were fitted to the \( N_{\text{min}} \) data and compared using PROC NLIN in conjunction with the Marquardt algorithm in SAS (SAS Institute, 2011). The model with the lowest AIC was chosen as the best fit. In PB at 30 and 60% WFPS, the models were fit when \( N_{\text{min}} \) started to increase on Day 6. Models were not tested at 90% WFPS, where negative mineralization was observed, since the models were proposed for positive net mineralization.

Nitrogen mineralization in PB was best described by a linear form of the three-half-order kinetic model (Trefry and Franzmann, 2003):

\[ N_{\text{min}} = N_0 \left[1 - \exp\left(-k_1 t - k_2 t^2/2\right)\right] + k_0 t \]

where \( N_0 \) (mg N kg⁻¹) is potentially mineralizable N in the easily decomposable pool at the start of incubation, \( k_1 \) is a first-order rate constant (d⁻¹), \( k_2 \) is a linear second-order microbial biomass growth rate term (d⁻²), and \( k_0 \) is a zero-order rate constant (mg kg⁻¹ d⁻¹).

Nitrogen mineralization in SB was best described by a first-order kinetic model:

\[ N_{\text{min}} = N_0 \left[1 - \exp(-k_1 t)\right] \]

where \( k_1 \) is the first-order rate constant (d⁻¹). Parameters for different treatments were considered significantly different if their 95% confidence intervals did not overlap.

Results and Discussion

Biosolids Properties

Total N concentration was significantly greater in PB than in SB (Table 1). The difference in N concentration in the biosolids was likely due to the different types of sewage sludge produced

<table>
<thead>
<tr>
<th>Property</th>
<th>PB†</th>
<th>SB‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total N (g kg⁻¹)</td>
<td>7.7</td>
<td>1.8</td>
</tr>
<tr>
<td>Organic N (g kg⁻¹)</td>
<td>7.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Total P (g kg⁻¹)</td>
<td>2.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Total C (g kg⁻¹)</td>
<td>151</td>
<td>72</td>
</tr>
<tr>
<td>C/N ratio</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>NO₃⁻-N (mg kg⁻¹)</td>
<td>532</td>
<td>183</td>
</tr>
<tr>
<td>NH₄⁺-N (mg kg⁻¹)</td>
<td>69</td>
<td>3.5</td>
</tr>
<tr>
<td>Olsen P (mg kg⁻¹)</td>
<td>251</td>
<td>143</td>
</tr>
<tr>
<td>pH</td>
<td>7.1</td>
<td>7.6</td>
</tr>
<tr>
<td>EC (dS cm⁻¹)</td>
<td>4.2</td>
<td>2.8</td>
</tr>
</tbody>
</table>

† PB, biosolids from the primary cell.
‡ SB, biosolids from the secondary cell.
§ EC, electrical conductivity.
during the wastewater treatment processes occurring in the primary and secondary lagoon cells. Primary wastewater treatment, which occurs in the primary cell, involves the removal of heavy solids from the wastewater by sedimentation, resulting in the accumulation of concentrated primary sludge, which is essentially fecal (USEPA, 1997; Haynes et al., 2009). Effluent containing lighter solids, soluble organic matter, reduced nutrients, and trace elements overflows to the secondary cell where most of the microbial biodegradation takes place. Secondary sludge is mainly composed of bacterial biomass (USEPA, 1997; Haynes et al., 2009). Therefore, PB, which was essentially composed of fecal matter, had greater N and C concentrations than SB, which was primarily composed of bacterial biomass (Haynes et al., 2009).

Both PB and SB had lower total N concentrations (7.7 and 1.8 g kg⁻¹, respectively) compared with total N concentrations reported in other studies. For example, Cogger et al. (2004) reported 23 g kg⁻¹ total N in biosolids stored for more than 15 yr. Biosolids, such as those used in our study, generated from sewage sludge stabilized in lagoons where the residence time spans several years are generally classified as low-N biosolids with total N ranging from 10 to 30 g kg⁻¹ (Brown and Henry, 2002). High-N biosolids include anaerobically digested, lime-stabilized, and heat-treated biosolids and have total N ranging from 30 to 60 g kg⁻¹ (Brown and Henry, 2002). About 8% of total N in PB and 10% of total N in SB in the present study was in the inorganic form.

