Biosolids Type, Rate, and Receiving Soil Affect Anaerobic Incubation Nitrogen Availability Coefficients

Seven-day anaerobic incubation can provide relatively quick and easy estimates of potentially available N (PAN), but has been little used to estimate N availability coefficients (NAC) of biosolids destined for land application. We hypothesized that waterlogged-incubation estimates of PAN and NAC depend on biosolids type, application rate, and receiving soil. We applied three dissimilar biosolids at five rates to four representative southeastern US soils and measured NH₄–N and NO₃–N after a 7-d laboratory waterlogged incubation. Target PAN rates were 0, 0.5, 1, 1.5, and 2 × a realistic yield expectation (RYE) rate, 127 kg N ha⁻¹, for tall fescue (Festuca arundinacea), a common biosolids-receiving grass. Biosolids application rates were based on biosolids types, associated book-value NACs, and biosolids total N. Anaerobic incubation of soil plus biosolids yielded predominantly NH₄–N. There were three-way biosolids × rate × soil interactions for NH₄–N, PAN, and NAC. The PAN differed substantially among biosolids, rates, and receiving soils, ranging from –12.1 to 146 mg kg⁻¹, while NAC ranged from -0.13 to 0.86. Negative values suggested N lost via denitrification or immobilization. The PAN trends reflected biosolids total N. At the highest application rate, soil had no detectable effect on the NAC; otherwise, soil affected NAC by as much as an order of magnitude. Presuming anaerobic incubation provides reasonable estimates of PAN, NAC of any particular biosolids might best be estimated via incubation with the receiving soil across an RYE-based range of N application rates, rather than relying on book value NAC.

Abbreviations: CP, Cary Pellets; NAC, N availability coefficient; NCDA&CS, North Carolina Department of Agriculture and Consumer Services; OC, OWASA cake; PAN, potentially available N; RP, Raleigh Plus; RYE, realistic yield expectation; WRRF, water resource recovery facility.

 Biosolids are the largely organic semi-solid residuals of municipal wastewater treated to meet the land application standards in the 1993 USEPA Title 40 Code of Federal Regulation Part 503 Rule (USEPA, 1994). Federal and state regulations require that biosolids be applied at agronomic rates based on the N need of the receiving crop, biosolids total N content, and an N availability coefficient (NAC), that is, the proportion of total N that is mineralizable to potentially available N (PAN; USEPA, 1995). Numerous studies have demonstrated that N mineralization from biosolids depends on many factors including treatment processes, N content and form, C/N ratio, receiving soil, application rate, and a host of environmental factors (Rigby et al., 2016). Thus, estimating biosolids NACs is a challenge.

Aerobic and anaerobic laboratory incubations of soil, organic N sources (e.g., manures, composts), and soil amended with organic N sources can provide reasonable estimates of N mineralization potential and thus NACs (Bundy and Meisinger, 1994). Aerobic incubations may better represent field conditions and typically last many weeks or months, with considerable effort for repeated sampling and analysis.
Long-term aerobic incubation has been widely used to estimate NAC from biosolids-amended soil (Rigby et al., 2016). Arguably the most important and influential study using aerobic incubation was by Sommers et al. (1981) because it became the basis for NAC recommendations in the USEPA Process Design Manual Land Application of Sewage Sludge and Domestic Septage (USEPA, 1983, 1995), the National Manual of Good Practice for Biosolids (Water Environment Research Foundation 2000, 2003, 2005, 2011), and regulations promulgated by many state agencies. In an un-refereed report, Sommers et al. (1981) recommended NACs of 0.40 for waste-activated sludges, 0.25 for raw and primary sludges, 0.15 for anaerobically digested sludges, and 0.08 for composted sludges. However, the study was conducted prior to the promulgation of Part 503, with a single soil, and all but the anaerobic digestion NAC were based on only two samples. No recommendations were made for lime stabilized nor thermally treated biosolids, two processes commonly used by water resource recovery facilities (WRRF, a.k.a., wastewater treatment plants) since promulgation of Part 503 (Jameson et al., 2016).

The Design Manual (USEPA, 1995) cautions that the NACs “are provided as examples only and may be quite different for different sewage sludges, soils, and climates. Therefore, site-specific data, or the best judgement of individuals familiar with N dynamics in the soil–plant system, should always be used in preference to these suggested Km values”. From comprehensive field and laboratory studies, Gilmour et al. (2003) reached a similar conclusion: it is inappropriate to base NACs on treatment processes unless the biosolids have been stabilized substantially, for example, via lagooning or composting. Across eight field trials, they found that field-observed PAN was linearly related to biosolids total N, but cautioned that the relationship may be specific to the studied biosolids and environments; it also varied substantially between individual sites biosolids combinations. In an exhaustive critical review of numerous studies of N mineralization in biosolids-amended soils, Rigby et al. (2016) found mean NAC of 0.47 for aerobic digestion biosolids, 0.40 for thermally dried biosolids, 0.34 for lime-treated biosolids, 0.30 for mesophilic anaerobic digestion biosolids, and 0.07 for composted biosolids. However, the ranges of each category overlapped substantially, again putting into question the validity of biosolids-process-based NACs.

