Residual Effects of Lime- and Clay-Amended Biosolids Applied to Coarse-Textured Pasture Soil

Sanjutha Shanmugam and Lynette K. Abbott

Soil Biology and Molecular Ecology Group, School of Earth and Environment and UWA Institute of Agriculture, Faculty of Science, The University of Western Australia, 35 Stirling Highway, M087, Crawley, WA 6009, Australia

Correspondence should be addressed to Sanjutha Shanmugam; sanjutha.shanmugam@gmail.com

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This study investigated whether there was residual effect of application of lime- and clay-amended biosolids (LaBC®) on ryegrass growth and soil microbial biomass in a coarse-textured, acid pasture soil. Reapplied LaBC® increased fertiliser-use efficiency and plant growth in this glasshouse experiment. Soil management history was established with a single application of LaBC® (50 t ha⁻¹ wet weight equivalent) with or without inorganic fertiliser (NPK) prior to growing annual ryegrass for 5 cycles. In cycle 6 there was no residual nutrient effect of the original application of LaBC® but there was a residual liming effect of the previously applied LaBC®. A nutrient effect of reapplied LaBC® in plant growth cycle 6, had little residual benefit in cycle 7. The residual concentration of inorganic N remaining in this coarse-textured acid soil after a single application of LaBC® was negligible and did not appear to be a risk to the environment when applied at 50 t ha⁻¹ wet weight equivalent.

1. Introduction

Soils on the Swan Coastal Plain in Western Australia (WA) are generally coarse-textured and nutrient deficient and need remedial measures to address soil conditions such as acidity [1]. In addition, these soils are characterised by water repellence that leads to poor water-infiltration and poor nutrient and water retention capabilities in soil that eventually can cause problems for the quality of nearby waterways [2]. Appropriate soil management and nutrient application practices are needed to provide better conditions for plant growth and to reduce the potential for nutrient leaching into waterways on the Swan Coastal Plain [3, 4]. It has been suggested that lime-amended biosolids further blended with clay (called LaBC®) could be incorporated as a soil amendment to remediate these sandy soils [5]. However, the nutrient release pattern for plant available N from LaBC® and considerations for a potential long-term nutrient release need to be monitored to ensure safe recycling of nutrients in this acid sandy soil [6].

Amending soil with biosolids can increase plant growth in degraded pastures associated with improvements in soil fertility [7–9]. Pasture productivity can be increased with application of both organic and inorganic sources of nutrients in such soils [10–13]. Liming, alone or in the form of alkaline stabilised sewage sludge (biosolid), has been used to alleviate soil acidity [14–16]. Various forms of biosolids are available for use in agriculture, including lime-stabilised biosolids [17, 18] and a modified clay-amended biosolid product (LaBC®) which was created for use on acid sandy soils of the Swan Coastal Plain in WA [5, 19]. LaBC® is similar to a biosolid product that was developed by mixing biosolids with river-sediments for application to native grasses in Illinois, USA [20].

Soil management practices that include amending soils with organic or inorganic fertilisers can release residual nutrients (N and P) for some time after their application [21, 22]. Several studies have demonstrated residual effects of applied amendments such as biosolids [23–26], inorganic fertilisers [27, 28], and lime [29–31] that increased soil macronutrients and microbial dynamics in a range of degraded and agricultural lands. A recent study in a sandy-loam soil demonstrated a differential effect on N availability for a repeated application of fertiliser and/or organic manures
in a 17-year experimental site [22]. Long-term N application stimulated gross nitrification more than 5.3 times and soil organic matter was 2.7 times greater in fertiliser applied treatments. A 2-year study concluded that sewage sludge used as an organic amendment was not a complete substitute for mineral fertilisers as inorganic nutrient inputs resulted in greater ryegrass growth on a land-fill site restored with London clay [32]. The N release pattern for the supply of plant available nutrients from biosolids or fertilisers needs to be independently assessed according to the rate applied and soil conditions, because a generalised approach for estimating nutrient supply has several limitations when budgeting nutrient release [33].

In our previous study, clay addition to lime-amended biosolids (LaBC\textsuperscript{®}) increased microbial biomass in an acid sandy soil, which was associated with an increase in soil pH when the amendments were incubated for 30 weeks [19]. It was also found that N was released over a longer period when clay was added to the biosolid with lime (as LaBC\textsuperscript{®}) rather than when either clay or lime was added independently [19]. In addition, a long-term effect of LaBC\textsuperscript{®} and its potential for supplying plant nutrients has been investigated using ryegrass at both field and glasshouse scales [6]. However, the residual effect of LaBC\textsuperscript{®} on soil fertility, especially in relation to microbial activities, is not known. Therefore, in order to study the residual effects of LaBC\textsuperscript{®} on soil fertility, LaBC\textsuperscript{®} was initially applied once as a basal amendment with initial and repeated applications of inorganic fertiliser containing N, P, K, Ca, and Mg before sowing 5 sequential cycles of ryegrass. This established a fertiliser history in the soil containing N, P, K, Ca, and Mg before sowing 5 sequential cycles with initial and repeated applications of inorganic fertiliser in order to study the residual effects of LaBC\textsuperscript{®} on soil fertility.

