Soil Carbon and Nitrogen Fraction Accumulation with Long-Term Biosolids Applications

Global sustainability depends on the recycling of organic wastes, containing carbon and plant nutrients, back into food production. Biosolids are capable of building total soil organic matter, but their ability to build stable organic C and N fractions is less well understood. The sequestration of stable soil C and replacement of energy-requiring commercial fertilizers both have implications for greenhouse gas mitigation. Our aim was to assess the effectiveness of biosolids amendments to store labile and stable soil C and N fractions while supplying crop N needs. Three rates of anaerobically digested biosolids were incorporated following wheat harvest every 4 yr in a wheat-fallow system over 20 yr, and compared to a no-fertilizer check and a standard commercial fertilizer treatment. Increases in total soil C and N correlated to cumulative application rates of total and acid-resistant, non-hydrolyzable (NH) C and N, with 91% of added biosolids C efficiently retained in soil. The soil accumulated light fraction (LF) C and N in biosolids treated plots, whereas the LF pools of the control plots decreased and LF pools in the commercial fertilizer plots stayed relatively constant. Total soil N correlated with cumulative biosolids N, with 35% of added biosolids N retained in the soil of which only 4% was stored in the soil NH N fraction. Anhydrous ammonia increased wheat yields by 27% over the 0-fertilizer check, without increasing soil N. Biosolids markedly elevated total, stable and LF soil C and N pools in semiarid conditions, while maintaining comparable wheat productivity to commercially fertilized wheat-fallow.

Abbreviations: LF, light fraction; NH, non-hydrolyzable.

A pplication of biosolids to food crops has several benefits. Biosolids recycling completes the cycle of nutrients through soil-plant-human systems (Brown et al., 2010), and it is an effective mitigation strategy for reducing greenhouse gas emissions by substituting for synthetic fertilizers and increasing organic matter (Bogner et al., 2007). Land application of biosolids supplies a full complement of plant nutrients (Barbarick et al., 2010; Cogger et al., 2013), reducing the need for synthetic fertilizers that require fossil fuel inputs and generate greenhouse gas emissions during fertilizer manufacturing (Wood and Cowie, 2004). Land application of biosolids also fosters soil C and N accumulation, thereby reducing greenhouse gas emissions otherwise incurred during more common biosolids disposal pathways such as incineration and landfilling (Brown et al., 2010).

Intensive cropping practices of cereal production systems have degraded agricultural soils, and the degradation is best represented by decreased soil organic matter due to erosion and accelerated soil respiration with intensive tillage (Reicowski et al., 1995). Land-applied organic amendments help to restore depleted soils (Gibson et al., 2002) and improve soil water holding capacity (Brown et al., 2011). Long-term experiments have globally illustrated that the application of organic soil amendments is most effective at re-building soil organic matter while...
supplying plant-available nutrients for human food production (Jenkinson, 1991; Katterer and Andren, 1998; Rasmussen et al., 1998). Manure has been shown to be far more effective at elevating soil organic matter than inorganic fertilizer inputs in an 80-yr experiment on semiarid wheat (Machado, 2011).

Land application of biosolids has potential for reducing greenhouse gas emissions with increased soil C storage and fertilizer replacement according to life cycle analysis (Brown et al., 2010). A key variable in these models is the estimation of biosolids C sequestration. Long-term application of lagoon-stored biosolids has increased total soil C at a rate of 1.7 Mg C ha\(^{-1}\) yr\(^{-1}\) compared to 0.1 Mg C ha\(^{-1}\) yr\(^{-1}\) in fertilized controls (Tian et al., 2009). Emissions of lagoon-stored biosolids displayed slower CO\(_2\) evolution, indicative of greater stabilized C forms relative to fresh biosolids (Gilmour et al., 2003). A model of total soil C accumulation with land application of biosolids uses a predicted storage efficiency of 25 Mg CO\(_2\)e per 100 Mg of dry biosolids (Brown et al., 2010).