Since promulgation of Part 503, there have been numerous studies using aerobic incubation to estimate organic matter mineralization and PAN from biosolids-amended soils (e.g., Gilmour et al., 1996, 2003; Gilmour and Skinner, 1999; Wang et al., 2003; Smith et al., 1998a, 1998b). These are among the numerous laboratory and field studies reviewed by Rigby et al. (2016), and we make no attempt to summarize them here. Notably lacking in Rigby et al. (2016) is any mention of anaerobic incubation as a means to estimate PAN in biosolids-amended soil, leading one to conclude that it has been little used to that end.

While the 7-d anaerobic incubation of Keeney (1982) was posited by Curtin et al. (2017) to be the most widely used N mineralization bioassay, we found relatively few reports of its use to determine potentially mineralizable N with biosolids-amended soil. It has been used to estimate mineralization in such soils (Speir et al., 2004; Wang et al., 2017; Chowdhury et al., 2016), but these studies did not measure potentially mineralizable N from biosolids. To that purpose, Stark and Clapp (1980) used a 16-wk anaerobic incubation (Smith and Stanford, 1971) to study N availability in a field trial of four sludges applied to a single soil. They found a potential mineralizable N pool of 126 to 1010 g N kg–1, which corresponded to NAC of >0.10 to 0.50. Nikolaidis et al. (1999) used anaerobic incubation to estimate N mineralization from three biosolids added to a glaciated soil. Pelleted biosolids had the greatest NAC, followed by composted and lime stabilized, with NACs of 0.90, 0.73, and 0.56, respectively. Debosz et al. (2002) used a 7-d anaerobic incubation to study the effects of one sewage sludge and household compost on soil properties in situ and in vitro. Other than stating that the NAC from compost was only 0.017, no other results regarding the anaerobic incubation were presented. Thangarajan et al. (2015) used a 7-d anaerobic incubation to study the effects of temperature on NH4 mineralized from three Australian soils amended with a single rate of biosolids of unspecified type. They found NACs ranging from 0.38 to 0.48 and excellent agreement (R2 = 0.99) between the anaerobic incubation and a 28-d aerobic incubation of a variety of organic materials including the biosolids. Gómez-Muñoz et al. (2017) used the anaerobic incubation of Lober and Reeder (1993) to study NH4–N mineralization in soil from a field that for 11 yr had received sewage sludge at an agronomic rate and at three times the agronomic rates, based on a statutory NAC of 0.45. In vitro, they also studied soil to which sludge was added at a rate of 5 mg total N g–1, approximately equivalent to an extraordinary 11 Mg N ha–1, intended to represent the total N applied over the 11 yr. For the agronomic and 3× rates, they found very low NAC of 0.018 and 0.022, respectively, for the field soil, while in the laboratory, the NAC for the fresh biosolids-soil mix was still only 0.044.

Research is needed to investigate the use of anaerobic incubation to estimate potential N mineralization from biosolids-amended soil. The relative simplicity and brevity of the procedure make it attractive for routine determination of NACs. Current NACs take no account of the characteristics of neither the receiving soil nor the application rate. Our objectives were to test the hypotheses that PAN and NACs from anaerobic incubation of biosolids-amended soils would depend on biosolids type, application rate, and receiving soil.
MATERIALS AND METHODS

Design

There were three treatment factors in a 3 × 4 × 4 factorial: biosolids by N rate by soil. The three biosolids were added at four non-zero rates to four soils, augmented with a common zero-N control. The factorial was implemented in a completely randomized design (CRD) and replicated four times.

Nitrogen Sources

USEPA standards define three biosolids classes: Class B, treated to meet maximum limits for pathogens, potentially toxic metals, and disease vector attractants; Class A: very low metal levels and undetectable pathogens; and Exceptional Quality (EQ): Class A biosolids that meet more stringent limits on metals. To meet regulatory requirements, facilitate biosolids recycling, and advance environmental stewardship, WRRFs are increasingly producing Class A and EQ biosolids (Jameson et al., 2016). Three contrasting biosolids representing the trends toward production of Class A EQ biosolids and Class B dewatered cake (rather than slurry) were compared. These were: “Raleigh Plus” (RP), Cary Pellets (CP; branded and sold as “Enviro Gems”), and “OWASA Cake” (OC). Raleigh Plus, a Class A EQ product of the Neuse River WRRF, Raleigh, NC, was a twice-dewatered, lime-stabilized (USEPA, 2000), and pasteurized cake originating from an aerobic treatment process with C-enhanced N removal. Cary Pellets, a Class A EQ product of the South Cary Water Reclamation Facility, Apex, NC, had been processed via biological nutrient removal, then heat treated to pasteurize and form a pellet ~4.5 mm in diameter. OWasa Cake, a Class B product of the Orange County Water and Sewage Authority’s Mason Farm Wastewater Treatment Plant, Carrboro, NC, was the result of anaerobic digestion via a batch process to achieve pathogen reduction then processed to cake via a gravity belt thickener. We collected fresh biosolids directly from the production facilities. OWasa Cake and RP were stored in a refrigerator at 4°C in polyethylene bags to maintain moisture content; CP was stored in a mesh sack at room temperature. A single subsample of each biosolids was analyzed for a suite of nutrients (total and mineral N [NO₃ + NO₂ + NH₄] and by difference organic N; P, K, Ca, Mg, SO₄, Fe, B, Mn, Zn, Cu, Na); pH; soluble salts; C; dry matter; and lime/CaCO₃ equivalent by the Plant/Waste/Solution/Analysis Section of the North Carolina Department of Agriculture and Consumer Services (NCDA&CS) Agronomic Services Division (McGinnis et al. (2011)).

