Research Article

N Mineralisation from Bioresources Incubated at 12.5°C

S. W. Ives,¹ L. A. Sparrow,¹ W. E. Cotching,² R. B. Doyle,³ and S. Lisson⁴

¹University of Tasmania, P.O. Box 46, Kings Meadows, TAS 7249, Australia
²Tasmanian Institute of Agriculture, University of Tasmania, P.O. Box 3523, Burnie, TAS 7320, Australia
³University of Tasmania, Private Bag 54, Hobart, TAS 7001, Australia
⁴CSIRO Sustainable Ecosystems University of Tasmania, Private Bag 54, Hobart, TAS 7001, Australia

Correspondence should be addressed to S. W. Ives; stephen.ives@utas.edu.au

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1. Introduction

The mineralisation and nitrification of nitrogen (N) in soil and in bioresources applied to soil depend on temperature and moisture [1–3]. In Australian cool-temperate climates, soil preparation for cropland, including application and incorporation of bioresources (e.g., composts, sewage sludge, and processing waste material), traditionally occurs in autumn and spring when average air temperatures range between 8 and 15°C and average soil temperatures range between 9 and 20°C (http://www.bom.gov.au). However, because crops in such climates are often not sown until spring, nitrogen release from the bioresources in these time periods may not be aligned with crop demand which is mostly in late spring or early summer, thus providing potential for either N loss (from the bioresources) or nutrient deficiency (of the crop).

Bergström and Brink [4] emphasised the importance of application rate and timing of inorganic fertilisers being calculated to meet crop demand, with new techniques such as organic resin coatings used to slow down the release of elemental N [5, 6]. Furthermore, stewardship programmes have been found to have a positive impact on water quality by preventing soluble nutrient losses through leaching or overland flow from agriculture [7].

Incubation experiments to investigate N mineralisation of various soil-applied bioresources have been conducted by Flavel and Murphy [8], Burgos et al. [9], and Hseu and Huang [10]. The incubation temperatures (and times) used for the amended soils were different for each study (15°C (142 days), 28°C (280 days), and 30°C (336 days), resp.). Although these studies were conducted for periods between 20 and 48 weeks, most changes occurred within the first 4 weeks following incorporation. N mineralisation studies conducted specifically on biosolids-amended soil have been incubated at 25°C [11, 12] and 20°C [13] with Smith et al. [12] concluding that biosolids type, soil temperature, and time from incorporation are dominant factors in determining N-release rate and nitrate formation.

Few studies have been conducted at soil temperatures typical of spring and autumn in temperate climates [14]. Furthermore, the Q10 principle, as described by Silvia and Machado [3], may not be adequate to predict mineralisation rates of bioresources at lower temperatures. Ågren and Bosatta [15] have suggested that soil organic matter (SOM) in cold climate soils mineralises faster when exposed to...
warmer temperatures than it does in warm climate soils where the SOM is much more resistant to change. However, introduced organic matter from a bioresource may alter this temperature effect on SOM mineralisation because of the overall changes in chemical and physical soil characteristics from incorporation of external material. This suggests that a seasonal appropriate temperature is required for incubation studies to emulate field conditions.

Organic materials such as animal manures, crop residues, composts, and sewage sludge have been used in agriculture since cultivation of crops began, to supply plant nutrients and improve soil properties. Traditional agriculture in India and China has always considered these products as part of the farming system and a natural cycling of nutrients [16]. However, most developed nations have regarded agricultural residues and by-products of urbanisation and industrialisation as waste products for disposal. Therefore, amendment availability and logistical limitations have often determined application timing and rate for agricultural use rather than the demand for nutrients and organic matter [17]. If there is to be a change from conventional inorganic fertiliser inputs to organic material amendments, or a fusion of the two, to increase or maintain soil organic matter, the products and mechanisms of nutrient release from organic amendments within the soil matrix need to be understood.