### Inorganic Nitrogen Concentration

There was a significant biosolids × moisture × time interaction (P < 0.001) for NO₃⁻N concentration (Table 2). In PB, NO₃⁻N concentration differed significantly among moisture levels at all sampling times except on Day 3 (Fig. 1a). Initial NO₃⁻N flushes in PB at 30 and 60% WFPS decreased fairly slowly from Day 6 to Day 70 but more sharply thereafter, likely as a result of increased anaerobicity and limited N immobilization observed in our study is unlikely to adversely affect plant growth.

The initial NO₃⁻N flushes in PB at 30 and 60% WFPS were likely due to inhibition of microbial activity as a result of reduced diffusion of substrates (Schjonning et al., 2003), reduced microbial mobility (Killham et al., 1993), and reduced microbial growth. In PB, NO₃⁻N concentration at 90% WFPS decreased continuously relative to 30 and 60% WFPS during the entire incubation period after the initial NO₃⁻N flush (Fig. 1a). This was likely due to low N_{min} under near-saturated conditions and N loss via denitrification. Previous studies have shown increases in N₂O emission with increasing soil water content, with emissions increasing most rapidly above 60% WFPS (Doran et al., 1990; Abbasi and Adams, 2000) and in biosolids-amended soil (Mendoza et al., 2006; Pu et al., 2010). In our study, NO₃⁻N concentration in PB at 90% WFPS decreased fairly slowly from Day 6 to Day 70 but more sharply thereafter, likely as a result of increased anaerobicity and reduced diffusivity of substrates (Schjonning et al., 2003).

### Nitrogen Mineralization

<table>
<thead>
<tr>
<th>Effect†</th>
<th>NO₃⁻N</th>
<th>NH₄⁺N</th>
<th>N_{min}‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg kg⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biosolids (B)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary biosolids</td>
<td>723</td>
<td>15</td>
<td>101</td>
</tr>
<tr>
<td>Secondary biosolids</td>
<td>173</td>
<td>3.6</td>
<td>25</td>
</tr>
<tr>
<td>Moisture (M)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30% WFPS</td>
<td>463</td>
<td>8.7</td>
<td>77</td>
</tr>
<tr>
<td>60% WFPS</td>
<td>514</td>
<td>7.8</td>
<td>128</td>
</tr>
<tr>
<td>90% WFPS</td>
<td>367</td>
<td>11.4</td>
<td>−16</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

† Time main effects are not presented for the 10 multiple sampling times. The interactions are presented in figures.

‡ N_{min} = (N_i)_{t} − (N_i)_{0}, where (N_i)_{t} is the inorganic N at time t (d) and (N_i)_{0} is the inorganic N concentration (mg kg⁻¹) at Day 0.

The biosolids used in the study were kept at room temperature (21 ± 2°C) until they were used. This probably maintained a high population of nitrifying bacteria (Agehara and Warncke, 2005), which proliferated on rewetting.

The initial (Days 0–3) NO₃⁻N flushes in PB at 30 and 60% WFPS were followed by a period of temporary net immobilization from Day 3 to 6 (Fig. 1a). The proliferation of microorganisms that rapidly decomposed substrate during the initial NO₃⁻N flush might have created a huge demand for available N. As a result, available N was assimilated into the microbial biomass, thus temporarily decreasing available N. The brief N immobilization observed in our study is unlikely to adversely affect plant growth.

Following the initial NO₃⁻N immobilization, NO₃⁻N concentration in PB at 60% WFPS increased by 268 mg kg⁻¹ (37%) from Day 6 to Day 50 and remained relatively constant thereafter, while NO₃⁻N concentration increased only slightly at 30% WFPS from Day 6 onward (Fig. 1a). Nitrate N concentrations were significantly greater in PB at 60% WFPS than at 30% WFPS at Day ≥50. The lower NO₃⁻N concentrations at 30% WFPS were likely due to inhibition of microbial activity as a result of reduced diffusion of substrates (Schjonning et al., 2003), reduced microbial mobility (Killham et al., 1993), and reduced microbial growth.