Application Rates

The target N application rates were 0.5, 1, 1.5, and 2× the RYE N rate, 127 kg N ha⁻¹, for tall fescue (Festuca arundinacea), a common biosolids-receiving crop (North Carolina Nutrient Management Workgroup, 2003). We used the existing NCDA&CS first-year NAC (Table 1; McGinnis et al., 2011) to estimate the amount of each biosolids that would be necessary to release these amounts. Nutrient availability of the biosolids was calculated as specified by NCDA&CS (McGinnis et al., 2011): Nutrient available (mg kg⁻¹) = nutrient concentration (mg kg⁻¹) × (Dry matter %/100) × NAC. The book-value NCDA&CS NAC differ somewhat based on the biosolids production process and were chosen accordingly for a broadcast application (Table 1). The RYE intervals, target PAN, and total N added for each RYE rate and N source are shown in Table 2. Conversions between concentrations (mg kg⁻¹) and rates (kg ha⁻¹) were made based on a 2.2 × 10⁻³ Mg ha⁻¹ furrow slice.

Soils

Through discussions with regional land appliers, we identified study soils typical of those permitted to receive biosolids in the US Southeast. Among these, we chose representative and diverse soils, two from the North Carolina Piedmont: Vance sandy clay loam (fine, mixed, semiactive, thermic Typic Hapludults) and Wedowee sandy loam (fine, kaolinitic, thermic Typic Kanhapludults); and two from the North Carolina Coastal Plain: Norfolk loamy sand (fine-loamy, kaolinitic, thermic Typic Kandiudults) and Noboco loamy sand (fine-loamy, Thermic Kandiudults).
siliceous, subactive, thermic Oxyaquic Paleudults). These soils are representative of ≈1.2 million ha in the US Southeast from Virginia to Florida (University of California-Davis, 2017). Using USDA-NRCS soil maps (Soil Survey Staff, 2011), we identified suitable sites for collecting samples. A Vance sandy loam, 6% slope (Soil Survey Staff, 2011), was collected under prior-managed sod at the Upper Piedmont Research Station, Reidsville, NC. A Wedowee sandy loam, 2% slope (Soil Survey Staff, 2011), was collected under fescue sod near Spring Hope, NC. A Norfolk loamy sand, 2% slope (Soil Survey Staff, 2011), was collected under prior-managed sod at the Central Crops Research Station, Clayton, NC. A Noboco loamy sand, 0% to 2% slope (Soil Survey Staff, 2011), was collected under prior-managed sod bordering a research field at the Williamsdale Biofuels Field Laboratory, Wallace, NC. From each site, we collected ≈130 L of soil through a depth of ≈20 cm. The soil consisted primarily of the surficial Ap horizon, but for the piedmont soils, included some of the clayey Bt horizon. We sieved the soil to pass a 2-mm screen and submitted a single moist sample of each to the NCDA&CS Soil Test Section laboratory for routine fertility and chemical analysis. (Hardy et al., 2014; Mehlich 3 (Mehlich, 1984a): P, K, Ca, Mg, S, Cu, Mn, Zn, Na; cation-exchange capacity and base saturation; pH/acidity/lime requirement (Mehlich et al., 1976); soil class (mineral, mineral-organic, organic); sieved weight-to-volume; and humic matter (Mehlich, 1984b). Humic matter as determined by the NCDA&CS method is strongly correlated with soil organic matter (Blumhorst et al., 1990; Gonese and Weber, 1998). The remainder of the soil was air dried prior to the incubations described below.

### Table 3. Chemical analyses of a single sample of study biosolids analyzed by the Plant/Waste/Solution/Media Analysis Section of the Agronomic Division of the NC Dep. Agric. & Consumer Services (McGinnis et al., 2011). Results are typical based on qualitative comparisons with periodic analyses performed for the water resource recovery facilities.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Biosolids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter, %</td>
<td>Cary Pellet (CP)</td>
</tr>
<tr>
<td>pH</td>
<td>5.8</td>
</tr>
<tr>
<td>Calcium carbonate equivalent, %</td>
<td>0</td>
</tr>
<tr>
<td>Agricultural lime equivalent, t</td>
<td>0</td>
</tr>
<tr>
<td>C/N</td>
<td>6.4</td>
</tr>
<tr>
<td>Total organic C</td>
<td>417 000</td>
</tr>
<tr>
<td>Total N</td>
<td>65 550</td>
</tr>
<tr>
<td>Organic N</td>
<td>62 207</td>
</tr>
<tr>
<td>Inorganic N</td>
<td>3296</td>
</tr>
<tr>
<td>NH₄</td>
<td>3290</td>
</tr>
<tr>
<td>NO₃ + NO₂</td>
<td>6</td>
</tr>
<tr>
<td>P</td>
<td>34 900</td>
</tr>
<tr>
<td>K</td>
<td>5660</td>
</tr>
<tr>
<td>Ca</td>
<td>16 100</td>
</tr>
<tr>
<td>Mg</td>
<td>5150</td>
</tr>
<tr>
<td>Na</td>
<td>1100</td>
</tr>
<tr>
<td>Fe</td>
<td>42 700</td>
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<tr>
<td>Cu</td>
<td>286</td>
</tr>
<tr>
<td>Zn</td>
<td>702</td>
</tr>
<tr>
<td>Cd</td>
<td>2</td>
</tr>
</tbody>
</table>