In Tasmania, Australia, biosolids, poppy mulch, and poppy seed waste are three organic matter products produced in sufficient quantity for application to agricultural land. Biosolids are by-products from the treatment of urban sewage, poppy mulch is the by-product of alkaloid production, and poppy seed waste is the residue from poppy seed oil production. Although the annual state production of biosolids is by far the largest (about 40 000 wet tonnes), poppy mulch (10 000 wet tonnes) and poppy seed waste (5 000 wet tonnes) also contribute significantly to the overall organic matter resource available in the state. Ives et al. [18] conducted 2-year field trials with these materials, assessing soil characteristic and plant growth changes in response to their application in both incorporated and unincorporated crop production systems (to reflect minimum and no-tillage cropping situations). The results showed no significant difference in crop yields, grain total N, and postcrop soil NO$_3^-$ over two growing seasons between incorporating and not incorporating lime-amended biosolids. However, it must be noted that while surface applied biosources may be used in minimum and no-tillage cropping systems and not incorporated by cultivation, the planting operation provides some form of incorporation and/or mixing with the topsoil. Either way, the timing and availability of N from applying these biosources under temperate soil/climatic conditions require further investigation.

The objectives of this study were as follows:

(i) to quantify the rate of N release from poppy mulch (PM), poppy seed waste (PSW), lime-amended biosolids (LAB), and anaerobically digested biosolids (ADB) when mixed with a sandy loam soil at a temperature typical of autumn and spring in a temperate climate,

(ii) to determine the peak mineralisation periods of the different products that may be used to influence application timing to match crop demand,

(iii) to determine the effect of CaCO$_3$ in LAB on N release.

2. Methods and Materials

An incubation study was undertaken in a growth chamber over 56 days at 12.5°C. This temperature was selected based on a calculated average air temperature obtained from http://www.bom.gov.au/climate/averages/ for five sites in the cropping regions of Tasmania, Australia (Cressy, Cambridge, Campbell Town, Ross, and Palmerston) for autumn and spring. A randomised complete block design with three replicates was used. Treatments included control (unamended), LAB, ADB, PM, and PSW. LAB was produced by Self’s Point Wastewater Treatment Plant and ADB was produced by Macquarie Point Wastewater Treatment Plant, Hobart, both now managed under one authority, TasWater. PM was supplied by J. S. Aitken, Longford, and PSW was supplied by Rob and Kathy Henry, Woodrising Farms, Cressy. Two other treatments of NaNO$_3$ and NH$_4$Cl at 1% w/w soil were included for observing denitrification and N mineralisation, respectively [19]. A further control soil plus lime treatment (CaCO$_3$ at 4% of LAB wet rate) was used to determine the effect (if any) of additional calcium on the release of nitrogen in the absence of the biosolids treatment (i.e., LAB). Each replicate comprised seven samples for removal and analysis at days 0, 3, 7, 14, 28, 42, and 56.

Treatment preparation was derived from Smith et al. [12] with application rates based on treatments being incorporated in the soil to a depth of 10 cm at a wet weight equivalent rate of 7.5 dry solid (DS) t/ha, assuming a bulk density of 1 Mg m$^{-3}$. Although measured bulk density for this soil in situ was 1.4 Mg m$^{-3}$, the lesser value was used to reflect the state of soil immediately following cultivation. Soil to a depth of 10 cm was collected from an agricultural site near Cressy, Tasmania, sieved to < 4 mm and stored at 4°C. The soil had been previously classified as a Brown Sodosol [20]. The textual size (analysis undertaken by CSBP Soil and Plant Laboratory, Western Australia) for the trial soil was 51% fine sand, 20% coarse sand, 16% silt, and 13% clay, with an exchangeable Na percentage of 2%. The gravimetric moisture content (GMC) of the soil at field capacity (FC) was determined using “Haines” apparatus [21] and calculated as 33%.

One and a half kilogram subsamples of field moist soil (20% GMC ≈ 61% FC) were spread loosely at an even thickness on 35 cm × 40 cm stainless steel trays. Each amendment was then evenly distributed over the soil samples at the required DS rate and mixed by hand using a broad spatula, turning the soil in a uniform motion. Both biosolids products were mixed into a slurry with 40 mL of distilled water before incorporating in the soil. A 40 mL aliquot of distilled water was added to all other treatments (including control) to ensure minimum soil water content of 70% field capacity at commencement of incubation. Subsamples (50 g each, seven for each replicate) were then placed in individual 125 mL
plastic bottles with loose fitted lids (for gaseous exchange) and incubated in the dark at 12.5 ± 0.5°C. The treated and untreated soils were gently tamped down in the bottles (7 light taps on a bench) to achieve a similar bulk density (i.e., similar height in container). No additional water was added to the samples over the incubation period due to minimal moisture loss (72% FC at day 0 decreasing to 65% FC by day 56). The same dry weight application rate was used for all bioresources in the incubation in an effort to maintain similar soil to product contact, regardless of total N in the product. The C:N ratio was not kept constant because it has not been found a reliable indicator of mineralization rates [22].