The aim was to determine whether there was a residual effect of LaBC\textsuperscript{®} application or an effect of reapplication of LaBC\textsuperscript{®} to soil that is with a history of LaBC\textsuperscript{®} application. This was assessed with and without the application of basal inorganic fertiliser to determine whether there is potential environmental risk from application of LaBC\textsuperscript{®}. It was hypothesised that (i) reaplication of LaBC\textsuperscript{®} would have an immediate effect of increasing soil pH regardless of prior history of LaBC\textsuperscript{®} and fertiliser application and (ii) application of LaBC\textsuperscript{®} to soil with a fertiliser history would release more N and increase ryegrass growth to a greater extent than an application of LaBC\textsuperscript{®} to soil without a fertiliser history.

2. Materials and Methods

2.1. Experimental Design

2.1.1. Pretreatment of Soil with LaBC\textsuperscript{®} and Fertilizer for Establishing Soil Management History. Soil used for this experiment was taken from pots that had been pretreated under controlled glasshouse conditions in the following way by the Water Corporation of Western Australia (WA). Prior to establishing the fertiliser history, a coarse-textured acidic sandy soil had been collected from a depth of 0–10 cm after removing coarse surface litter. The field was adjacent to a site with natural woodland vegetation at Bullsbrook (31° 40' 0" South, 116° 0' 0" East), WA, and represented the Swan Coastal Plain-Bassendean sands of the Ellen Brook catchment (pale deep, bleached-Orthic Tenosol [34]). The soil had been air-dried and passed through a 4 mm sieve. It was dominated by sand (98%; 20 \mu m–2 mm) with a small silt fraction (2%; <20 \mu m). Soil chemical properties were pH (CaCl\textsubscript{2}) 5.1, total carbon 1.3%, total nitrogen 0.1%, total oxidisable carbon 0.15 mg g\textsuperscript{-1}, and inorganic nitrogen 0.8 lg g\textsuperscript{-1}.

Free draining pots (175 mm in diameter, 2.8 L) had been filled with 5 kg of soil, packed to a bulk density of 1.6 g cm\textsuperscript{-3} (Water Corporation of WA). The pots had been placed inside a bucket to simulate leaching after irrigation under controlled glasshouse conditions. Dry LaBC\textsuperscript{®} (50 t ha\textsuperscript{-1} wet weight equivalent, 2 to 4 mm) had been applied in cycle 1,
Table 2: Single basal and reapplication of LaBC® during 7 cycles of ryegrass grown in pots of coarse-textured, acid soil under glasshouse conditions to determine the residual effects of LaBC®.

<table>
<thead>
<tr>
<th>Ryegrass cycles 1 to 5</th>
<th>Ryegrass cycle 6</th>
<th>Ryegrass cycle 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of single basal application of LaBC®+–fertiliser</td>
<td>LaBC® reapplied to soil from half of each pot after cycle 5 (+LaBC®)</td>
<td>No LaBC® added in this cycle (to all prior treatments)</td>
</tr>
<tr>
<td>Control</td>
<td>+/- LaBC®</td>
<td>-LaBC®</td>
</tr>
<tr>
<td>aLaBC®</td>
<td>+/- LaBC®</td>
<td>-LaBC®</td>
</tr>
<tr>
<td>bFertiliser</td>
<td>+/- LaBC®</td>
<td>-LaBC®</td>
</tr>
<tr>
<td>LaBC® + fertiliser</td>
<td>+/- LaBC®</td>
<td>-LaBC®</td>
</tr>
</tbody>
</table>

aNLaBC® = 50 t ha−1 of wet weight equivalent applied in cycle 6.

bFertiliser = macronutrient fertiliser applied according to district practice at each cycle.

and macronutrient fertiliser treatments (district practice) had been applied in each cycle according to the treatment schedule (Table 1). The amendments had been fully incorporated through the soil prior to planting ryegrass seeds. Macronutrient fertiliser had been applied on the soil surface prior to planting ryegrass where applicable in each of the 5 cycles (Table 1). The macronutrients had been applied at district farming practice rates equivalent to Flexi N (11.88 kg ha−1), triple superphosphate (20.03 kg ha−1), K2SO4 (54.51 kg ha−1), CaCl2 (30.67 kg ha−1), and MgSO4 (26.62 kg ha−1). Each treatment had been replicated 3 times and pots watered with deionised water had been maintained at 60% field capacity throughout this pretreatment experimental phase.