**Anaerobic (Waterlogged) Incubation**

Laboratory procedures were adapted from Bundy and Meisinger (1994). Bulk samples were made using 100 g of soil in 17.7 × 20.3 cm slider-sealable 0.045 mm-thick plastic bags. Amendments were added at the rates described above and mixed by hand to yield a homogeneous mixture. From the bulk mixture, 15 ± 0.01 g of the soil-plus-N-source mixture were placed into a 120-mL extraction cup. Fifty milliliters of deionized H₂O was added and swirled gently to minimize adhesion of the mixture to the walls of the container. The cups were sealed and placed in an incubator for 7 d at 40 ± 1°C. The samples were removed from the incubator and extracted with 50 mL of 2 mol L⁻¹ KCl and poured through a No. 42 Whatman filter paper. Samples were decanted into 20-mL scintillation vials, sealed, and placed in a freezer until analysis of inorganic-N (NH₄ + NO₃) of the filtrate on a Lachat QuikChem 8000 Flow Injection Analysis system (Lachat Instruments, Loveland, CO). The NAC was calculated as the inorganic N recovered as a percentage of the total N added (Table 2).

**Statistical Analysis**

For ANOVA, we used PROC MIXED (Littell et al., 2006) in SAS Version 9.4 (SAS Institute, 2017). Results from the initial three-way ANOVA, biosolids × rate × soil, were examined for interaction. If present, the analyses were broken out to examine all possible two-way interactions, and if present, simple effect means. Mean separation was performed using the PDMIX800 macro (Saxton, 1998) based on Tukey’s Method for all simple-effect pairwise means comparisons (Westfall et al., 1999). Statistical significance was judged at p ≤ 0.05; we use “significant” and its derivatives only to declare statistically significant differences. For the ANOVA, anaerobic incubation values of the unamended-soil controls were subtracted from the amended samples to isolate the effects of the amendments. By “no difference” or “no effect,” we mean none was detected.

**RESULTS AND DISCUSSION**

**Biosolids Analysis**

Numerically, CP contained the greatest amount of total N, followed by OC and RP (Table 3). Raleigh Plus had substantially lower total N than the other two biosolids, which likely reflects: (i) the effectiveness of the C-augmented denitrification process at the Neuse River WRRF, (ii) NH₄ lost via the addition of lime kiln dust (CaO + CaCO₃) used for pasteurization and disease vector reduction, and (iii) dilution by the lime. Generally, the greater the biosolids processing, the higher the C/N and the lower the N content and mineralization (Sommers et al., 1981). Total organic C
Table 4. North Carolina Dep. of Agric. and Consumer Services analyses (Hardy et al., 2014) of a single sample of each of the four soils used in the anaerobic incubation of biosolids; and study analyses of NH$_4$–N, NO$_3$–N, and total inorganic N (NH$_4$–N + NO$_3$–N) from anaerobic incubation of four replicates of unmanaged control soils.

<table>
<thead>
<tr>
<th>Soil series</th>
<th>Property</th>
<th>Noboco LS†</th>
<th>Norfolk LS</th>
<th>Vance SCL</th>
<th>Wedowee SL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pan</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NH$_4$–N</td>
<td>21.5 ± 0.43</td>
<td>17.9 ± 0.79</td>
<td>12.9 ± 3.4</td>
<td>41.7 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>NO$_3$–N</td>
<td>0.66 ± 0.35</td>
<td>1.82 ± 0.45</td>
<td>11.4 ± 3.5</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>Total inorganic N (NH$_4$–N + NO$_3$–N)</td>
<td>22.1 ± 0.71</td>
<td>19.7 ± 0.60</td>
<td>12.9 ± 3.4</td>
<td>41.7 ± 2.7</td>
</tr>
</tbody>
</table>

† LS, loamy sand; SCL, sandy clay loam; SL, sandy loam.
‡ Organic matter = (1.27 × humic matter) + 0.849 (Weber and Peter, 1982; Gonese and Weber, 1998).
§ Within rows, means followed by the same letter are not significantly different according to Tukey's method, p ≤ 0.05.

Potential Organic Matter (OM) and Available N

<table>
<thead>
<tr>
<th>Soil series</th>
<th>Property</th>
<th>F</th>
<th>Pr &gt; F</th>
<th>F</th>
<th>Pr &gt; F</th>
<th>F</th>
<th>Pr &gt; F</th>
<th>F</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NH$_4$–N</td>
<td>634</td>
<td>&lt;0.0001</td>
<td>32.9</td>
<td>&lt;0.0001</td>
<td>239</td>
<td>&lt;0.0001</td>
<td>239</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>NO$_3$–N</td>
<td>239</td>
<td>&lt;0.0001</td>
<td>2.23</td>
<td>0.087</td>
<td>41.3</td>
<td>&lt;0.0001</td>
<td>64.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>NAC</td>
<td>239</td>
<td>&lt;0.0001</td>
<td>4.11</td>
<td>0.0008</td>
<td>47</td>
<td>&lt;0.0001</td>
<td>11.8</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 5. Three-way ANOVA for the effects of three biosolids applied at four rates to four soils on anaerobic incubation potentially available N (Pan = NH$_4$–N + NO$_3$–N), NH$_4$–N; NO$_3$–N; and the N availability coefficient (NAC: PAN/total N). Target N rates were 64, 127, 191, and 254 kg ha$^{-1}$ and based on biosolids N content and book-value NAC (Table 1). To isolate the effects of biosolids, values of unamended control soils were subtracted from each rate.
(Table 6), biosolids × rate within each soil (Table 7), and rate × soil within each biosolids (Table 8). All two-way interactions were significant except for Rate × Soil with CP and OC. We present simple effect means comparisons in lieu of showing all of the simple effect ANOVA.