In PB, NO₃⁻N concentration at 90% WFPS decreased continuously relative to 30 and 60% WFPS during the entire incubation period after the initial NO₃⁻N flush (Fig. 1a). This was likely due to low N_{min} under near-saturated conditions and N loss via denitrification. Previous studies have shown increases in N₂O emission with increasing soil water content, with emissions increasing most rapidly above 60% WFPS (Doran et al., 1990; Abbasi and Adams, 2000) and in biosolids-amended soil (Mendoza et al., 2006; Pu et al., 2010). In our study, NO₃⁻N concentration in PB at 90% WFPS decreased fairly slowly from Day 6 to Day 70 but more sharply thereafter, likely as a result of increased anaerobicity...
as microbial activity depleted oxygen. Therefore, restricted Nmin or loss of N through denitrification in end-of-life lagoons is expected during long periods of saturation, such as during spring snowmelt or periods of heavy rain. Although, from a remediation perspective, N is lost from end-of-life lagoons during long periods of saturation, low N availability under such conditions reduces plant productivity, hence phytoextraction of other contaminants.

Nitrate N concentrations did not differ significantly among moisture levels in SB at all sampling times (Fig. 1b), suggesting that the organic N pool in SB was more resistant to mineralization than that in PB. Similarly, NO3–N concentration in SB did not vary significantly with sampling time, regardless of moisture level. The labile organic pool in SB might have been biodegraded during long-term storage, leaving a recalcitrant organic N fraction possibly composed of stable bacterial cell walls (Haynes et al., 2009). Mineralization becomes less sensitive to water content as the substrate becomes less decomposable (Paul et al., 2003).

On average, NH4–N accounted for less than 3% of total inorganic N in the biosolids during the incubation. Nonetheless, there was a significant moisture × biosolids × time interaction (P < 0.004) for NH4–N concentration (Table 2). A distinct, rapid decline in NH4–N concentration occurred in PB at all moisture levels during the first 3 d of incubation (Fig. 2a). This rapid decline in NH4–N concentration coincided with the initial NO3–N flush reported above (Fig. 1a) and was likely due to rapid nitrification, hence rapid consumption of NH4–N. The rapid decline in NH4–N concentration was followed by a slight increase in NH4–N concentration, after which the NH4–N concentration decreased to less than 4 mg kg⁻¹ on Day 100 at all moisture contents (Fig. 2a). Ammonium N concentration then increased to a mean of about 12 mg kg⁻¹ from Day 100 to Day 130. The reason for this increase is not clear; however, although statistically significant, the biophysical significance of the observed increase in NH4–N concentration was minimal, considering that more than 90% of the inorganic N was in the nitrate form (Fig. 1). Trace amounts of NH4–N (<5.5 mg kg⁻¹) were measured in SB throughout the sampling period with no significant temporal changes.

The NH4–N/NO3–N ratio observed in PB at 90% WFPS was lower than expected, considering that ammonification is mediated by heterotrophic microorganisms that function in both aerobic and anaerobic conditions (Nugroho and Kuwatsuka, 1990; Vymazal, 2007). In contrast, nitrifying bacteria are obligate aerobes and their activities are restricted in near-saturated conditions, resulting in limited nitrification (Sierra et al., 2001). The low NH4–N concentrations measured at 90% WFPS in our study suggest that biosolids were aerobic enough under the near-saturated conditions to allow nitrification to occur to the extent that it caused measurable decreases in NH4–N concentration. The subsequent decrease in NO3–N concentrations can be attributed to denitrification. Alternatively, our results may suggest that ammonium loss occurred in the form of N2O through nitrifier denitrification by autotrophic NH3 oxidizers (Webster and Hopkins, 1996; Wrage et al., 2001).