For the pretreatment conducted by the Water Corporation of WA, seeds of annual ryegrass (Lolium multiflorum, cultivar Winter Star) had been pregerminated in Petri dishes and 12 seedlings of uniform size transplanted into each pot as 1-week-old seedlings. Plants had been grown for six weeks after transplanting and the plant biomass was harvested at the end of each growth cycle. Following each harvest, pots had been returned to the glasshouse with a simulated fallow of approximately 2 weeks without irrigation. Following this 2-week period, all contents had been emptied from pots and passed through a sieve (4 mm) to remove coarse root debris. After each fallow period, the pots had been planted with new set of 1-week-old seedlings and macronutrient fertilisers were applied to the soil surface according to treatment schedule (Table 1). The same method had been repeated in total for five cycles of ryegrass growth and harvests. The entire process of establishing the soil history is summarised in the flow diagram (Figure 1).

2.1.2. Reapplication of LaBC® (+–) to Soil for Cycles 6 and 7 of Ryegrass. Soil from each pot, which had been exposed to pretreatment cycles 1 to 5, was divided and placed into 2 equal smaller pots (115 mm in diameter, 1.5 L) prior to reapplication of treatments (Figure 1). LaBC® was reapplied at the start of cycle 6 (Table 2).

For cycles 6 and 7, pots were filled with 1.65 kg of soil collected from the pretreated soil and packed to a bulk density of 1.6 g cm−3. Dry LaBC® (2 to 4 mm fraction) was applied according to the treatment schedule (Table 2) and fully incorporated throughout the soil prior to planting. Pots were irrigated with deionised water and maintained at 60% field capacity level throughout the experiment. Plants were sown with ryegrass and harvested after 6 weeks in cycles 6 and 7 as described above for the 5 pretreatment cycles. Each treatment was replicated 3 times.

2.2. Characteristics of LaBC®. The LaBC® blend was air dried at 40°C and homogenised using a mechanical crusher. The amendment was then passed through a sieve that captured particles between 2 and 4 mm before application. Basic characteristics of the amendment were analysed using standard procedures [35]. The pH (CaCl2) was 8.2. Oxidisable C and carbonate contents were measured on 0.5 M K2SO4 extracts (sample–solution ratio of 1:4) using an autosampler (Shimadzu, ASI-5000A) and TOC analyser (Shimadzu, TOC 5000A) based on wet combustion method [36]. LaBC® had 3% total C, 0.3% total N, 2.4 mg g−1 oxidisable C, 0.9 mg g−1 carbonate, 46 µg g−1 total inorganic N, and traces of micronutrients such as Zn and Cu (mg kg−1, dry weight basis).

2.3. Sampling and Analyses. Plants were harvested after 6 weeks in cycles 6 and 7. Shoots were cut just above the soil surface and their base was washed with deionised water to remove any adhering soil particles. Soil from each pot was then transferred to separate trays to collect root biomass; larger roots were gently removed by hand whereas the finer roots were removed by sieving through a 2 mm sieve. The soil was returned into the respective pots after removing the roots and root fragments. All plant samples were dried to a constant weight at 40°C for 7 days in a hot air oven and weighed to record dry weight biomass.

Soil subsamples (approximately 50 g) were collected at the end of each harvest, after removing the finer root fragments and homogenising the soil from each pot. Soil pH (1:5 v/v ratio of soil/0.01 M CaCl2 suspension) was measured after shaking for 1 hour continuously in a mechanical end-over-end shaker [35].

Soil inorganic N (NO3−-N + NH4+-N) was determined using fresh soil (10 g dry weight equivalent) extracts of 0.5 M K2SO4 (40 mL) after shaking in a mechanical end-over-end shaker for 1 hour and microbial biomass N (MBN)
Table 3: Shoot dry biomass (g pot\(^{-1}\)) in ryegrass cycles 6 and 7 with reapplied LaBC\(\textregistered\) (+/−) as influenced by residual effects of soil amendments from previous management history.