The PAN ranged from -12.1 for RP with the Wedowee soil to 146 mg kg⁻¹ for CP with the Norfolk soil. Negative values resulted when the 41.7 mg kg⁻¹ PAN of the unamended Wedowee soil control was subtracted from the RP PAN. This suggests that N was lost via denitrification or immobilized when RP was added to the Wedowee. In general, PAN trends reflected the total N of the biosolids (Table 3). Within all rates and soils, there was no difference (A vs. B column means in Table 9) in PAN between CP and OC. However, their PAN was greater than RP for all Rate × Soil combinations except: (i) at the lowest rate with the Noboco and Norfolk soils, where the same trend was apparent, and (ii) at the lowest rate with the Vance soil, where RP had about twice as much PAN as CP and OC. The latter was unexpected, so we reran the incubation, but similar results were obtained. Among the four soils, the Vance had the lowest humic matter (Table 3) and the least PAN in the control (Table 4). Mineralization may have been primed by the addition of RP (Dalenberg and Jager, 1989). Increasing the rate may have resulted in immobilization of PAN in microbial biomass due perhaps to alleviation of a constraint that had hindered microbial growth and reproduction prior to the addition of RP (Soriano-Disla et al., 2010).

The PAN as a proportion of the target rates ranged from -41 to 281%. In all cases except for CP on the Wedowee, PAN of CP and OC was greater than the target N rates, on average.
~25%, indicating that their disparate book-value NACs (Table 1) resulted in very similar underestimations of PAN. With the notable exception of the Vance soil, PAN of RP was consistently lower than the target N rate, indicating that its NAC tended to overestimate PAN, on average ~51%.

Within rates and biosolids (row means: a, b, c), soil had no effect on OC PAN at any rate, nor on CP PAN at the three lowest rates. At the highest rate, PAN of CP with the Norfolk soil was greater than with the Wedowee, with the other soils intermediate. In contrast, RP PAN with the Vance soil exceeded that on the Wedowee at all rates. At 64 and 191 kg N ha⁻¹, RP PAN with the Vance also exceeded that with the other soils. At 127 kg N ha⁻¹, RP PAN with the Vance was greater than with the Norfolk and Wedowee and tended to exceed Noboco PAN. At the highest rate, RP PAN with the Vance tended to exceed that with the Noboco and Norfolk. Except on the Vance, the response of RP reflected the contrast of its chemical properties with those of CP and RP. For example, RP had very low total and organic N, lower total organic C, very high pH, and a relatively high C/N ratio, 18.5, albeit one where net N mineralization would be expected (Vigil and Kissel, 1991). The organic matter in biosolids typically has two fractions, readily mineralizeable and refractory (Torrì et al., 2014). The high C/N ratio of RP compared with CP and RP (6.4 and 6.3, respectively) is reflective of an older, recalcitrant organic fraction.

As to be expected, within biosolids and soils, PAN of CP and OC tended to increase with increasing rate (Table 9 X, Y, Z). For CP and OC, the rate means separations were identical: the PAN at the highest rate always exceeded that at the lowest rate while intermediate rates were not significantly different from each other nor from the lowest and highest rates. On all soils except the Vance, PAN of RP tended to increase with rate, while with the Vance, no trend was apparent.