Mineralized Nitrogen Concentration

The effects of moisture level on Nmin concentration varied with biosolids type and sampling time, as indicated by the significant biosolids × moisture level × sampling time interaction (Table 2). In PB, Nmin concentration was positive for 30 and 60% WFPS at all sampling times, while Nmin concentration decreased...
at 90% WFPS (Fig. 3a). Net mineralized N concentration in PB was significantly greater at 60% WFPS than at 30% WFPS on Day ≥50. The low \( N_{\text{min}} \) at 30% WFPS suggests that N availability in end-of-life lagoons during long periods of drought may not sustain healthy plant growth of high-N-demanding plants. Low plant available N reduces the phytoextraction of N and other contaminants because of reduced biomass yields. The apparent negative \( N_{\text{min}} \) at 90% WFPS in PB, which was probably due to denitrification, suggests that N deficiency may adversely affect the development and biomass yields of plants in near-saturated biosolids, thus reducing the effectiveness of phytoextraction in cases where the plants are used for phytoremediation. Despite the low \( N_{\text{min}} \) at 30% WFPS and decreasing \( N_{\text{min}} \) at 90% WFPS in PB, total plant available N concentrations at the end of the experiment (880 and 390 mg kg\(^{-1}\), respectively), were still sufficient for plant growth.

Moisture level had no significant effect on \( N_{\text{min}} \) in SB, regardless of sampling time (Fig. 3b). Additionally, \( N_{\text{min}} \) did not differ significantly among sampling times, regardless of moisture level. The lack of treatment effects in SB can be attributed to a nonlabile pool of organic N. Secondary biosolids will likely provide inadequate available N to support high biomass yields during periods of rapid growth, regardless of moisture level. Low plant productivity reduces the phytoextraction of N and other contaminants. Harvesting of plants may be limited to a single cut per season instead of multiple harvesting because low \( N_{\text{min}} \) in SB may not replenish available N fast enough to support a robust regrowth during the growing season.

Plants such as switchgrass (\( Pani\)cum \( \text{virgatum} \) L.) that can produce high biomass yields in N limiting conditions would likely successfully remediate N and other contaminants in SB. Switchgrass was selected by the US Department of Energy’s Bioenergy Feedstock Development Program as a model crop because of its high biomass yield and low water and nutrient requirements (McLaughlin, 1992; McLaughlin and Walsh, 1998). Giannoulis and Danalatos (2014) studied nutrient-use efficiency and uptake characteristics of switchgrass and reported that switchgrass has low N requirements and can be grown in less fertile soils and still produce high biomass yields.

**Nitrogen Mineralization Kinetics**

**Primary Cell Biosolids**

Nitrogen mineralization in PB incubated at 30 and 60% WFPS followed three-half-order kinetics (Table 3; Fig. 4). This suggests that PB contained two N pools with different degrees of biodegradability. The first-order kinetic component of the three-half-order kinetic model describes mineralization of the labile organic N fraction and takes into account microbial growth (Trefry and Franzmann, 2003), while the zero-order component describes mineralization of the less labile N fraction. The slow \( N_{\text{min}} \) described by the zero-order component may also be caused by depletion of essential nutrients or cessation of microbial growth (Trefry and Franzmann, 2003).

Several laboratory incubation studies have shown that \( N_{\text{min}} \) in biosolids is characterized by at least two N pools: a rapid- and a slow-release pool (Lerch et al., 1992; Smith et al., 1998; Gil et al., 2011).

![Fig. 3. Net mineralized N (\( N_{\text{min}} \)) concentration in (a) primary cell biosolids (PB) and (b) secondary cell biosolids (SB) as affected by moisture content. Vertical bars represent standard errors of the mean.](Image)

![Fig. 4. Net mineralized N (\( N_{\text{min}} \)) concentration in primary cell biosolids (PB) at 30 and 60% water-filled pore space (WFPS) as described by a three-half-order kinetic model.](Image)

**Table 3. Three-half-order kinetic model parameter estimates for net mineralized N (\( N_{\text{min}} \)) concentration in primary cell biosolids incubated at 30 and 60% water-filled pore space (WFPS).†**