<table>
<thead>
<tr>
<th>Soil management history (cycles 1 to 5)</th>
<th>Shoot dry biomass (g pot(^{-1}))</th>
<th>Ryegrass cycle 6</th>
<th>Current treatment (+/−LaBC(\textregistered))</th>
<th>Ryegrass cycle 7</th>
<th>Treatment (+/−LaBC(\textregistered))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.03 ± 0.03(^a)</td>
<td>0.33 ± 0.03(^b)</td>
<td>0.11 ± 0.02(^d)</td>
<td>0.38 ± 0.04(^b)</td>
<td></td>
</tr>
<tr>
<td>(\textsuperscript{a})LaBC(\textregistered)</td>
<td>0.10 ± 0.00(^a)</td>
<td>0.39 ± 0.01(^b)</td>
<td>0.09 ± 0.01(^d)</td>
<td>0.30 ± 0.02(^a)</td>
<td></td>
</tr>
<tr>
<td>(\textsuperscript{b})Fertiliser</td>
<td>0.28 ± 0.04(^b)</td>
<td>0.40 ± 0.06(^a)</td>
<td>0.15 ± 0.00(^d)</td>
<td>0.47 ± 0.04(^a)</td>
<td></td>
</tr>
<tr>
<td>LaBC(\textregistered) + fertiliser</td>
<td>0.33 ± 0.03(^b)</td>
<td>0.60 ± 0.06(^a)</td>
<td>0.11 ± 0.02(^d)</td>
<td>0.40 ± 0.04(^ab)</td>
<td></td>
</tr>
</tbody>
</table>

\(\textsuperscript{a}\)LaBC\(\textregistered\) = 50 t ha\(^{-1}\) of wet weight equivalent.

\(\textsuperscript{b}\)Fertiliser = macronutrient fertilisers (N, P, K, Ca, and Mg) applied according to district practice.

Values are mean ± SE (\(n = 3\)). The letters followed by mean ± SE were based on LSD (\(P < 0.05\)).

The main and interaction effects of treatments with the same letters, within the same column and between columns within each cycle, respectively, were not significant (\(P > 0.05\)).

3. Results

3.1. Shoot Dry Biomass (g pot\(^{-1}\)). Shoot biomass was significantly influenced (\(P < 0.05\)) only by fertiliser from the basal history but not by the previously applied LaBC\(\textregistered\), after 5 cycles of ryegrass planting (Table 3). When LaBC\(\textregistered\) was reapplied at 50 t ha\(^{-1}\) in cycle 6, shoot biomass was increased (by >100%) compared to non-reapplied LaBC\(\textregistered\). A residual effect of reapplied LaBC\(\textregistered\) from cycle 6 was carried over to cycle 7.

3.2. Root Dry Biomass (g pot\(^{-1}\)). Root biomass was significantly influenced (\(P < 0.05\)) only by fertiliser from the basal history but not by the previously applied LaBC\(\textregistered\), after 5 cycles of ryegrass planting (Table 4). It was increased 3-fold by the fertiliser from the prior history. When LaBC\(\textregistered\) was reapplied at 50 t ha\(^{-1}\) in cycle 6, it did not have a significant effect regardless of the soil history. However, a residual effect of reapplied LaBC\(\textregistered\) from cycle 6 was carried over to cycle 7.

3.3. Root: Shoot. Root: shoot was significantly increased (\(P < 0.05\)) by pretreatment of soil with both LaBC\(\textregistered\) and fertiliser and in combination when assessed in cycle 6 (Table 5). A residual effect of fertiliser but not LaBC\(\textregistered\) lasted through to cycle 7. When LaBC\(\textregistered\) was reapplied at 50 t ha\(^{-1}\) in cycle 6, it did not have a significant (\(P > 0.05\)) effect on root: shoot regardless of the soil history and the same pattern was also observed at cycle 7 (Table 5). Overall, the residual effect of fertiliser history significantly (\(P < 0.05\)) influenced the root: shoot but only in the absence of reapplied LaBC\(\textregistered\).

3.4. Shoot N Concentration (%). Shoot N concentration was not influenced (\(P > 0.05\)) by LaBC\(\textregistered\) and/or fertiliser applied to the pretreated soil when assessed in cycle 6 (Table 6). When LaBC\(\textregistered\) was reapplied at 50 t ha\(^{-1}\) in cycle 6, it did not have a significant (\(P > 0.05\)) effect regardless of the soil history and no residual effect of reapplied LaBC\(\textregistered\) was carried over to cycle 7.

3.5. Soil pH. Soil pH (CaCl\(_2\)) was significantly influenced (\(P < 0.05\)) by LaBC\(\textregistered\) applied to the pretreated soil when assessed in cycle 6 (Table 7). When LaBC\(\textregistered\) was reapplied at 50 t ha\(^{-1}\) in cycle 6, there was a further significant (\(P < 0.05\)) increase (by 1-2 units) in soil pH regardless of the soil history. The residual effect of reapplied LaBC\(\textregistered\) from cycle 6 was carried over to cycle 7 and the pH increased by 0.5 units.

3.6. Soil Total Inorganic N. Soil total inorganic N was significantly (\(P < 0.05\)) increased by fertiliser only application with or without reapplied LaBC\(\textregistered\) in cycle 6 but not when LaBC\(\textregistered\) was reapplied without fertiliser (Table 8). There was no residual N effect of reapplied LaBC\(\textregistered\) carried from cycle 6 on to cycle 7.