The light fraction of soil organic matter of agricultural soils typically represents a labile C pool of partially decomposed plant residues, and not a pool generally considered stable. This fraction might serve to be an indicator of long-term impacts of cropping practices (Jenzen et al., 1992). It is isolated by flotation on high specific gravity solutions containing soluble salts such as NaI (Nyborg et al., 1999). Increased soil organic matter with conversion from conventional tillage to no-tillage systems is mainly attributable to increased labile fraction (LF) C in fine soil (Tian et al., 2007) and wheat (Jenzen et al., 1992), eliciting a distinction between soil C accretion and sequestration when crop residues are the primary source of organic inputs into a semiarid cropping system (Gollany et al., 2013).

The heavy fraction typically contains more modified and stable soil organic matter (Golchin et al., 1995). Analysis of biosolids revealed fatty acids dominated the isolated polar fraction, with smaller quantities of aliphatic acids and sterols. Stable sterols were speculated to contribute to the recalcitrant C pool (Torri and Alberti, 2012).

The chemical forms of organic matter that are added and subsequently formed may influence the efficiency of long-term soil C and N sequestration. Chemical fractions of soil C and N are theorized to correlate with their stability and half-life. Hydrolysis of soil organic matter with strong acid is a long-standing method for isolating stable organic compounds (Bremner, 1949; Plante et al., 2006) by releasing chemically labile proteins, nucleic acids and polysaccharides, while leaving recalcitrant long-chain aliphatics and aromatics (Martel & Paul, 1974; Schnitzer & Preston, 1983). The acid recalcitrant (non-hydrolyzable [NH] C) and soil humin fractions are correlated to the oldest C fraction determined with radiocarbon dating (Helfrich et al., 2007; Pessenda et al., 2001). The NH C was more associated with silt than clay-sized soil particles (Plante et al., 2006).

Biosolids had been applied at three rates every 4 yr over 20 yr, supplying ample N and P to produce wheat grain yields comparable to plots receiving commercial fertilizer in a semiarid system (Cogger et al., 2013). Ultimate impacts of biosolids land application are dependent on prospects for long-term buildup of soil C and N storage pools, which have been represented by acid hydrolysis and density fractionations. The present study was conducted to (i) determine soil total, non-hydrolyzable, hydrolyzable, and light fraction C and N accumulation rates with repeated land application of biosolids when compared to anhydrous ammonia, (ii) assess apparent N recoveries into grain and soil from biosolids and standard fertilizer inputs, and based on these findings, (iii) assess potential implications of biosolids use in wheat-fallow on soil C and N storage pools with varying prospects for long-term greenhouse gas mitigation.

**MATERIALS AND METHODS**

**Field Site and Experiment**

A long-term biosolids experiment has been conducted on a commercial farm field since 1994 in northcentral Washington where biosolids have been applied to a semiarid winter wheat-fallow cropping system (Cogger et al., 2013). The climate is cold and wet in winter, hot and dry in summer where summer fallow is typically practiced. Summer fallow was managed with shallow chisel plowing, disked and rodweeded to 10- to 15-cm depth. Biosolids were produced in a King County, WA, sewage treatment plant at Renton, WA, that anaerobically digests sewage sludge at 37°C and dewatered it with a belt press to total solids contents of 18.3 to 22.0%. The biosolids were applied every 4 yr, in the fall following wheat harvest beginning in 1994. Five treatments included a no amendment control, conventional standard fertilizer, and three rates of biosolids. Biosolids were disked into the top 10 cm, at rates of 0, 4.8, 6.9 and 9.0 dry Mt ha\(^{-1}\), compared to wheat fallow conventionally fertilized with 56 kg N ha\(^{-1}\) shanked to a 5- to 10-cm depth of every 2 yr as anhydrous ammonia and ammonium thiosulfate at overall N to S ratio of 5:1. Phosphorus and potassium soil test levels were sufficient. Plots 15 m × 275 m arranged in a randomized complete block design were established on Timentwa ash fine sandy loam (mesic Vitrandic Haploxeroll) near Waterville, WA, with 270 mm mean annual precipitation.

Winter wheat (*Triticum aestivum* L. ‘Eltan’) was planted during the previous August of the 1994 to 2014 alternating growing seasons with fallow. Grain was combine harvested within a 7.5 m × 275 m center of each plot, truck weighed and subsampled for grain N analysis (Cogger et al., 2013).