<table>
<thead>
<tr>
<th>WFPS</th>
<th>( N_0 )</th>
<th>( k_0 )</th>
<th>( k_1 )</th>
<th>( k_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>mg kg(^{-1})</td>
<td>mg kg(^{-1}) d(^{-1})</td>
<td>d(^{-1})</td>
<td>d(^{2})</td>
</tr>
<tr>
<td>30</td>
<td>30b‡</td>
<td>0.995a</td>
<td>0.024a</td>
<td>0.004a</td>
</tr>
<tr>
<td>60</td>
<td>222a</td>
<td>0.672a</td>
<td>0.012a</td>
<td>0.003a</td>
</tr>
</tbody>
</table>

† \( N_{\text{min}} = N_0 \left[1 - \exp(-k_1 t - k_2 t/2)\right] + k_0 t \), where \( N_0 \) is the potentially mineralizable N pool at the start of incubation, \( k_1 \) is a first-order rate constant, \( k_2 \) is a linear second-order microbial biomass growth rate term, and \( k_0 \) is the zero-order rate constant.

‡ Means in a column followed by the same letter are not significantly different. Differences were considered significant if 95% confidence intervals of the means did not overlap.
Potentially mineralizable N concentration in PB was greater at 60% WFPS (222 mg kg\(^{-1}\)) than at 30% WFPS (30 mg kg\(^{-1}\)) (Table 3), indicating that the full mineralization potential was not attained at 30% WFPS. The lower N\(_0\) at 30% WFPS was probably due to a smaller microbial population under the low moisture conditions. Schimel et al. (1999) and Bottner (1985) reported low microbial diversity under dry soil conditions, with microbial communities better adapted to the drier conditions thriving. Agehara and Warncke (2005) attributed the increase in N\(_0\) with increasing moisture content to a shift in microbial populations, with those microbes that thrive at higher moisture content metabolizing substrates not utilized at lower moisture contents. Therefore, at 30% WFPS, limited microbial diversity likely resulted in a lower fraction of the total mineralizable N being mineralized while a greater microbial diversity at 60% WFPS produced a greater N\(_0\) and total mineralized N concentration. Rate constants (k\(_0\), k\(_1\), and k\(_2\)) did not differ significantly between 30% (0.995 mg kg\(^{-1}\) d\(^{-1}\), 0.024 d\(^{-1}\), and 0.004 d\(^{-2}\)) and 60% (0.672 mg kg\(^{-1}\) d\(^{-1}\), 0.012 d\(^{-1}\), and 0.003 d\(^{-2}\)) WFPS in PB (Table 3), which is likely a result of the large standard errors associated with rate constant estimates (Benbi and Richter, 2002).

The microbial term of a three-half-order model can have a linear or exponential component and the components describe the rate at which the bioavailable substrate fraction is depleted (Treffry and Franzmann, 2003). The better fit of N\(_{\text{min}}\) data to a linear rather than exponential growth component in the three-half-order kinetic model was unexpected since microbial growth typically follows exponential growth (Brunner and Focht, 1984). Brunner and Focht (1984) suggested that linear rather than exponential microbial growth can be attributed to restricted diffusion of substrate or nutrients to microbes. Diffusion of substrate to microbes can be limited because of the medium matrix or more prominently by the distribution of microbes on the medium surface (Zobell, 1943; Marshall, 1971). When microbial cell density exceeds the surface that the microbes can occupy because of a large microbial community, multilayers of cells are formed on the surface and diffusion of substrate to the inner cells is limited (Brunner and Focht, 1984). Comparable results from published literature are currently nonexistent since the three-half-order kinetic model has not been widely used to describe N\(_{\text{min}}\) data. The model has instead been widely fitted to C mineralization and organic compound biodegradation data (Treffry and Franzmann, 2003).

**Secondary Cell Biosolids**

Nitrogen mineralization in SB was adequately described by the first-order kinetic model at all moisture levels (Fig. 5), suggesting the existence of a single pool of N\(_0\) that mineralized at a rate proportional to its concentration (Stanford and Smith, 1972). The three-half-order kinetic model failed to converge for SB. First-order kinetics are fitting when the half-life is independent of time and concentration, indicating that the population of degrader microorganisms does not change with time (Johnsen et al., 2013). At 60 and 90% WFPS, N\(_{\text{min}}\) in SB increased from 0 to 60 mg kg\(^{-1}\) during the entire incubation period, while a rapid N release was observed at 30% WFPS during the first 30 d, after which it plateaued at about 30 mg kg\(^{-1}\) (Fig. 5). Potentially mineralizable N in SB was significantly greater at 60 (68.4 mg kg\(^{-1}\)) and 90% WFPS (94.1 mg kg\(^{-1}\)) than at 30% WFPS (32 mg kg\(^{-1}\)) (Table 4). The lower microbial activity as a result of limited substrate diffusion and microbial mobility in SB at 30% WFPS (Stark and Firestone, 1995; Schimel et al., 2007) likely resulted in the N\(_{\text{min}}\) plateau and low N\(_0\).