3.7. Soil Microbial Biomass N. Soil microbial biomass N was not significantly influenced (\(P > 0.05\)) by LaBC\(\textregistered\) and/or fertiliser applied to the pretreated soil when assessed in cycle 6 (Table 9). In cycle 6, when LaBC\(\textregistered\) was reapplied, there was no influence on soil MBN regardless of soil history, and there was no residual effect in cycle 7 (Table 9).

4. Discussion

In the absence of reapplied LaBC\(\textregistered\), there was no residual nutrient effect of a single basal application of LaBC\(\textregistered\).
Table 4: Root dry biomass (g pot\(^{-1}\)) in ryegrass cycles 6 and 7 with reapplied LaBC\(^+(+/-)\) as influenced by residual effects of soil amendments from previous management history.

<table>
<thead>
<tr>
<th>Soil management history (cycles 1 to 5)</th>
<th>Ryegrass cycle 6</th>
<th>Current treatment (+/−LaBC(^+))</th>
<th>Ryegrass cycle 7</th>
<th>Treatment +LaBC(^+) from cycle 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−LaBC(^+)</td>
<td>+LaBC(^+) (50 t ha(^{-1}))</td>
<td>Treatment −LaBC(^+) from cycle 6</td>
<td>Treatment +LaBC(^+) from cycle 6</td>
</tr>
<tr>
<td>Control</td>
<td>0.23 ± 0.03(^d)</td>
<td>0.53 ± 0.13(^d)</td>
<td>0.10 ± 0.02(^c)</td>
<td>0.50 ± 0.10(^b)</td>
</tr>
<tr>
<td>(^a)LaBC(^+)</td>
<td>0.34 ± 0.08(^d)</td>
<td>0.58 ± 0.06(^d)</td>
<td>0.31 ± 0.09(^bc)</td>
<td>0.45 ± 0.06(^b)</td>
</tr>
<tr>
<td>(^b)Fertiliser</td>
<td>1.08 ± 0.22(^a)</td>
<td>0.57 ± 0.15(^d)</td>
<td>1.03 ± 0.20(^a)</td>
<td>0.59 ± 0.14(^b)</td>
</tr>
<tr>
<td>LaBC(^+) + fertiliser</td>
<td>1.03 ± 0.12(^d)</td>
<td>0.67 ± 0.09(^d)</td>
<td>0.95 ± 0.11(^a)</td>
<td>0.52 ± 0.09(^b)</td>
</tr>
</tbody>
</table>

\(^a\)LaBC\(^+\) = 50 t ha\(^{-1}\) of wet weight equivalent.

\(^b\)Fertiliser = macronutrient fertilisers (N, P, K, Ca, and Mg) applied according to district practice.

Values are mean ± SE (n = 3). The letters followed by mean ± SE were based on LSD (P < 0.05).

The main and interaction effects of treatments with the same letters, within the same column and between columns within each cycle, respectively, were not significant (P > 0.05), based on 2-way ANOVA.

Table 5: Root: shoot in ryegrass cycles 6 and 7 with reapplied LaBC\(^+(+/-)\) as influenced by residual effects of soil amendments from previous management history.

<table>
<thead>
<tr>
<th>Soil management history (cycles 1 to 5)</th>
<th>Ryegrass cycle 6</th>
<th>Current treatment (+/−LaBC(^+))</th>
<th>Ryegrass cycle 7</th>
<th>Treatment +LaBC(^+) from cycle 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−LaBC(^+)</td>
<td>+LaBC(^+) (50 t ha(^{-1}))</td>
<td>Treatment −LaBC(^+) from cycle 6</td>
<td>Treatment +LaBC(^+) from cycle 6</td>
</tr>
<tr>
<td>Control</td>
<td>0.67 ± 0.67(^d)</td>
<td>1.56 ± 0.22(^bcd)</td>
<td>0.89 ± 0.21(^b)</td>
<td>1.38 ± 0.32(^b)</td>
</tr>
<tr>
<td>(^a)LaBC(^+)</td>
<td>3.33 ± 0.88(^ab)</td>
<td>1.50 ± 0.14(^bcd)</td>
<td>3.22 ± 0.75(^b)</td>
<td>1.48 ± 0.13(^b)</td>
</tr>
<tr>
<td>(^b)Fertiliser</td>
<td>4.28 ± 1.62(^a)</td>
<td>1.45 ± 0.37(^bcd)</td>
<td>6.64 ± 1.13(^b)</td>
<td>1.29 ± 0.36(^b)</td>
</tr>
<tr>
<td>LaBC(^+) + fertiliser</td>
<td>3.14 ± 0.43(^abc)</td>
<td>1.13 ± 0.16(^d)</td>
<td>8.97 ± 1.63(^a)</td>
<td>1.34 ± 0.31(^b)</td>
</tr>
</tbody>
</table>

\(^a\)LaBC\(^+\) = 50 t ha\(^{-1}\) of wet weight equivalent.