On each sampling day (i.e., 3, 7, 14, 28, 42, and 56) a sample bottle from each treatment was removed, the soil placed in individual plastic bags and frozen at −19°C until analysis. Samples for day 0 were bagged and frozen immediately after mixing.

Frozen samples were thawed to room temperature before subsampling (10–15 g), drying at 105°C for 24 hours and reweighing to determine GMC. Five grams of each moist sample was also weighed into a 125 mL PPE screw top container and shaken with 2M KCl solution at a 1:10 ratio (w/v) for 1 hour. Extracts were then filtered through Whatman number 42 filter paper, analysed colorimetrically by CSBP Laboratories for NH₄⁺ and NO₃⁻, with results corrected for moisture using GMC.

The total inorganic N content was calculated as the sum of NH₄⁺ and NO₃⁻ extracted from each sample throughout the incubation and the net N mineralised from the applied products was calculated as the difference between inorganic N in each treatment and the control soil [9]. Reported values are actual concentrations on each respective sampling day. Extract concentrations in mg/L were converted to mg/kg using the following formula.

In the following formula, CA = concentration of analyte, CE = concentration in extract, EV = extract volume, and SDW = sample dry weight:

\[ CA (\text{mg/kg}) = \frac{CE (\text{mg/L}) \times EV (L)}{SDW (kg)}. \] (1)

The chemical compositions of LAB, ADB, PM, and PSW, together with the soil used in the trial, are shown in Table 1. Analysis was undertaken by Analytical Services Tasmania, with results shown as a Dry Solid basis.

### 3. Results and Discussion

#### 3.1. N Mineralisation

The NO₃⁻ and NH₄⁺ concentrations of treated soils are shown in Tables 2 and 3, respectively. The moist control soil contained 8.5 mg/kg DS of NO₃⁻ at day 0, and after 56 days of incubation at 12.5°C in the dark it contained 48.3 mg/kg DS NO₃⁻. The ammonium chloride (1% NH₄Cl = 3372 mg/kg NH₄⁺) treatment still contained 2626 mg/kg (78%) of applied NH₄⁺ as NH₄⁺ by day 56 (Table 3) but its NO₃⁻ concentration (Table 2) was 41 mg/kg less than the control by day 56. This suggests that the NH₄⁺ added in the NH₄Cl treatment inhibited rather than stimulated nitrification. The soil with added sodium nitrate (1% NaNO₃ = 7295 mg/kg NO₃⁻) still contained 1745 mg/kg (24%) of the added NO₃⁻ as NO₃⁻ by day 56 (Table 2), concomitant with an increase in NH₄⁺ from 19.2 mg/kg to 46.1 mg/kg. In a similar study Rouch et al. [19] found after 70 days of soil incubation at 20°C in the dark that 84% of added NH₄⁺ was converted to NO₃⁻, whilst NO₃⁻ concentrations only increased by 8.7% in NaNO₃-amended soil. The differences between our results and those of Rouch et al. [19] are probably because of the different incubation temperatures (12.5°C and 20°C, resp.) and they demonstrate the potential effects on mineralisation from applying bioresources in cooler periods.

The concentration of NH₄⁺ in the lime treatment (CaCO₃) was not significantly different than that of the control or LAB (that contains lime as CaO). However, the concentration of NO₃⁻ in LAB at day 56 was significantly higher than the lime treatment. This difference may be due to the different adsorption rates of Ca²⁺ from the two different liming materials onto the colloidal complex, increasing base saturation and ultimately increasing soil pH. Lyngstad [23] found an increase in N mineralisation over a 3-year period as a result of adding CaCO₃ lime, whilst Mühlbachová and Tlustos [24] found that although soil microbial activity initially decreased after application of CaO compared to
Conversely, there was a decrease in soil NO$_3^-$ concentration at day 14 and the reverse at days 28 and 42. The ADB treatment significantly higher than those in the other treatments by the end of the incubation. This does not explain why the PSW treatment, which had a preapplication C:N ratio of 7:1, exhibited a similar negative priming effect to the PM treatment (CN = 16:1). Furthermore, they suggested that if the C:N ratio exceeds 25:1, the microbes would not show an initial increase in NH$_4^+$ concentration and was not significantly different from the other treatments by the end of the incubation.