**Soil Sampling and Processing**

Soil samples were taken after each wheat harvest, and shortly before the application of biosolids in 1998, 2002, 2006, and 2014. Ten samples were taken from the top 10 cm of soil and composited for each plot. Bulk density was determined by collecting intact soil cores using a hammer-driven core sampler (three per plot), determining the moisture content of a subsample and applying this value to solve for mass of dry soil in the 146 cm\(^3\) soil sample. Composite samples were screened through a 2-mm sieve and air dried at 40°C, and ground for total C and N dry combustion analysis (LECO corporation, model ‘Truspec’, St. Joseph, MO).
Acid Hydrolysis

Acid hydrolysis of archived soils (Cogger et al., 2013), additional soils in 2014, and biosolids samples archived from 2006 were analyzed for recalcitrant or NH C and N remaining in the sample after strong acid digestion. A 1-g subsample of the sieved soil or a 1 to 3 g of dried biosolids was put into a digestion tube with 25 mL of 6 mol L⁻¹ HCl, capped with a large glass marble, and refluxed at 115°C for 16 h. Samples were then filtered, air dried, and weighed to quantify mass loss due to hydrolysis. Then 0.25 g of the soil remaining after hydrolysis was weighed for dry combustion analysis. Non-hydrolyzable C concentration was calculated by:

\[
\text{NH C} = \left( \frac{\text{g kg sample}}{\text{g sample ash}} \right) \times \left( \frac{\text{g sample ash}}{\text{g sample wet}} \right)
\]

as in Plante et al. (2006). The same method was used to calculate NH N. Hydrolyzable C and N were calculated as the difference between total and NH C and N.

Light Fraction

The light fraction (LF) C and N were determined through density fractionation, in which 22.5 g of dried soil was brought up to 10% moisture (to reflect the field conditions) with 2.5 g of deionized water. A 50-mL aliquot of 1.7 specific gravity NaI was added to each sample. Samples were shaken for 1 h, transferred to a 125-mL Erlenmeyer flask and the heavy fraction settled for 24 h. After the settling period the LF was skimmed off the top of the NaI solution and into a Buchner funnel lined with a grade F glass-fiber filter. Filtered samples were rinsed 3 times with CaCl₂ and 3 times with deionized water before being air dried at 40°C for 24 h. A 0.1-g sample of the LF was hand ground and weighed for LECO combustion analysis. The soil from the Waterville Plateau contain pumice, which has a density similar to the LF. The pumice floated with the LF, and this would have skewed the results if it were not removed. Pumice was quantified by burning approximately 0.5 g of sample for 12 h at 375°C in a muffle furnace. Samples were weighed after ignition to determine the ratio of fresh and partially decomposed residues and humus to pumice. This ratio was used to correct the floating fraction so that just LF was being quantified.

Apparent Fertilizer N Recovery

Apparent fertilizer recovery in the wheat grain (AFRg) and soil (AFRr) from anhydrous ammonia and the three biosolids application rates were estimated (Raun and Johnson, 1999) as:

\[
\text{AFR}_g = \frac{(\text{fertilized grain N}) - (\text{untreated grain N})}{(\text{total fertilizer N applied})}
\]

\[
\text{AFR}_r = \frac{(\text{fertilized final soil N}) - (\text{untreated final soil N})}{(\text{fertilizer N applied})}
\]

\[
\text{AFRt} = \text{AFR}_g + \text{AFR}_r
\]

Linear regressions between soil total C and total N, NH C and N, and hydrolyzable C and N in samples collected over the 20 yr to obtain the slopes, intercepts and correlation coefficients (Fig. 1) using Sigmaplot (Systat Software, Inc., 2016). The total soil-hydrolyzable and non-hydrolyzable fractional C and N accumulation were also regressed against biosolids C and N cumulative inputs (Fig. 2). Means of three replicates and standard errors are presented for these linear relationships.