Table 8. Two-way ANOVA for the effects of four N rates × four soils within each of three biosolids on anaerobic incubation potentially available N (PAN = NH₄–N + NO₃–N); NH₄–N; NO₃–N; and the N availability coefficient (NAC: PAN/total N). Target N rates were 64, 127, 191, and 254 kg ha⁻¹ and based on biosolids N content and book-value NAC (Table 1). To isolate the effects of biosolids, values of unamended control soils were subtracted from the N parameters: PAN, NH₄–N, and NO₃–N.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Effect</th>
<th>df</th>
<th>F</th>
<th>Pr &gt; F</th>
<th>F</th>
<th>Pr &gt; F</th>
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</thead>
<tbody>
<tr>
<td>PAN</td>
<td>N rate</td>
<td>3</td>
<td>295</td>
<td>&lt;0.0001</td>
<td>90.84</td>
<td>&lt;0.0001</td>
<td>2.45</td>
<td>0.075</td>
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<tr>
<td></td>
<td>Soil</td>
<td>3</td>
<td>27.3</td>
<td>&lt;0.0001</td>
<td>0.59</td>
<td>0.62</td>
<td>95.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>N rate × soil</td>
<td>9</td>
<td>1.07</td>
<td>0.40</td>
<td>0.51</td>
<td>0.86</td>
<td>3.48</td>
<td>0.002</td>
</tr>
<tr>
<td>NH₄–N</td>
<td>N rate</td>
<td>3</td>
<td>347</td>
<td>&lt;0.0001</td>
<td>109</td>
<td>&lt;0.0001</td>
<td>1.50</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>Soil</td>
<td>3</td>
<td>49.2</td>
<td>&lt;0.0001</td>
<td>3.38</td>
<td>0.026</td>
<td>93.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>N rate × soil</td>
<td>9</td>
<td>1.23</td>
<td>0.30</td>
<td>0.62</td>
<td>0.77</td>
<td>5.77</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NO₃–N</td>
<td>N rate</td>
<td>3</td>
<td>0.01</td>
<td>1</td>
<td>0.01</td>
<td>0.99</td>
<td>6.33</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Soil</td>
<td>3</td>
<td>37</td>
<td>&lt;0.0001</td>
<td>37.5</td>
<td>&lt;0.0001</td>
<td>0.43</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>N rate × soil</td>
<td>9</td>
<td>0.01</td>
<td>1</td>
<td>0.01</td>
<td>1</td>
<td>3.58</td>
<td>0.001</td>
</tr>
<tr>
<td>NAC</td>
<td>N rate</td>
<td>3</td>
<td>13.2</td>
<td>&lt;0.0001</td>
<td>5.8</td>
<td>0.002</td>
<td>25.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Soil</td>
<td>3</td>
<td>25.6</td>
<td>&lt;0.0001</td>
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<td>0.93</td>
<td>104</td>
<td>&lt;0.0001</td>
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<td></td>
<td>N rate × soil</td>
<td>9</td>
<td>1.04</td>
<td>0.42</td>
<td>0.28</td>
<td>0.98</td>
<td>25.8</td>
<td>&lt;0.0001</td>
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</table>

Table 9. Simple effect mean potentially available N (PAN = NH₄–N + NO₃–N) from anaerobic incubation of three biosolids applied at four rates to four soils. Target N rates were based on biosolids N content and book-value N availability coefficients (Table 1). To isolate the effects of biosolids, the PAN of unamended control soil was subtracted, which in some cases resulted in negative values, implying incubation loss due to denitrification or immobilization. SE = 0.87.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Target N rate</th>
<th>Biosolids</th>
<th>Noboco</th>
<th>Norfolk</th>
<th>Vance</th>
<th>Wedowee</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg ha⁻¹</td>
<td>mg kg⁻¹</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>64</td>
<td>29.1</td>
<td>39.3</td>
<td>35.5</td>
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<td>74.6</td>
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<tr>
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<td>35.8</td>
<td>35.8</td>
<td>35.8</td>
<td>35.8</td>
</tr>
</tbody>
</table>

† Conversion: mg N kg⁻¹ = kg N ha⁻¹/2.2.
‡ Within a soil and rate, biosolids means followed by the same capital letter (A or B) are not significantly different according to Tukey’s method, p ≤ 0.05.
§ Within a rate and biosolids, soil (row) means followed by the same lowercase are not significantly different according to Tukey’s method, p ≤ 0.05.
¶ Within a biosolids and soil, rate means followed by the same capital letter (X, Y, Z) are not significantly different according to Tukey’s method, p ≤ 0.05.
Within all biosolids, means followed by the same lowercase letter are not significantly different according to Tukey’s method, $p \leq 0.05$.

Within a rate and soil, biosolids means followed by the same capital letter are not significantly different according to Tukey’s method, $p \leq 0.05$.

Within a biosolids and soil, Rate means followed by the same capital letter (X, Y, Z) are not significantly different according to Tukey’s method, $p \leq 0.05$.

Within a rate and biosolids, soil (row) means followed by the same lowercase letter are not significantly different according to Tukey’s method, $p \leq 0.05$.

Within a soil and rate, biosolids means followed by the same capital letter (A or B) are not significantly different according to Tukey’s method, $p \leq 0.05$.

Within a biosolids and soil, Rate means followed by the same lowercase letter are not significantly different according to Tukey’s method, $p \leq 0.05$.

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Within a biosolids and soil, Rate means followed by the same lowercase letter are not significantly different according to Tukey’s method, $p \leq 0.05$.

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Within a soil and rate, biosolids means followed by the same capital letter (A or B) are not significantly different according to Tukey’s method, $p \leq 0.05$.

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Within a biosolids and soil, Rate means followed by the same lowercase letter are not significantly different according to Tukey’s method, $p \leq 0.05$.

Within a rate and biosolids, soil (row) means followed by the same lowercase letter are not significantly different according to Tukey’s method, $p \leq 0.05$.

Within a soil and rate, biosolids means followed by the same capital letter (A or B) are not significantly different according to Tukey’s method, $p \leq 0.05$.

Within a biosolids and soil, Rate means followed by the same lowercase letter are not significantly different according to Tukey’s method, $p \leq 0.05$.

Within a rate and biosolids, soil (row) means followed by the same lowercase letter are not significantly different according to Tukey’s method, $p \leq 0.05$.

Within a soil and rate, biosolids means followed by the same capital letter (A or B) are not significantly different according to Tukey’s method, $p \leq 0.05$.