The first-order rate constant for N\(_{\text{min}}\) in SB was significantly greater at 30% WFPS (0.069 d\(^{-1}\)) than at 60 (0.013 d\(^{-1}\)) and 90% WFPS (0.008 d\(^{-1}\)) (Table 4). Rapid N\(_{\text{min}}\) at 30% WFPS during the first 20 d, followed by a plateau thereafter (Fig. 5), most likely resulted in the higher k\(_0\) value than those at 60 and 90% WFPS. Similarly, the half-life of N\(_{\text{min}}\) in SB at 30% WFPS (10 d) was lower than the half-life of N\(_{\text{min}}\) in SB at 60 (53 d) and 90% WFPS (87 d) because of the low N\(_0\) (32 mg kg\(^{-1}\)) that plateaued at about Day 20.

**Conclusions**

Moisture content influenced N\(_{\text{min}}\) differently in PB and SB. Our results indicate that PB can provide adequate N supply for high biomass plants under conditions close to field capacity. Plant available N supply from PB under relatively dry and near-saturated conditions in end-of-life lagoons may fail to support a healthy plant population that produces high biomass yields, thus reducing the effectiveness of phytoextraction as a strategy for nutrient and trace element removal from

**Table 4. First-order kinetic model parameter estimates for net mineralized N (N\(_{\text{min}}\)) concentration in secondary biosolids incubated at 30, 60, and 90% water-filled pore space (WFPS).†**

<table>
<thead>
<tr>
<th>WFPS</th>
<th>N(_0)</th>
<th>k(_0)</th>
<th>t(_{1/2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>mg kg(^{-1})</td>
<td>d(^{-1})</td>
<td>d</td>
</tr>
<tr>
<td>30</td>
<td>32.0b</td>
<td>0.069a†</td>
<td>10</td>
</tr>
<tr>
<td>60</td>
<td>68.4a</td>
<td>0.013b</td>
<td>53</td>
</tr>
<tr>
<td>90</td>
<td>94.1a</td>
<td>0.008b</td>
<td>87</td>
</tr>
</tbody>
</table>

† N\(_{\text{min}}\) = N\(_0\) [1 − exp(−k\(_0\)t)], where N\(_{\text{min}}\) is the net mineralized N concentration at time t, N\(_0\) is the potentially mineralizable N pool, k\(_0\) is the first-order rate constant and t\(_{1/2}\) = 0.693/k\(_0\) is the time required to mineralize one half of the organic N pool (half-life).

† Means in a column followed by the same letter are not significantly different. Differences were considered significant if 95% confidence intervals of the means did not overlap.
the biosolids. However, unlike in the near-saturated microcosms of our study, which had no plants growing, in the presence of plant roots, higher dissolved oxygen concentrations are expected because of the presence of plant roots in the rhizosphere, which should enhance mineralization. Moisture level had no significant effect on \( N_{\text{min}} \) in SB. The low \( N_{\text{min}} \) in SB suggests that high-N-demanding plants would have limited effectiveness to remediate SB. Phytorextraction in SB may be enhanced by using plant species such as switchgrass that can produce appreciable biomass yields in low N conditions. High biomass plants that have high N requirements can be grown in PB to phytorextract high concentrations of mineralized N and other contaminants. Multiple harvesting of plants during the growing season to increase total biomass yield, and, hence, contaminant uptake is likely feasible in PB because of the high mineralization rates. Low mineralization rates in SB may not provide sufficient available N concentrations to support plant regrowth, restricting harvesting to a single cut per season.

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References