RESULTS AND DISCUSSION

Repeated biosolids application to a wheat-fallow rotation every 4 yr supplied sufficient available N and other nutrients to produce wheat yields and grain N equal to or higher than wheat that was fertilized during summer fallow with anhydrous ammonia (Cogger et al., 2013). A method for estimating apparent fertilizer N recovery (Raun and Johnson, 1999) in grain and soil was applied to published data from this experiment (Table 1). This method is based on proportional increase in N recovery in grain and soil N of fertilized vs. untreated check plots. Greater apparent grain and soil N efficiencies of ammonia fertilizer than biosolids was observed. High recovery of anhydrous ammonia N applied during summer fallow of winter wheat was previously observed (Pan et al., 2001). Applied N not captured in grain and top 10 cm of soil was subject to nitrification beyond the root zone, ammonia volatilization and denitrification. Impacts of land applied biosolids on nitrous oxide and methane greenhouse gas emissions have yet to be well-documented in this type of cropping system. Soil analyses conducted to 30-cm depth in 2012 showed some nitrate leaching, but no significant increases in total C and N in 10- to 30-cm depths (Cogger et al., 2013). Biosolids increased grain and soil N relative to the control, while anhydrous ammonia mostly increased grain N. Grain N production was higher in the medium and high biosolids rates compared to the standard fertilizer treatment (Table 1).

Analysis of archived and new 0 to 10 cm soil samples from this experiment enabled an assessment of changes in soil organic C and N fractions that contribute reactive and stable pools of the increases in total soil C and N. Biosolids application rates affected the linear rate of total soil C and N accumulation (Fig. 1). Time series of soil C and N annual accumulation over 20 yr shows that applications of biosolids every 4 yr were associated with incremental increases in total C and N, with slopes ranking by treatment as follows: 9.0 Mg ha⁻¹ > 6.9 Mg ha⁻¹ > 4.8 Mg ha⁻¹ > anhydrous ammonia = 0-fertilizer check (Fig. 1; Table 2). Total soil C and N of anhydrous ammonia and untreated check plots were indistinguishable until the last sampling. The high biosolids treatment resulted in total soil C accumulation at a rate of 0.77 Mg C ha⁻¹ yr⁻¹, less than the 1.7 Mg C ha⁻¹ yr⁻¹ earlier reported for lagoon stored biosolids on entisols and alfisols of the US Midwest (Tian et al., 2009).

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Total soil C increased by 0.91 Mg C per Mg of added C in biosolids (0.186 Mg C per Mg dry biosolids, 67 Mg CO₂e per 100 Mg dry biosolids) added over the course of the experiment (Fig. 2). This is a higher rate of soil C retention of land.
applied biosolids than the 25 Mg CO₂ per 100 Mg dry biosolids previously observed and modeled (Brown et al., 2010), but lower than the 140 Mg CO₂e per 100 Mg dry biosolids estimated by Trlica and Brown (2013). If only stable C, as represented by non-hydrolyzable C, is credited for C sequestration, then the 0.34 Mg C in soil per Mg of added C in biosolids (0.07 Mg C per Mg dry biosolids; 25 Mg CO₂e per 100 Mg dry biosolids) rate of C accumulation should be used, a value that is in accordance with Brown et al. (2010).

Fig. 1. Linear regressions of (a) total soil C, (b) light fraction C, (c) total soil N and (d) light fraction N accumulation over time in untreated control, anhydrous ammonia (AA), and three rates of biosolids, after initiation of the experiment in 1994. Symbols and error bars represent treatment means of three replicates ± standard error. Regression statistics of annual accumulation rates are presented in Table 2.

Fig. 2. Cumulative gains in (a) soil hydrolyzable C, non-hydrolyzable C as a function of total biosolids C added, and (b) soil hydrolyzable N, non-hydrolyzable N as a function of total biosolids N added over 20 yr. Symbols and error bars represent treatment means of three replicates ± standard error.
In contrast, total soil N only increased by 0.41 Mg soil N per Mg dry biosolids N applied (Fig. 2), while large portions of applied biosolids N were being removed with grain harvest (Table 1). Only a slight increase in non-hydrolyzable soil N was observed, at an accumulation rate of 0.06 Mg soil NH N per Mg dry biosolids N applied.

