Sequestering C in Stable Soil Organic Matter Fractions: How important is fertilizer-N in sequestering C?

Chris van Kessel¹, Johan Six¹, and Haegeun Chung¹

Summary

Fertilizer-N addition has the potential to increase soil C and N sequestration through its interaction with incorporated residue. In a previous incubation study, we found that mineral-N addition promoted the transformation of residue-C to humin-C. Moreover, N was sequestered more when both fertilizer and residue was applied than with the addition of mineral-N or residue alone. Based on our previous results, we hypothesized that C and N sequestration in stable C and N pools will be highest in an agroecosystem in which both N fertilizer and residue are applied. We tested our hypothesis in the cropping systems at the Center for Integrated Farming Systems (CIFS) of the University of California, Davis. The main cropping system is a two-year rotation of corn and tomatoes, and is under conventional, integrated, and organic management. In the conventional system, N fertilizer is applied, and in the integrated system, fertilizer is added and residue is incorporated in alternating years. Under the organic management, residue is incorporated and manure is applied. We employed extracellular enzyme assay, soil incubation, and alkali extraction and acid precipitation to determine whether the integrated system has the highest proportion of C and N sequestered in stable organic matter fractions.

The organic system sequestered the greatest level of C and N in long-term stabilized C and N pools. Cellulolytic potential was highest in the organic system, and this likely is the mechanistic link to the greatest cumulative respiration observed in this farming system. Phenol oxidase activity, on the other hand, was lowest in the organic system, which could be due to relatively low recalcitrance of the organic substrates as suggested by the lowest soil C:N ratio observed in this system. The efficiency of C and N sequestration expressed as total soil C and N residing in stable C and N pools did not differ between the conventional, integrated, and the organic farming systems, which suggests that the combined effect of biological and chemical SOM sequestration mechanisms are similar across the three farming systems. Our results demonstrate that long-term C and N stabilization is greatest in the organic system, likely through the high organic substrate input.

Objectives

1) To determine how residue-N and fertilizer-N interact to enhance C and N sequestration in stable organic matter pools under field conditions.

2) To investigate the biological and chemical mechanisms underlying soil C and N sequestration in conventional, low-input, and organic farming systems.

In this study, we aim to investigate N sequestration in stable organic matter pools as well as C sequestration, and to compare not only conventional and organic farming systems but also the

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integrated system in which chemical fertilizer is applied and residue is incorporated in alternate years.

**Approaches and Procedures**

**Experimental design and soil sampling**

Our research was conducted at the CIFS (Center for Integrated Farming Systems) experiment at the University of California, Davis. CIFS was established in 1993 to study agronomic, biological, and economic aspects of conventional and alternative farming systems in the Sacramento Valley in California. The region has a Mediterranean climate with most rainfall occurring during the winter months (December – March) and relatively little during the growing season. Total annual rainfall is typically 400-500 mm, and daytime temperatures during the growing season average 30-35 °C. The soils at this site are classified as Yolo silt loam (fine-silty, mixed, non-acid, thermic Typic Xerorthents) and Reiff loam (coarse-loamy, mixed, non-acid, thermic Mollic Xerofluvents).

The cropping system is a two-year rotation, seeded to corn and tomatoes in each of the 64-m x 64-m plot, and is managed with conventional, integrated, and organic practices. There are two phases (the odd and the even years) of each cropping system, and each phase of a cropping system has three replicates. The conventional system receives fertilization twice a year. In the integrated system, cover crop residue is incorporated during the odd years before corn is planted, and moderate rates of N fertilizer are applied during the even years before tomatoes are planted. In the organic system, cover crop residue and manure are incorporated before every cash crop. Starting in the year 2002, each plot of the cropping systems was split into two half of the plot was randomly assigned to standard tillage (ST), whereas the other half of the plot was subjected to conservation tillage (CT). In November 2005 and August 2006, soil samples were collected from tomato and corn plots that are subjected to ST under conventional, integrated, and organic farming systems. Soil samples collected in November 2005 were analyzed for their humin and humic acid contents, and soil samples from August 2006 were subjected to enzyme analysis and incubation.