Within a biosolids and soil, Rate means followed by the same lowercase letter are not significantly different according to Tukey’s method, $p \leq 0.05$.
to the other N parameters, no three-way interaction was detected (Table 5). However, rate \(\times\) biosolids and biosolids \(\times\) soil interactions were significant while no rate \(\times\) soil interaction was detected. Within rates (Table 6), there was a biosolids \(\times\) soil interaction and significant main effects of biosolids and soils at all but the lowest rate. Within soils (Table 7), no biosolids \(\times\) rate interactions nor main effects of rate were detected. The main effect of biosolids was significant within all soils. Despite the lack of a Rate \(\times\) Soil interaction in the three-way ANOVA, to be consistent with the other analyses we examined the effects of Rate \(\times\) Soil within the biosolids (Table 8), which revealed a Rate \(\times\) Soil interaction for RP. For CP and OC but not for RP, the main effect of soil was significant, while the main effect of rate was significant for RP but not for CP and OC. Within rates and soils (Table 11, A vs. B), no differences among biosolids were detected with the Noboco and Norfolk soils. With the Vance soil, RP had more NO\(_3^–\)-N than CP and OC. The main effect of biosolids was not significant, while the main effect of rate was significant for RP but not for CP and OC. Within rates and soils (Table 11, A vs. B), no differences among biosolids were detected with the Noboco and Norfolk soils. With the Vance soil, RP had more NO\(_3^–\)-N than CP and OC at the 127 kg N ha\(^{-1}\) rate. These results reflected the initial chemical constitution of the biosolids, where NO\(_3^–\)-N and NO\(_2^–\)-N of CP and OC were negligible, while they constituted about 13% of the mineral N of RP. For the effects of soil within rates and biosolids (Table 11, row a,b,c): when differences were detected, the Vance soil had the least (most negative) NO\(_3^–\)-N due to the relatively large amount of NO\(_3^–\)-N measured in the incubated unamended soil. We have no hypotheses as to why the dynamics of NO\(_3^–\) mineralization on the Vance soil were different from all other treatments. Among the soils, the only distinguishing characteristics of the Vance were lowest humic matter and sieved weight/volume, and greatest K, Cu, and Zn.

**Nitrogen Availability Coefficients**

The NAC (\(=\) PAN/Total N applied) ranged from -0.13 for RP with the Wedowee soil to 0.86 for RP with the Vance (Table 12), substantially beyond the range of book-value NAC (Table 1) used to determine the experimental N rates. The three-way and all two-way interactions as well as all main effects of biosolids, rate, and soil were significant (Table 5). There was a biosolids \(\times\) soil interaction at all rates (Table 6), where the main effects of these factors were also significant. Within soils (Table 7), there were biosolids \(\times\) rate interactions with the Vance and Wedowee soils, but not with the Noboco and Norfolk (Table 7). The main effect of biosolids was significant for all soils, while that for Rate was significant with the Noboco, Norfolk, and Vance, but not the Wedowee. Within biosolids (Table 8), there was a Rate \(\times\) Soil interaction for RP but not for CP nor OC. The main effect of Rate was significant for all biosolids, while that for Soil was significant for CP and RP but not OC. Within rates and soils and for all but the three lower rates on the Vance soil (Table 12), the NAC of CP always exceeded that of RP, with the NAC of OC intermediate between these two. Within biosolids at the highest rate, soil had no detectable effect on the NAC of any of the biosolids. At the two intermediate rates, soil had no effect on the NAC of CP and OC. At these rates, the NAC of RP with the Vance soil exceeded or tended to exceed that with the other three soils, which did not differ among themselves. Within biosolids at the lowest rate, the NAC of CP with the Norfolk soil was greater than with the Wedowee soil, with the two other soils intermediate. At the lowest rate, soil did not affect the NAC of OC. At this rate, the NAC of RP with the Vance soil exceeded that with all other soils, while the Noboco and Norfolk soils did not differ but were greater than with the Wedowee. Consistent with PAN, the NAC of CP and OC were greater than the book values (Table 1) used to calculate the target N rates, that is, the book values underestimated PAN, while for RP, the opposite was true. Within biosolids and soils, the only rate effect detected was for RP on the Vance soil, where the NAC at the lowest rate exceeded those at all of the other rates. On all soils but the Wedowee, the NAC tended to decrease as rate increased, suggesting decreasing mineralization efficiency. On average, the NAC in the present study were less than half those determined by Nikolaides et al. (1999) via anaerobic incubation with a glaciated soil of biosolids derived from similar processes: heat-treated pelleted biosolids, 0.37 vs. 0.90; lime stabilized, 0.15 vs. 0.56. These differences may have been

### Table 12. Simple effect mean N availability coefficients (NAC = \([\text{PAN}] / \text{[total N applied]}\)) \(\text{PAN} = \text{NH}_4^+ + \text{NO}_3^–\)-N from anaerobic incubation of three biosolids applied at four rates to four soils. Target N rates were based on biosolids N content and book-value NAC (Table 1). To isolate the effects of biosolids, the PAN of unamended control soil was subtracted from that of soil plus biosolids, which in some cases resulted in negative values, implying incubation loss due to denitrification or immobilization. SE = 0.004.