Relative contributions of hydrolyzable and non-hydrolyzable fractions to total soil C and N accumulation are shown in Fig. 3. The amount of soil NH C in the system receiving 9.0 Mg ha\(^{-1}\) biosolids increased by 97% over the course of 20 yr (Fig. 2), while the zero amendment check plots increased in NH C by only 29%. The added biosolids C efficiently accumulated at 0.9 Mg soil C per Mg C added, with a majority buildup of hydrolyzable C, but significant buildup of NH C. In contrast, soil N retention efficiency was only 0.41 Mg soil N per Mg N added (Fig. 2) due to biosolids contribution to grain N removal (Table 1). A large fraction of the total gain in soil N was attributed to a buildup in hydrolyzable N. The microbial processes during the anaerobic digestion of the biosolids at the sewage plant likely contributed to increasing the acid resistant fraction in the biosolids. Mantovi et al. (2005) demonstrated that composted sewage sludge was more effective than liquid or dewatered raw sewage sludge in building soil organic matter per unit of carbon input. Similarly in this study, the application of anaerobically digested biosolids supplied the soil with acid resistant NH C that sufficiently accounted for the buildup of acid resistant soil C and N over 20 yr. Biosolids and manure were also found to be more effective at building soil organic C compared to legume and wheat residues in continuous wheat or fallow systems (Weust and Gollany, 2013), and these elevated levels were sustained several years after the applications (Weust and Reardon, 2016).

The C to N ratio of soil fractions has been a standard indicator of lability of N in organic matter (Nicolardot et al., 2001). The relationships between soil C and soil N among all treatments were linear, and incremental increases in total soil C was accompanied by a correlated increase in total soil N at a ratio of 12.4:1 C to N (Fig. 3a), supporting the conclusion of others that buildup of soil C requires a concomitant in total soil N (Kirkby et al., 2011). This soil C to N ratio compares to a lower 5.7:1 C to N of the applied biosolids (Table 3), reflecting (i) a larger soil storage of biosolids C compared to N due to biosolids N mineralization and use by the wheat crop (Table 1), and (ii) potential soil N losses greater than soil C losses.

The LF organic matter increased over treatments and years at a C to N slope ratio of 14.7:1 (Fig. 3b), while the slope of the hydrolyzable fraction increased at a 9.2:1 slope ratio (Fig. 3c), and the non-hydrolyzable organic fraction increased at the highest C to N ratio of 21.5:1 (Fig. 3d). The LF accumulated at a C to N ratio between the ratios of the hydrolyzable and non-hydrolyzable fractions in the biosolids, suggesting that the new LF was composed of a mixture of high C:N from NH C and lower C:N from hydrolyzable C. The C to N ratios of all soil fractions likely correlate with their N mineralization potential (Nicolardot et al., 2001). Cogger et al. (2013) reported narrow ranges of C (310 to 360 g C kg\(^{-1}\)) and N (52 to 65 N g kg\(^{-1}\)) concentrations in the biosolids used in this experiment. The biosolids were all sourced from the same sewage processing facility that used anaerobic digestion. Fractionation of the anaerobically digested biosolids archived from 2006 revealed 99% of the total biosolids had a low density that classified as LF organic matter, of which 32% was non-hydrolyzable. This non-hydrolyzable fraction of the biosolids samples had a 39.4 C to N ratio, in stark contrast to the 4.7

Table 2. Slopes and correlation coefficients for linear gains in total cumulative soil C and N in 0- to 10-cm depth in the control, three biosolid rates, and anhydrous ammonia (AA). The slope represents annual accumulation (Mg ha\(^{-1}\) yr\(^{-1}\)) over the 20-yr experiment.

<table>
<thead>
<tr>
<th>Soil treatment</th>
<th>Total C accumulation over time</th>
<th>Total N accumulation over time</th>
<th>LF† C accumulation over time</th>
<th>LF† N accumulation over time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope</td>
<td>r(^2)</td>
<td>Slope</td>
<td>r(^2)</td>
</tr>
<tr>
<td>0 C or N added</td>
<td>0.158</td>
<td>0.69</td>
<td>0.003</td>
<td>0.10</td>
</tr>
<tr>
<td>4.8 Mg ha(^{-1}) biosolids</td>
<td>0.394</td>
<td>0.57</td>
<td>0.027</td>
<td>0.59</td>
</tr>
<tr>
<td>6.9 Mg ha(^{-1}) biosolids</td>
<td>0.478</td>
<td>0.77</td>
<td>0.035</td>
<td>0.72</td>
</tr>
<tr>
<td>9.0 Mg ha(^{-1}) biosolids</td>
<td>0.772</td>
<td>0.96</td>
<td>0.064</td>
<td>0.97</td>
</tr>
<tr>
<td>AA</td>
<td>0.314</td>
<td>0.59</td>
<td>0.017</td>
<td>0.39</td>
</tr>
</tbody>
</table>