**Enzyme analysis**

Fluorometric assays using methylumbelliferone (MUB)-linked substrates were performed to determine the activities of 1,4-β-glucosidase and cellobiohydrolase, which are enzymes that degrade cellulose. Two grams of each composite soil sample were combined with 125 mL of sodium acetate buffer (pH 5.0). The soil slurry was loaded on a 96-well microplate and for each enzyme assay, there were eight analytical replicates. We combined 200 µL of soil slurry and 50 µL of substrate specific for each enzyme. Both enzyme assays were incubated at 21 °C for two hours. Fluorescence was measured using an f-Max fluorometer (Molecular Devices Corp., Sunnydale, CA); excitation energy was 355 nm and emission was measured at 460 nm. Enzyme activity was expressed as nmol 4-MUB g⁻¹h⁻¹.

The activity of phenol oxidase and peroxidase was measured through colorimetric assay using 25 mM L-3,4-dihydroxy-phenylalanine (L-DOPA) as the substrate (Saiya-Cork et al. 2002). Procedures for these colorimetric assays were similar to those of the fluorometric assays described above. For each soil sample, there were 16 analytical replicates for both phenol
oxidase and peroxidase assays. The 96-well microplates were incubated at 21 °C for 24 hours, and absorbance was measured at 450 nm on EL-800 plate reader (Biotek Instruments, Inc., Winooski, VT). Activity was expressed in nmol L-DOPA oxidized g⁻¹h⁻¹.

**Soil incubation**

A 50-g subsample of the air-dried soil from each plot was incubated at 37°C in plastic filters. A glass fiber filter, and ‘extra thick’ glass fiber prefilter was placed beneath the soil. The filter units were sealed in airtight 2L jars fitted with septa. Approximately 20 ml of deionized water was placed in the bottom of each jar to prevent soil from drying. To determine the labile N pool size, the soil was leached at 1, 8, 24, 44, 66, 94 days with a solution containing all essential nutrients except N (Nadelhoffer 1990). At each leaching, 100 ml of the N-free leaching solution was added to the top of the filter, and allowed to equilibrate with the soil for 30 minutes. The leaching solution was then drawn through the filter with a weak vacuum, and the vacuum was applied until leachate ceased to drip from the filter. The leachates were frozen until analysis for NH₄⁺ and NO₃⁻ by spectrophotometric reading. The labile N pool was defined as the sum of NH₄⁺ and NO₃⁻. The stable N pool was determined by subtracting the labile N pool from the total soil N pool. The N content was converted to mg N per gram of soil using leachate volume and initial dry mass of the incubated soil.

**CO₂ analysis**

To determine the size of the labile C pool, CO₂ in the headspace of the incubation jars were analyzed. To equilibrate the CO₂ concentration in the jars with that of the atmosphere, the jars were fanned with atmospheric air for 30 minutes before sealing. The jars were sealed from one day at the start of the incubation to three weeks during incubation. We sampled 12ml of headspace gas and analyzed the CO₂ concentration using gas chromatography. To determine the background CO₂ concentration, headspace gas from two empty jars that were each incubated with a plastic filter without soil were also sampled and analyzed. Cumulative CO₂ respiration at the end of the incubation was defined as the labile C pool. The stable C pool was determined by subtracting the labile C pool from the total soil C pool. Atmospheric pressure, jar volume, sampled gas volume, air temperature, and dry mass of the incubated soil were used to convert headspace CO₂ concentration to CO₂-C per gram of soil.