3.2. C:N Ratio. Using the assumption that the microbial activity and subsequent N mineralisation are inversely proportional to the C:N ratio of residues added to soil [27, 28], the N mineralisation rates of the treatments should follow the sequence ADB > LAB > PSW > PM, with C:N ratios of 3:1, 5:1, 7:1, and 16:1, respectively. However, the results in this experiment showed the extent and rate sequence of N mineralisation of the organic amendments to be in the order of LAB > PSW > ADB > PM. The initial loss of NO$_3^-$ from PSW and PM (Figure 3) could have been due to denitrification or a negative priming effect (N drawdown) associated with the introduction of organic residues to soil [28]. The C:N ratio has been used to predict short-term N availability from solid manure amendments [29]; however Griffin and Hutchinson [22] found that the C:N ratio was poorly correlated with the rate and extent of mineralisation from soil-applied organic materials. Qian and Schoenau [29] found limited release of nitrogen over 67 days from cattle manure with a C:N ratio of between 13 and 15, which is close to the C:N ratio for PM (16:1). Furthermore, they suggested that if the C:N ratio exceeds 25:1, the microbes would source nitrogen from soil reserves (N drawdown, or negative priming). This does not explain why the PSW treatment, which had a preapplication C:N ratio of 7:1, exhibited a similar negative priming effect to the PM treatment (CN =

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**Table 2: NO$_3^-$ concentration of treated soils (dry weight) after incubation at 12.5°C for 56 days.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 0 (mg/kg)</th>
<th>Day 3 (mg/kg)</th>
<th>Day 7 (mg/kg)</th>
<th>Day 14 (mg/kg)</th>
<th>Day 28 (mg/kg)</th>
<th>Day 42 (mg/kg)</th>
<th>Day 56 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADB</td>
<td>9.75 ± 0.2</td>
<td>14.4 ± 2.1</td>
<td>19.6 ± 11</td>
<td>73.8 ± 4.0</td>
<td>96.0 ± 59</td>
<td>135 ± 1.4</td>
<td>169 ± 15</td>
</tr>
<tr>
<td>Control</td>
<td>8.47 ± 1.4</td>
<td>12.0 ± 1.6</td>
<td>14.6 ± 3.4</td>
<td>19.2 ± 2.3</td>
<td>31.5 ± 8.7</td>
<td>37.3 ± 7.6</td>
<td>48.3 ± 5.9</td>
</tr>
<tr>
<td>LAB</td>
<td>9.37 ± 1.4</td>
<td>11.6 ± 3.7</td>
<td>14.1 ± 9.9</td>
<td>52.0 ± 37</td>
<td>130 ± 29</td>
<td>167 ± 8.9</td>
<td>187 ± 16</td>
</tr>
<tr>
<td>Lime</td>
<td>9.49 ± 0.2</td>
<td>13.9 ± 0.5</td>
<td>17.7 ± 1.3</td>
<td>25.5 ± 4.1</td>
<td>33.4 ± 9.0</td>
<td>41.5 ± 1.4</td>
<td>48.2 ± 4.5</td>
</tr>
<tr>
<td>NH$_4$Cl</td>
<td>9.28 ± 0.5</td>
<td>8.45 ± 0.6</td>
<td>8.96 ± 0.4</td>
<td>3.33 ± 0.4</td>
<td>8.20 ± 1.2</td>
<td>7.90 ± 0.5</td>
<td>7.32 ± 0.8</td>
</tr>
<tr>
<td>PM</td>
<td>9.57 ± 1.2</td>
<td>5.68 ± 1.7</td>
<td>0.49 ± 0.2</td>
<td>3.84 ± 4.7</td>
<td>2.50 ± 0.1</td>
<td>14.0 ± 6.8</td>
<td>29.0 ± 9.7</td>
</tr>
<tr>
<td>PSW</td>
<td>9.79 ± 0.7</td>
<td>0.79 ± 0.6</td>
<td>1.10 ± 0.3</td>
<td>33.9 ± 5.7</td>
<td>168 ± 7.5</td>
<td>231 ± 13</td>
<td>235 ± 15</td>
</tr>
<tr>
<td>NaNO$_3$</td>
<td>1919 ± 55</td>
<td>2052 ± 179</td>
<td>1892 ± 287</td>
<td>1781 ± 268</td>
<td>1710 ± 80</td>
<td>1882 ± 46</td>
<td>1745 ± 61</td>
</tr>
</tbody>
</table>

Note: different letters indicate significant differences between treatment means within the same row (LSD = 0.05, P < 0.001).