\(^b\)Fertiliser = macronutrient fertilisers (N, P, K, Ca, and Mg) applied according to district practice.

Values are mean ± SE (n = 3). The letters followed by mean ± SE were based on LSD (P < 0.05).

The main and interaction effects of treatments with the same letters, within the same column and between columns within each cycle, respectively, were not significant (P > 0.05), based on 2-way ANOVA.

Table 6: Shoot N concentration (%) in ryegrass cycles 6 and 7 with reapplied LaBC\(^+(+/-)\) as influenced by residual effects of soil amendments from previous management history.

<table>
<thead>
<tr>
<th>Soil management history (cycles 1 to 5)</th>
<th>Ryegrass cycle 6</th>
<th>Current treatment (+/−LaBC(^+))</th>
<th>Ryegrass cycle 7</th>
<th>Treatment +LaBC(^+) from cycle 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−LaBC(^+)</td>
<td>+LaBC(^+) (50 t ha(^{-1}))</td>
<td>Treatment −LaBC(^+) from cycle 6</td>
<td>Treatment +LaBC(^+) from cycle 6</td>
</tr>
<tr>
<td>Control</td>
<td>1.79 ± 0.12(^d)</td>
<td>2.11 ± 0.05(^bcd)</td>
<td>1.46 ± 0.13(^a)</td>
<td>1.33 ± 0.06(^c)</td>
</tr>
<tr>
<td>(^a)LaBC(^+)</td>
<td>1.78 ± 0.04(^d)</td>
<td>2.18 ± 0.13(^ab)</td>
<td>1.58 ± 0.06(^a)</td>
<td>1.52 ± 0.03(^a)</td>
</tr>
<tr>
<td>(^b)Fertiliser</td>
<td>1.86 ± 0.1(^bcd)</td>
<td>2.28 ± 0.22(^a)</td>
<td>1.49 ± 0.03(^b)</td>
<td>1.44 ± 0.04(^b)</td>
</tr>
<tr>
<td>LaBC(^+) + fertiliser</td>
<td>1.75 ± 0.1(^d)</td>
<td>2.35 ± 0.07(^a)</td>
<td>1.46 ± 0.08(^a)</td>
<td>1.54 ± 0.01(^a)</td>
</tr>
</tbody>
</table>

\(^a\)LaBC\(^+\) = 50 t ha\(^{-1}\) of wet weight equivalent.

\(^b\)Fertiliser = macronutrient fertilisers (N, P, K, Ca, and Mg) applied according to district practice.

Values are mean ± SE (n = 3). The letters followed by mean ± SE were based on LSD (P < 0.05).

The main and interaction effects of treatments with the same letters, within the same column and between columns within each cycle, respectively, were not significant (P > 0.05), based on 2-way ANOVA.
Table 7: Soil pH in ryegrass cycles 6 and 7 with reapplied LaBC® (+/-) as influenced by residual effects of soil amendments from previous management history.

<table>
<thead>
<tr>
<th>Soil management history (cycles 1 to 5)</th>
<th>Ryegrass cycle 6</th>
<th>Current treatment (+/− LaBC®)</th>
<th>Ryegrass cycle 7</th>
<th>Treatment (+/− LaBC®)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>− LaBC®</td>
<td>+ LaBC® (50 t ha⁻¹)</td>
<td>Treatment − LaBC® from cycle 6</td>
<td>Treatment + LaBC® from cycle 6</td>
</tr>
<tr>
<td>Control</td>
<td>5.0 ± 0.03 d</td>
<td>6.6 ± 0.06 b</td>
<td>4.9 ± 0.01</td>
<td>7.1 ± 0.09 b</td>
</tr>
<tr>
<td>aLaBC®</td>
<td>5.4 ± 0.00 c</td>
<td>6.8 ± 0.06 a</td>
<td>5.4 ± 0.03 i</td>
<td>7.4 ± 0.07 a</td>
</tr>
<tr>
<td>bFertiliser</td>
<td>4.7 ± 0.03 e</td>
<td>6.5 ± 0.12 b</td>
<td>4.6 ± 0.03 i</td>
<td>7.1 ± 0.06 b</td>
</tr>
<tr>
<td>LaBC® + fertiliser</td>
<td>5.3 ± 0.07 e</td>
<td>6.9 ± 0.09 e</td>
<td>5.2 ± 0.06 d</td>
<td>7.2 ± 0.06 b</td>
</tr>
</tbody>
</table>

^a LaBC® = 50 t ha⁻¹ of wet weight equivalent.