**Alkali extraction and acid precipitation of humin and humic acids.**

Thirty grams of air-dried bulk soil was subject to an alkali extraction of fulvic and humic acids from the humin, followed by acid precipitation of the humic acid from the fulvic acid fraction (adapted from Bird et al. 2002). 0.4N NaOH was added to the soil, and was followed by purging with nitrogen. Soil samples were shaken for 12 hours on a reciprocal shaker, and were centrifuged at 15,200 x g at 25°C for 15 min. The dark-colored supernatant which contains humic and fulvic acids was saved, and 100 ml of 0.4N NaOH was added and purged with nitrogen. This step was repeated six times until the supernatant became translucent. The alkali insoluble residue (humin) was neutralized with concentrated HCl and was oven-dried. Humic acid was precipitated from the supernatant (fulvic acid) by adding concentrated HCl until the pH dropped to 1.5, and was centrifuged at 25,600xg at 10°C for 15 minutes. Bulk soil, humin, and humic acid were ground to fine powder, packed in tin capsules, and analyzed for C and N.
Sequestering C in Stable Soil Organic Matter Fractions: How important is fertilizer-N in sequestering C?—van Kessel

centrations using a PDZ Europa 20-20 Stable Isotope Analyzer (Europa Scientific, Crewe, UK) at the University of California, Davis, Stable Isotope Facility.

**Statistical analysis**

We analyzed the measured variables using an analysis of variance (ANOVA) to test the effect of management practice and crop. Tukey’s honestly significant difference (HSD) test was performed to assess which group means differ from other means within the group. SAS 9.1 was used for the statistical analyses, and significance was accepted at α = 0.05 level of significance.

**Results**

The activity of cellobiohydrolase was highest in the organic system and lowest in the conventional system (P = 0.02) (fig 1). β-glucosidase activity also showed this trend, but the results were not significant (P = 0.17). The activity of phenol oxidase was highest in the integrated system and lowest in the organic system (P = 0.02). Phenol oxidase activity was significantly higher under corn than under tomato cultivation (P = 0.03; 166.93 nmol g⁻¹ h⁻¹ under corn vs. 81.83 nmol g⁻¹ h⁻¹ under tomato cultivation). The activity of peroxidase was not different among the three farming systems (P = 0.78) (fig 1).

**Figure 1.** Extracellular enzyme activity under the three farming systems. The activity of each enzymes was averaged for corn and tomato. Error bars indicate one standard error of the mean (n = 9). CON = conventional, INT = integrated, and ORG = organic.
Sequestering C in Stable Soil Organic Matter Fractions: How important is fertilizer-N in sequestering C?—van Kessel

Soils under organic farming had the highest whole soil C (P < 0.01) and N concentrations (P < 0.01). On the other hand, soil C:N ratio was lowest in the organic system (P < 0.01). There was a significant crop by management interaction effect on whole soil C (P = 0.05), and the whole soil C under the corn cultivation was highest in the organic system and not different between the other two systems. Under the tomato cultivation, whole soil C was highest under the organic system, intermediate in the integrated system, and lowest in the conventional system (table 1).

*Table 1.* Carbon and N concentration and C:N ratio in whole soil under corn and tomato cultivation in the three management systems. The units for C and N concentration are g C kg$^{-1}$ soil and g N kg$^{-1}$ soil, respectively. Standard errors are shown in parentheses (n = 3). Means followed by different letters within a row under a crop are significantly different at α = 0.05. CON = conventional, INT = integrated, and ORG = organic.

<table>
<thead>
<tr>
<th></th>
<th>Corn</th>
<th>Tomato</th>
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<tbody>
<tr>
<td></td>
<td>CON</td>
<td>INT</td>
</tr>
<tr>
<td>C</td>
<td>10.06a</td>
<td>9.58a</td>
</tr>
<tr>
<td></td>
<td>(0.23)</td>
<td>(0.48)</td>
</tr>
<tr>
<td>N</td>
<td>1.08a</td>
<td>1.06a</td>
</tr>
<tr>
<td></td>
<td>(0.02)</td>
<td>(0.04)</td>
</tr>
<tr>
<td>C:N</td>
<td>9.35b</td>
<td>9.01b</td>
</tr>
<tr>
<td></td>
<td>(0.09)</td>
<td>(0.19)</td>
</tr>
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The cumulative CO$_2$ respiration by the 94th day of incubation was greatest in the organic system (P = 0.01), and was not different between the conventional and the integrated systems (fig. 2). Stable C pool size was significantly affected by crop by management interaction effect (P = 0.05), and it was highest in the organic system and not different between the other two systems under the corn cultivation. On the other hand, stable C pool size was highest under the organic system, intermediate in the integrated system, and lowest in the conventional system under the tomato cultivation (table 2). However, the proportion of stable C pool in the whole soil C pool was not different between the three farming systems (P = 0.24) (table 2).