<table>
<thead>
<tr>
<th>Target N rate</th>
<th>Biosolids</th>
<th>Noboco</th>
<th>Norfolk</th>
<th>Vance</th>
<th>Wedowee</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-\text{kg ha}^{-1})</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>64</td>
<td>Cary Pellet</td>
<td>0.41 A(<em>{\text{f}}) a(</em>{\text{b}})</td>
<td>0.52 A a</td>
<td>0.42 B ab</td>
<td>0.32 A b</td>
</tr>
<tr>
<td></td>
<td>OWASA cake</td>
<td>0.27 A B a</td>
<td>0.32 B a</td>
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<td>0.30 A a</td>
</tr>
<tr>
<td></td>
<td>Raleigh Plus</td>
<td>0.18 B b</td>
<td>0.18 B b</td>
<td>0.06 A a</td>
<td>-0.13 B c</td>
</tr>
<tr>
<td>127</td>
<td>Cary Pellet</td>
<td>0.34 A a</td>
<td>0.44 A a</td>
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</tr>
<tr>
<td></td>
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<td>0.24 B a</td>
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</tr>
<tr>
<td></td>
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<td>0.12 B b</td>
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<tr>
<td>191</td>
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<td>0.40 A a</td>
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<td>0.29 A a</td>
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<tr>
<td></td>
<td>OWASA cake</td>
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<td>0.23 A a</td>
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<td>0.22 A a</td>
</tr>
<tr>
<td></td>
<td>Raleigh Plus</td>
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<td>0.02 B bc</td>
</tr>
<tr>
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<td>Cary Pellet</td>
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<td>0.38 A a</td>
<td>0.35 A a</td>
<td>0.28 A a</td>
</tr>
<tr>
<td></td>
<td>OWASA cake</td>
<td>0.23 A B a</td>
<td>0.22AB a a</td>
<td>0.22 AB a</td>
<td>0.19 AB a</td>
</tr>
<tr>
<td></td>
<td>Raleigh Plus</td>
<td>0.094 B a</td>
<td>0.083 B a</td>
<td>0.14 B a</td>
<td>0.033 B a</td>
</tr>
</tbody>
</table>

- Within a rate and soil, biosolids means followed by the same capital letter are not significantly different according to Tykuey’s method, \(p \leq 0.05\).
- Within a rate and biosolids, soil (row) means followed by the same lowercase letter are not significantly different according to Tykuey’s method, \(p \leq 0.05\).
- Within all biosolids \(\times\) soil combinations, the only Rate effect detected was for the lowest rate of Raleigh Plus with the Vance soil, which had the greatest NAC compared to other rates in that biosolids \(\times\) soil combination.
due to the contrasting soils used: in the present study, A horizons of Ultisols, vs. Nikolaidis et al., the B horizon of an Inceptisol.

Presuming that anaerobic incubation is a reliable indicator of NAC, results of the present study indicate that NAC, and thus PAN, might best be estimated by incubating a particular biosolids with the receiving soil. The evidence for this is substantial: (i) within all application rates, the biosolids × soil interaction was highly significant, and (ii) the NAC of two biosolids, CP and RP, varied significantly across soils at one or more rates, RP by as much as an order of magnitude. Such incubations would likely provide better estimates of PAN than would a generic book-value NAC based solely on the treatment processes used to produce a biosolids and independent of the characteristics of the receiving soil.

Whether incubations need be conducted across a range of potential application rates is, perhaps, less clear, but the results provide evidence that they should be. In addition to the significant biosolids × rate × soil interaction, there was a biosolids × rate interaction with the Vance and Wedowee soils, and a rate × soil interaction for RP. While there was only one biosolids-soil combination where a significant rate effect on the NAC was detected, that is, RP with the Vance, whether this would be the case for any particular biosolids–soil combination would not be known a priori. Our study provides little guidance in this regard: we examined only a single exemplar of each biosolids type and two soil pairs distinguished by their origins: Coastal Plain vs. Piedmont. For all parameters examined and among the 12 biosolids–soil combinations, we discerned no associations with regions, that is, Coastal Plain vs. Piedmont. In fact, the two most comparable soils were the Noboco and the Wedowee, the former from the Coastal Plain and the latter from the Piedmont. Relative to the other two soils, the Noboco and Wedowee shared some characteristic and not others. Of the four soils, they had the greatest CEC, base saturation, pH, Ca, and Cu; the lowest acidity and S; and intermediate sieved weight per volume and K. Their humic matter, Mg, Mn, and Zn were substantially different. The biosolids types were distinctly different both in the WRRF processes used to produce them and their ultimate characteristics. Among them, CP might be the preferable N source: it had the greatest initial N content and tended to have the greatest NAC; and had the lowest moisture content and pellet form, which make handling, transport, and application easier and less expensive than the other biosolids. However, CP had the lowest N/P ratio: if applied based on an agronomic N rate, it would be the most likely to result in excessive P application (Jameson et al., 2016).

To investigate whether biosolids and soil characteristics can be used to discern biosolids–soil combinations for which NAC are independent of rate would require a substantially larger study comprising multiple exemplars of individual biosolids types and soils incubated at multiple rates. Current book values presume that a single NAC well represents a treatment-process-based biosolids type across all soils and all N application rates. Based on our anaerobic incubation results, this presumption is unwarranted. If anaerobic incubation provides reasonable PAN and NAC estimates, NAC of any particular biosolids might best be determined via anaerobic incubation with the receiving soil across an RYE-based range of N application rates. A compromise might be to use an appropriate book-value NAC to estimate an application rate, then refine the NAC by conducting an aerobic incubation of the biosolids with the receiving soil at the estimated rate.

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REFERENCES