^b Fertiliser = macronutrient fertilisers (N, P, K, Ca, and Mg) applied according to district practice.

Values are mean ± SE (n = 3). The letters followed by mean ± SE were based on LSD (P < 0.05).

The main and interaction effects of treatments with the same letters, within the same column and between columns within each cycle, respectively, were not significant (P > 0.05), based on 2-way ANOVA.

Table 8: Total inorganic N (µg g⁻¹ soil) in ryegrass cycles 6 and 7 with reapplied LaBC® (+/-) as influenced by residual effects of soil amendments from previous management history.

<table>
<thead>
<tr>
<th>Soil management history (cycles 1 to 5)</th>
<th>Ryegrass cycle 6</th>
<th>Current treatment (+/− LaBC®)</th>
<th>Ryegrass cycle 7</th>
<th>Treatment (+/− LaBC®)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>− LaBC®</td>
<td>+ LaBC® (50 t ha⁻¹)</td>
<td>Treatment − LaBC® from cycle 6</td>
<td>Treatment + LaBC® from cycle 6</td>
</tr>
<tr>
<td>Control</td>
<td>0 ± 0.01 d</td>
<td>0.68 ± 0.33 d</td>
<td>0.20 ± 0.06 b</td>
<td>0.33 ± 0.17 ab</td>
</tr>
<tr>
<td>aLaBC®</td>
<td>0.25 ± 0.25 d</td>
<td>0.92 ± 0.04 ac</td>
<td>0.20 ± 0.15 b</td>
<td>0.60 ± 0.09</td>
</tr>
<tr>
<td>bFertiliser</td>
<td>0.09 ± 0.09 d</td>
<td>1.70 ± 0.37 a</td>
<td>0.37 ± 0.09 ab</td>
<td>0.63 ± 0.07 a</td>
</tr>
<tr>
<td>LaBC® + fertiliser</td>
<td>0.29 ± 0.14 d</td>
<td>1.50 ± 0.43 ab</td>
<td>0.37 ± 0.12 ab</td>
<td>0.43 ± 0.15 ab</td>
</tr>
</tbody>
</table>

^a LaBC® = 50 t ha⁻¹ of wet weight equivalent.

^b Fertiliser = macronutrient fertilisers (N, P, K, Ca, and Mg) applied according to district practice.

Values are mean ± SE (n = 3). The letters followed by mean ± SE were based on LSD (P < 0.05).

The main and interaction effects of treatments with the same letters, within the same column and between columns within each cycle, respectively, were not significant (P > 0.05), based on 2-way ANOVA.

Table 9: Microbial biomass N (µg g⁻¹ soil) in ryegrass cycles 6 and 7 with reapplied LaBC® (+/-) as influenced by residual effects of soil amendments from previous management history.

<table>
<thead>
<tr>
<th>Soil management history (cycles 1 to 5)</th>
<th>Ryegrass cycle 6</th>
<th>Current treatment (+/− LaBC®)</th>
<th>Ryegrass cycle 7</th>
<th>Treatment (+/− LaBC®)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>− LaBC®</td>
<td>+ LaBC® (50 t ha⁻¹)</td>
<td>Treatment − LaBC® from cycle 6</td>
<td>Treatment + LaBC® from cycle 6</td>
</tr>
<tr>
<td>Control</td>
<td>7.60 ± 7.60 a</td>
<td>0 ± 0.01</td>
<td>7.67 ± 5.55 ab</td>
<td>3.10 ± 1.84 b</td>
</tr>
<tr>
<td>aLaBC®</td>
<td>3.20 ± 3.20 a</td>
<td>3.90 ± 3.90 a</td>
<td>2.54 ± 2.54 b</td>
<td>7.20 ± 3.69 b</td>
</tr>
<tr>
<td>bFertiliser</td>
<td>7.63 ± 6.83 a</td>
<td>15.85 ± 14.77 a</td>
<td>7.61 ± 5.08 ab</td>
<td>22.94 ± 9.33 a</td>
</tr>
<tr>
<td>LaBC® + fertiliser</td>
<td>4.42 ± 2.37 a</td>
<td>09.77 ± 4.89 a</td>
<td>10.70 ± 2.74 ab</td>
<td>10.85 ± 6.13 ab</td>
</tr>
</tbody>
</table>

^a LaBC® = 50 t ha⁻¹ of wet weight equivalent.

^b Fertiliser = macronutrient fertilisers (N, P, K, Ca, and Mg) applied according to district practice.

Values are mean ± SE (n = 3). The letters followed by mean ± SE were based on LSD (P < 0.05).