Labile N leached by the 94th day of incubation was significantly higher under tomato cultivation when compared to that under corn cultivation (P = 0.03; 61.20 µg N kg$^{-1}$ soil under tomato vs. 33.11 µg N kg$^{-1}$ soil under corn cultivation), but it was not different among the three farming systems (P = 0.13) (fig. 3). The size of the stable N pool was greatest in the organic system (P = 0.01). The proportion of stable N pool over the whole soil N pool, however, was not different between the three farming systems (P = 0.78) (table 2).

Humin-C accounted for 79 to 85% of the total soil C (table 3). The concentration of C associated with humin was not different between the three farming systems (P = 0.38). Proportion of humic acid-C ranged from 8 to 14%, and it was not different between the three farming systems (P = 0.65) (table 3). Nitrogen concentration in humin was highest in the organic system soil (P = 0.01). However, N content in humic acid fraction was not significantly different between the three agroecosystems (P = 0.15) (table 3). Humin-N accounted for 69 to 72% of total soil N, and was not significantly different between the three management practices (P =
Sequestering C in Stable Soil Organic Matter Fractions: How important is fertilizer-N in sequestering C?—van Kessel

0.12) (table 3). Humic acid-N was in the range of 8 to 14% of the total soil N, and was not significantly different between the three farming systems (P = 0.68) (table 3).

**Figure 2.** Cumulative CO$_2$ respiration over 94 days of incubation. The cumulative CO$_2$ respiration was averaged for corn and tomato. Error bars indicate one standard error of the mean ($n = 9$). Means followed by different letters are significantly different at $\alpha = 0.05$. CON = conventional, INT = integrated, and ORG = organic.

**Figure 3.** Labile N generation over 94 days of incubation. The cumulative CO$_2$ respiration was averaged for corn and tomato. Error bars indicate one standard error of the mean ($n = 9$). CON = conventional, INT = integrated, and ORG = organic.
Table 3. Carbon and N concentration and the percent of total C and N residing in each soil chemical fraction under the three farming systems. The units for C and N concentration of humin and humic acid are g C kg\(^{-1}\) soil and g N kg\(^{-1}\) soil, respectively. Standard errors are shown in parentheses (n = 3). Means followed by different letters are significantly different at \(\alpha = 0.05\).

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>INT</th>
<th>ORG</th>
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<tbody>
<tr>
<td>Humin-C</td>
<td>7.74a (0.30)</td>
<td>7.88a (0.81)</td>
<td>9.04a (0.43)</td>
</tr>
<tr>
<td>Humic acid-C</td>
<td>167.43a (47.91)</td>
<td>201.49a (13.40)</td>
<td>239.35a (14.54)</td>
</tr>
<tr>
<td>Humin-C %</td>
<td>82.54a (2.46)</td>
<td>84.73a (4.06)</td>
<td>78.74a (1.24)</td>
</tr>
<tr>
<td>Humic acid-C %</td>
<td>14.17a (7.03)</td>
<td>10.51a (4.27)</td>
<td>7.63a (1.58)</td>
</tr>
<tr>
<td>Humin-N</td>
<td>0.67a (0.01)</td>
<td>0.68a (0.04)</td>
<td>0.89b (0.04)</td>
</tr>
<tr>
<td>Humic acid-N</td>
<td>16.75a (4.83)</td>
<td>21.21a (1.87)</td>
<td>28.10a (3.23)</td>
</tr>
<tr>
<td>Humin-N %</td>
<td>71.39a (0.74)</td>
<td>71.89a (1.33)</td>
<td>68.98a (0.27)</td>
</tr>
<tr>
<td>Humic acid-N %</td>
<td>14.19a (7.24)</td>
<td>10.24a (3.66)</td>
<td>8.14a (2.10)</td>
</tr>
</tbody>
</table>