**Table 3: NH$_4^+$ concentration of treated soils (dry weight) after incubation at 12.5°C for 56 days.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 0 (mg/kg)</th>
<th>Day 3 (mg/kg)</th>
<th>Day 7 (mg/kg)</th>
<th>Day 14 (mg/kg)</th>
<th>Day 28 (mg/kg)</th>
<th>Day 42 (mg/kg)</th>
<th>Day 56 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADB</td>
<td>65.2 ± 2.7</td>
<td>70.0 ± 1.9</td>
<td>80.7 ± 15</td>
<td>23.2 ± 2.0</td>
<td>8.32 ± 1.5</td>
<td>41.4 ± 4.1</td>
<td>8.47 ± 11</td>
</tr>
<tr>
<td>Control</td>
<td>20.0 ± 1.8</td>
<td>22.5 ± 0.8</td>
<td>22.6 ± 6.5</td>
<td>16.6 ± 0.6</td>
<td>8.33 ± 1.4</td>
<td>7.01 ± 0.8</td>
<td>14.0 ± 0.9</td>
</tr>
<tr>
<td>LAB</td>
<td>34.9 ± 2.8</td>
<td>80.7 ± 17</td>
<td>97.9 ± 9.8</td>
<td>69.6 ± 1.9</td>
<td>25.7 ± 0.9</td>
<td>11.2 ± 1.4</td>
<td>8.65 ± 1.6</td>
</tr>
<tr>
<td>Lime</td>
<td>23.2 ± 3.8</td>
<td>22.0 ± 0.3</td>
<td>25.1 ± 10</td>
<td>10.4 ± 2.1</td>
<td>8.80 ± 1.1</td>
<td>9.63 ± 0.2</td>
<td>8.72 ± 4.5</td>
</tr>
<tr>
<td>NaNO$_3$</td>
<td>19.2 ± 2.5</td>
<td>31.0 ± 3.2</td>
<td>41.0 ± 6.5</td>
<td>31.3 ± 6.0</td>
<td>46.1 ± 14</td>
<td>51.4 ± 18</td>
<td>46.1 ± 17</td>
</tr>
<tr>
<td>PM</td>
<td>22.7 ± 0.5</td>
<td>23.0 ± 1.0</td>
<td>23.4 ± 4.4</td>
<td>14.2 ± 6.7</td>
<td>19.5 ± 9.9</td>
<td>17.5 ± 11</td>
<td>21.1 ± 14</td>
</tr>
<tr>
<td>PSW</td>
<td>22.5 ± 1.4</td>
<td>29.5 ± 3.1</td>
<td>50.9 ± 11</td>
<td>109 ± 5.8</td>
<td>34.5 ± 7.3</td>
<td>11.5 ± 0.5</td>
<td>8.68 ± 0.4</td>
</tr>
<tr>
<td>NH$_4$Cl</td>
<td>2578 ± 293</td>
<td>2632 ± 203</td>
<td>2330 ± 41</td>
<td>2534 ± 114</td>
<td>2630 ± 67</td>
<td>2633 ± 25</td>
<td>2626 ± 78</td>
</tr>
</tbody>
</table>

Note: different letters indicate significant differences between treatment means within the same row (LSD = 19.3, P < 0.001).
The disparity between treatments with regard to their C:N ratios and subsequent mineralization may be due to the C:N ratio of the soil, as mineralization of the treatments would not occur in isolation.