† LF, light fraction.
While humic substance-like fractions contained in processed organic amendments chemically differ from humic substances of native soils, they assimilate in form over time (Senesi et al., 2007). Typically, the LF in agricultural soils contains relatively less stable plant materials, microbial biomass such as fungal hyphae and spores, largely representing an intermediate pool between undecomposed biological materials and humified organic matter (Gregorich and Janzen, 1996). However, the present results suggest anaerobically digested biosolids applied to soils adds significant amounts of NH₃-C and N in the light fraction.

Similarly, microbial decomposition of organic materials during composting increases humic acids in compost (Inbar et al., 1990; Zinati et al., 2001). Buildup of humic acids, 9 mo after sludge applied to a calcareous sandy soil, eventually dissipated 36 mo after application (García-Gil et al., 2004). Zinati et al. (2001) demonstrated a buildup of humic compounds following application of composted municipal solid waste and biosolids.

The municipal solid waste buildup of humic substances has been demonstrated in arid soils (Pascual et al., 1999).

The total quantity of NH₃-N in the added biosolids over the course of this experiment can only be estimated since biosolids were not archived from each application. Based on the assumption that all of the biosolids had the same C and N fractionation measured in the archived 2006 samples, a major portion of biosolids NH₃-N added over time could account for much of the total increases in soil NH₃-N observed in the biosolids plots.

**CONCLUSION**

Recycling biosolids as soil amendments constitutes a potential climate change mitigation strategy due to the high retention of soil C demonstrated in this long-term study, and to a reduction in synthetic fertilizer needs (Ippolito et al., 2007; Koenig et al., 2011). High soil C accumulation efficiency may be related to the temperate semiarid climate, where surface soil drying during hot summers may slow organic decomposition.
Table 3. Contributions of the hydrolyzable and non-hydrolyzable components to the total biosolids gravimetric mass, C and N concentrations in the total biosolids mass and in each fraction (in parentheses), and the C to N ratio of each fraction. Means and standard deviations of 8 samples are presented.

<table>
<thead>
<tr>
<th>Biosolids fraction</th>
<th>Mass (g per 100 g biosolids)</th>
<th>Carbon (g per 100 g total dry mass)</th>
<th>Nitrogen (g per 100 g fraction)</th>
<th>C to N ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolyzable</td>
<td>67.8 ± 1.1†</td>
<td>20.4 ± 0.5 (30.3%)</td>
<td>4.29 ± 0.03 (6.40%)</td>
<td>4.76 ± 0.09</td>
</tr>
<tr>
<td>Non-hydrolyzable</td>
<td>32.2 ± 1.1</td>
<td>15.9 ± 0.5 (49.3%)</td>
<td>0.40 ± 0.03 (1.25%)</td>
<td>39.47 ± 1.89</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>36.3</td>
<td>4.70</td>
<td>7.72</td>
</tr>
</tbody>
</table>

† Treatment means followed by the same letter are not significantly different at p < 0.05 type I error.

While recalcitrant fractions are the more stable forms of sequestered carbon, estimates of greenhouse gas reductions have been based on changes in total organic matter. Since the soil NH C is recognized as a long-term storage pool that is slow to respond to management changes, future life cycle analyses may need to consider this soil C pool as more conservative estimate of long-term reduction of greenhouse gas emissions. Nevertheless, the hydrolyzable C pool was a major contributor to the long-term buildup of total C in soil regularly amended with biosolids every 4 yr. Buildup of soil LF C and N in the biosolids amended soils was above and beyond LF levels with standard fertilization. This observation, coupled with the finding that the biosolids sample analyzed was a light fraction material, supports the conjecture that light fraction compounds persist in soil. These findings also suggest that some of the soil NH C and N buildup resulting from land application of biosolids could originate from recalcitrant compounds originally present in the light density, anaerobically digested biosolids.

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