Discussion

Management practices that promote C and N sequestration in SOM will help farming systems to be more environmentally and economically sustainable. With increasingly more land use being converted to agriculture and intensification of cultivation (Matson et al. 1997), it is important to identify management practices that maximize soil C and N stabilization capacity in order to regulate CO\(_2\) emission and reduce the loss of N from agroecosystems. Our study shows that C and N stabilization in long-term SOM pools is highest in the organic system, and that this is likely due to greatest substrate input to this farming system. The proportion of total soil C and N residing in stable C and N pools determined biologically and chemically did not differ across the conventional, integrated, and the organic systems, which suggests that the combined effect of biological and chemical mechanisms of C and N sequestration in the three farming systems may not be different.

The high cellobiohydrolase activity in the organic system is likely to be the mechanism for greatest respiration observed in this farming system. It is likely that cellulose, which is relatively labile compared to lignin or humic material in soil organic matter, is decomposed mainly by cellobiohydrolase in the organic system, and this results in the largest labile C pool. The production of cellulose-degrading enzymes is induced by the amount of substrate available (Lynd et al. 2002), and highest cellobiohydrolase activity in the organic system is likely to be a result of greatest C entering the organic system. A neighboring experiment in which organic and conventional systems were compared, microbial biomass was significantly larger in the organic system (Lundquist et al. 1999). If this is also the case in our experiment, this could lead to the highest respiration in the organic system.

The organic system had the lowest phenol oxidase activity, and this could be due to the biochemical composition of the substrates in this farming system. Soil C:N ratio was lowest in the organic system, which suggests that the C source for microbial metabolism in this farming system is least recalcitrant and therefore the need for phenol oxidase for decomposition is low.
Significantly higher phenol oxidase under corn cultivation when compared to tomato cultivation also is likely due to higher lignin concentration in corn than tomato (Lewis and Yamamoto 1990). Therefore, the biochemistry of substrates is likely to be the main determinant of phenol oxidase in this experiment.

**Table 2. Stable soil C and N pools under corn and tomato cultivation in the three management systems.** The units for stable C pool and stable N pool are g C kg⁻¹ soil and g N kg⁻¹ soil, respectively. Standard errors are shown in parentheses (n = 3). Means followed by different letters within a row under a crop are significantly different at α = 0.05. CON = conventional, INT = integrated, and ORG = organic.

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>INT</th>
<th>ORG</th>
<th>CON</th>
<th>INT</th>
<th>ORG</th>
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<tbody>
<tr>
<td>Stable C pool size</td>
<td>9.86a</td>
<td>9.38a</td>
<td>12.28b</td>
<td>8.88a</td>
<td>10.54b</td>
<td>12.56c</td>
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<td></td>
<td>(0.26)</td>
<td>(0.40)</td>
<td>(0.61)</td>
<td>(0.43)</td>
<td>(0.21)</td>
<td>(0.23)</td>
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<tr>
<td>Stable N pool size</td>
<td>1.05a</td>
<td>1.03a</td>
<td>1.46b</td>
<td>0.97a</td>
<td>1.14a</td>
<td>1.46b</td>
</tr>
<tr>
<td></td>
<td>(0.02)</td>
<td>(0.04)</td>
<td>(0.04)</td>
<td>(0.05)</td>
<td>(0.03)</td>
<td>(0.05)</td>
</tr>
<tr>
<td>Stable C pool %</td>
<td>98.00a</td>
<td>98.08a</td>
<td>97.39a</td>
<td>97.68a</td>
<td>98.13a</td>
<td>97.02a</td>
</tr>
<tr>
<td></td>
<td>(0.49)</td>
<td>(0.84)</td>
<td>(0.51)</td>
<td>(0.57)</td>
<td>(0.23)</td>
<td>(0.09)</td>
</tr>
<tr>
<td>Stable N pool %</td>
<td>97.31a</td>
<td>96.71a</td>
<td>97.50a</td>
<td>95.86a</td>
<td>95.37a</td>
<td>94.24a</td>
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<tr>
<td></td>
<td>(0.37)</td>
<td>(0.40)</td>
<td>(1.54)</td>
<td>(1.44)</td>
<td>(1.02)</td>
<td>(0.93)</td>
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It is likely that cellulolytic and ligninolytic enzyme activity is not directly regulated by N addition in this experiment. Greater N input to soil can hasten the decomposition of labile litter and inhibit lignin-degrading enzymes produced by white-rot basidiomycetes, actinomycetes and bacteria (Fog 1988; Carreiro et al. 2000; Saiya-Cork et al. 2002). However, N fertilization in conventional and integrated systems did not increase cellulolytic potential or suppress ligninolytic potential when compared to organic systems, which could be due to the uptake of N by plants. Because crop production increased in response to N fertilization, the direct effect of N addition on microbial communities may have been negligible similar to observations of other studies in which plant production was not altered by N amendments (Johnson et al. 1998; Ajwa et al. 1999).