3.3. % Total N Released. In order to compare between mineralization rates of ADB, LAB, PM, and LAB, the results were corrected relative to the total N contained in each product after mixing with soil. Results are shown as a percentage of total N of the product for NO$_3^-$, NH$_4^+$ and plant-available N (PAN = NO$_3^-$ + NH$_4^+$) concentrations, respectively, and are corrected by subtraction for N from the control soil. Regardless of total N concentration, the percentage of total N present as NO$_3^-$ (Figure 1) and NH$_4^+$ (Figure 2) followed similar trends to those of the dry weight concentrations of NO$_3^-$ and NH$_4^+$ in the soil, when products were applied at the same dry weight rate. There was a 7-day lag time in %NO$_3^-$ release for ADB and LAB with an estimated 10-day lag time in %NO$_3^-$ release from PSW. There was a steady decline in %NO$_3^-$ for the PM treatment until day 28, before a slight recovery to day 56. However, values for PM were still below 0, indicating that NO$_3^-$ was either denitrified or taken up by microbial biomass. The %NH$_4^+$ concentration for LAB (33.5%) was significantly higher than for ADB (16.8%) at their respective peaks after 7 days of incubation. The peak for NH$_4^+$ as a percentage of total N for the PSW treatment did not occur until day 14, whilst for PM the peak, or plateau, began at day 28 but was not significantly different from any of the other treatments at that time.

The PAN results (Figure 3) show that 45%, 36%, 25%, and –8% of total N applied in LAB, PSW, ADB, and PM, respectively, were recovered as PAN at day 56, with the negative values in the PM treatment indicating a significant N drawdown for the whole period. The implications of this drawdown from the PM treatment include determining application timing (either before crop is planted or when crop nutrient demand is low) and the application timing and amount of additional fertiliser N to satisfy plant requirements. Application timing may also need to be changed to satisfy plant demand when using PSW to take advantage of the early availability of N from the product. The practical limitations of shifting application to a more suitable time for plant uptake may increase risks associated with the season. For example, summer application may be suitable for autumn...
nutrient release but may not be suitable for cultivation. Furthermore winter application may be suitable for spring nutrient release but paddocks may not be accessible at this time due to waterlogging or the risk of compaction of over wet soils and the increased risk of denitrification of mineralised N. The results for LAB in this study support the suggestion by Rigby et al. [30] that current biosolids guidelines do not reflect actual N release. This assertion was based on their study that found up to 65% of total N was released as PAN in the first season after application of lime-amended biosolids to sandy soils in Western Australia. Al-Dhumri et al. [31] also found that 39% of total N was mineralised 120 days after application of anaerobically digested biosolids to Sodosols in Victoria. However, the results of this incubation experiment contrast with the Victorian Biosolids Reuse guidelines that suggest only about 20% of total nitrogen in the product is released in the first twelve months following application [32]. Furthermore, the results of Rigby et al. [20] indicated that applying biosolids at guideline rates in autumn and spring may produce mineral nitrogen in excess of plant requirements at those times of year and increase the potential for leaching and denitrification. Similar to assertions by Al-Dhumri et al. [31] regarding the Victorian biosolids guidelines, Eldridge et al. [33] also questioned the adequacy of the current New South Wales biosolids guidelines [34] for calculating application rates.

4. Conclusion

The results of this study confirm that N mineralisation from different organic amendments is far from uniform and that predictions of mineralisation rates may not be reliably based on the C:N ratio of the applied product, at least for sandy loam soils as used here. Results also showed that, despite being incubated at lower than optimum mineralisation temperature, nitrogen mineralisation continued to occur, with 45%, 36%, and 25% of total N from LAB, PSW, and ADB, respectively, released as PAN by the end of the incubation period. The difference in N mineralisation between LAB and ADB may be due to the water soluble Ca$^{2+}$ from LAB stimulating microbial activity and accelerating decomposition. The mineralisation rates at the temperature used suggest that application timing is critical to ensure that mineralisation of nitrogen from the applied products coincides with plant nutrient requirements and that mineralised N is not exposed to leaching loss and denitrification. These situations can potentially occur in the winter/early spring period in temperate climates such as Tasmania when rainfall is high and evapotranspiration is low, suggesting that autumn and early spring applications may not be appropriate. Although there are potential risks of nutrient build-up (i.e., phosphorus from PSW) associated with annual or periodical applications of PSW and PM (which are not regulated by EPA guidelines), it is suggested that regular soil tests be undertaken to detect any nutrient imbalances. The results also demonstrated that further work is required to understand the relationship between N mineralisation and composition of bioresources and whether the interaction of bioresources with sandy soils is similar with other soil types.

Conflict of Interests

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

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