The main and interaction effects of treatments with the same letters, within the same column and between columns within each cycle, respectively, were not significant (P > 0.05), based on 2-way ANOVA.
However, soil pH was maintained at a significantly higher level, indicating a residual liming effect of the previously applied LaBC® in this acid soil. Furthermore, increased ryegrass shoot and root weight were associated with a residual effect of fertiliser history in addition to the direct nutrient release from reapplied LaBC®. The effect of reapplied LaBC® was generally confined to plant growth cycle. Where there were benefits of LaBC®, there was no corresponding change in microbial biomass N.

Combined application of organic amendments and inorganic mineral fertilisers is a balanced approach while budgeting plant nutrient management and carbon sequestration practices [37–41]. Inorganic soluble N fertilisation effects on vegetation cover, plant biomass, and yield [42, 43] can be immediately noticeable, significantly higher, and long-lasting [39, 44–48]. For example, application of inorganic N (37 kg ha⁻¹) and P (94 kg ha⁻¹) fertilisers increased growth of both shoot and root biomass and increased the root:shoot ratio of cool-season wheatgrass (Agropyron sp.) when grown in a low fertile strip mine soil under glasshouse and field conditions [49]. In a 3-year study [46], a single application of inorganic fertilisers (100 kg N and 35 kg P ha⁻¹) on a degraded semiarid ecosystem continuously supported a significantly higher native plant cover with shrubs and grasses (to 29%) after 24 months compared with a single application of screened and unscreened biosolid and municipal waste composts (each at 40 t ha⁻¹), supplied a range of nutrients (10–54 kg N and 6–68 kg P ha⁻¹). The root biomass and root length did not differ significantly between treatments, but the root:shoot was significantly lower (2.1) with inorganic fertiliser application associated with increased shoot biomass. However, the effect was less pronounced after 3 years. Similarly, in the evaluation of residual effects of the biosolid product LaBC® and fertiliser in our study, residual effects of LaBC® were minimal compared with the residual effects of fertiliser.

LaBC® had a residual effect on increasing soil pH, primarily due to the incorporation of lime into this biosolids byproduct. In contrast, continuous application of mineral fertilisers, especially inorganic N, can acidify the soil environment [50] as was demonstrated in cycles 6 and 7 of our study where there was a decrease in soil pH (CaCl₂) (to 4.6) associated with inorganic fertiliser application. An increase in soil pH can potentially increase N mineralisation and organic substrate availability thereby increasing microbial biomass and its activity [51]. Similar liming effects have been shown in an incubation study over 56 days [31].

In contrast with the residual liming effect, inorganic N released from LaBC® was not sufficient to carry over to a sixth cycle of ryegrass growth after a single application. Soil N mineralisation is considered to be a crucial factor while determining agronomical values of organic amendments [52]. Soil inorganic N was significantly higher only when LaBC® was reapplied in soils that had received either mineral fertiliser alone or mineral fertiliser in combination with LaBC® application in the past. In addition to the residual effect of inorganic fertilisers, the added slow release nutrients from reapplied LaBC® contributed to plant available nutrients only within one ryegrass growth cycle. This implies that LaBC® reapplied after previous basal application of LaBC® (50 t ha⁻¹ wet weight equivalent) would contribute little towards the potential of N leaching into the immediate environment.

Increased soil microbial biomass and activities indicate improved biological components of soil fertility status after application of organic amendments [53, 54]. Nevertheless, in the acid sandy soil used in this study, reapplied LaBC® did not increase microbial biomass N although soil pH and mineralisation based on soil inorganic N were higher with reapplied LaBC®. In contrast, microbial biomass N changed over time with application of LaBC® in the absence of plants [19]; the presence of plants may have introduced competition for N at the level of LaBC® applied (50 t ha⁻¹).

5. Conclusions

(i) As hypothesised, reaplication of a lime- and clay-amended biosolids product, LaBC®, had an immediate effect of increasing soil pH regardless of prior history of LaBC® and fertiliser application. The main benefit of LaBC® was to increase soil pH (CaCl₂) which was maintained at about 5.4 even after 6 cycles of ryegrass growth following a single application.

(ii) The second hypothesis is that the application of LaBC® to soil with a fertiliser history would release more N and increase ryegrass growth to a greater extent than an application of LaBC® to soil without a fertiliser history; this was not supported.

(iii) The release of inorganic N with reapplied LaBC® appeared to be confined to the current growth cycle, with little or no residual effect.

(iv) Our investigation demonstrates that both reapplied and previously applied LaBC® at 50 t ha⁻¹ wet weight equivalent are unlikely to pose a risk of leachable N in this sandy soil, based on the available soil inorganic N measured.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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