The greatest whole soil C concentration and stable C pool size in the organic system is likely to be driven by the high C input to this system (Clark et al. 1998; Clark et al. 1999). Although the conventional system had higher yield than the integrated or the organic system (Dennison et al. 2004), the organic system received the greatest total C input through plant residue, manure addition, and plant production, and this is followed by the conventional system, and then the integrated system in this experiment (Kong et al. 2005). The organic system also had the highest whole soil N concentration and stable N pool, and this indicates a tight coupling of C and N cycling. Nitrogen input is also highest in the organic system when compared across the three
Sequestering C in Stable Soil Organic Matter Fractions: How important is fertilizer-N in sequestering C?—van Kessel

farming systems (Reed et al. 2006), which likely contributes to the greatest soil N sequestration observed in the organic system (Clark et al. 1998; Clark et al. 1999).

We initially hypothesized that the integrated farming system in which both residue and N fertilizer are applied would have the highest percentage of C and N sequestered in stable organic matter fractions. In our previous residue incubation study, mineral N and residue addition together resulted in the greatest fraction of C transformed to humin when compared to residue or mineral N addition alone (Moran et al. 2005). Moreover, humin-derived N was also greatest when mineral N and residue were both added, rather than under incubation with only mineral N or residue (Moran et al. 2005). These results indicated that mineral N addition, together with residue incorporation, could enhance C and N sequestration though recalcitrant organic matter formation. Azam et al. (1985) also found that more fertilizer-N was retained in soils through incorporation by microorganisms and humus formation when legume residues were added together with the N fertilizer. These studies suggested that C and N sequestration could be maximized in integrated agroecosystems in which both residue and fertilizer are applied. However, highest sequestration of C and N in the organic system shows that the quantity of C and N input plays a more important role in C and N sequestration.

No difference observed in the proportion of stable C and N pools suggests that the combined effect of different mechanisms of C and N stabilization across the three farming systems contribute similarly to C and N stabilization in long-term SOM pools. Microbial uptake of C and N and their subsequent turnover can lead to stable soil organic matter formation (Voroney et al. 1989; Stark and Hart 1997; Kaye et al. 2002). Voroney et al. (1989) found that the C remaining after seven years in the field following glucose amendment was more stable than C derived from wheat straw, which indicates that microbial products can be more recalcitrant than lignin and can contribute significantly to C sequestration. It has also been reported that microbial turnover can foster N stabilization in soil (Stark and Hart 1997; Kaye et al. 2002). Nitrogen fertilization can also increase C and N sequestration by chemically binding to resistant fractions of plant residues and making them more recalcitrant (Berg and McClaugherty 2003). It is likely that the combined effect of these biological and chemical C and N sequestration mechanisms do not differ among the three farming systems.

In summary, we have found that the organic system sequesters greatest level of C and N in long-term C and N pools. Moreover, the organic system showed the highest respiration, and this could be due to the greatest cellobiohydrolase activity observed in this farming system. Highest phenol oxidase activity in the organic system is likely due to lower recalcitrance of the organic substrates entering this system, as suggested by the lowest soil C:N ratio. Although the efficiency of C and N sequestration expressed by total soil C and N residing in stable C and N pools did not differ among the three farming systems, our results demonstrate that long-term C and N stabilization is greatest in the organic system, likely through high organic substrate input.
References


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