Total and permanganate-oxidizable organic carbon in the corn rooting zone of US Coastal Plain soils as affected by forage radish cover crops and N fertilizer

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Forage radish (\textit{Raphanus sativus} L. var. \textit{longipinnatus}) is a relatively new winter cover crop becoming widely grown in humid temperate North America. Little is known about how the use of this fall/winter cover crop may influence carbon sequestration and distribution in the soil profile in corn silage production system. The objectives of this study were to determine quantities and distribution in the soil profile of total organic carbon (TOC) and permanganate oxidizable carbon (POXC) as affected by forage radish cover crops and to examine the relationship between TOC and POXC in the profile. While there was no significant difference in TOC between radish (RAD) and no cover crop (NC) treatments for each depth interval at each site, the TOC in RAD (10.3 g C/kg) was higher compared with NC (9.3 g C/kg) in surface soil depth (0–30 cm) when analyzed across all site years. Forage radish impacts on POXC were observed not only for surface horizons (0–15 cm), but also for deep horizons (90–105 cm). Banded nitrogen fertilizer affected the soil C:N ratio deep in the soil profile at both sites (at 90–105 cm in RAD and at 60–75 cm in NC). Where N fertilizer was applied, soil POXC in 0–30 cm was significantly greater following radish (535.7 mg POXC/kg) than following no cover crop (418.2 mg POXC/kg). Additionally, strong positive linear relationships between POXC and TOC were observed (\textit{P} < 0.05), with a much steeper regression slope (higher POXC/TOC ratio) in the 60–105 cm layer (POXC/TOC ratio = 0.22) was much steeper than for the surface soil (0–30 cm) with POXC/TOC ratio = 0.05. We speculate that the higher POXC levels may have resulted from increased rooting and exudation by both corn and radish where nitrogen fertilizer was placed. Using forage radish cover crops show potential for mitigating against soil C depletion.

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1. Introduction

Soil organic matter (SOM) is a key factor of soil quality since it influences nutrient holding and cycling, soil structure, erosion resistance, and soil biological processes (Weil and Magdoff, 2004; Lucas and Weil, 2012). Levels of SOM are most often estimated by measuring total organic carbon (SOC). Because SOC is such a large pool of C and mainly comprised of relatively stable material protected from decomposition, the effects of contrasting soil management practices may take many years to become apparent in SOC measurements (Weil et al., 2003). It remains difficult to measure small quantitative changes in SOC pools caused by variations in soil management practices over short time scales of a few years, despite the fact that these changes may impose significant effects on soil properties and associated microbial processes (Weil et al., 2003). Alternatively, labile soil organic carbon (LOC) is a relatively small fraction of TOC that has a short half life in soils and responds quickly to changes in soil management and fertilization practices (Weil and Magdoff, 2004). The LOC fraction is an important component that determines soil quality because of its involvement in soil aggregate stabilization (Tisdall and Oades, 1982) and its direct link to soil carbon (C) and nitrogen (N) mineralization (Gunapala and Scow, 1998).

Recently Culman et al. (2012), in a meta-analysis of 12 studies, presented evidence that the LOC reactive with a dilute (0.02 M) potassium permanganate solution (Weil et al., 2003) is a microbial
processed pool of labile soil C that often exhibits greater sensitivity to changes in management or environmental variation than other commonly measured parameters such as particulate organic carbon (POC), microbial biomass carbon (MBC) or total TOC. They recommended that this fraction be termed permanganate oxidizable carbon (POXC). Several recent studies have reported that POXC was one of the most sensitive and reliable indicators for evaluating the short- and long-term impacts of soil management practices on soil quality (Awale et al., 2013; Chen et al., 2009; DuPont et al., 2010; Melero et al., 2009; Morrow et al., 2016; Plaza-Bonilla et al., 2014; Spargo et al., 2011; Veen et al., 2014). Studies found that POXC quantified by a modified potassium permanganate method (Weil et al., 2003) is sensitive to changes in SOC content induced by organic amendments (Miles and Brown, 2011), cover crop treatments (Jokela et al., 2009), and high-residue cropping systems (Miles and Brown, 2011). Lucas and Weil (2012) reported that POXC determination is useful for identifying soils where improved SOC management is likely to increase grain productivity and further contribute to soil quality interpretations for producers. Measurement of the POXC content of a soil is also a very simple, inexpensive and non-hazardous method for estimating the LOC fraction (Culman et al., 2012; Morrow et al., 2016; Lucas and Weil, 2012).

Forage radish (Raphanus sativus L.) is a unique fall/winter cover crop that is relatively new but becoming rapidly adopted in temperate, humid North America. Forage radish performs a number of unique and desirable functions, including alleviating soil compaction through effective bio-drilling (Chen and Weil, 2010), and efficient capture of N from deep soil layers. The N capture function prevents excess N from leaching into natural waters (Kristensen and Thorup-Kristensen, 2004; Dean and Weil, 2009). The radish has also been reported to increase soil test phosphorous (White and Weil, 2011) and very effectively suppress early spring weeds (Lawley et al., 2011).

Given two months of favorable growing conditions in fall (600+ growing degree days), radish cover crops typically produce 3–8 metric tons/ha of dry matter (approximately 20–30% of which is in the fleshy, partially above ground root). Because of its rapid growth in fall, a forage radish cover crop can add significant quantities of organic carbon to the soil (Mute et al., 2011, 2013; Dean and Weil, 2009). It is important to keep in mind however that forage radish biomass is highly decomposable so the carbon added to the soil system after radish cover crops has a rapid turnover rate (Kremen and Weil, 2006). More sensitive measures of SOC (e.g., POXC) may be able to detect changes in SOC resulting from radish cover cropping but we could find no published studies to date investigating radish effects on labile soil organic carbon. Moreover, the vast majority of studies measuring cover crop and management effects on SOC have investigated only the upper 10–30 cm of soil, but the few studies that have looked deeper point to the importance of carbon changes in the deep subsoil layers (Baker et al., 2007; Jandl et al., 2014).

This study investigates corn silage production with and without the use of forage radish as a fall/winter cover crop planted immediately after corn is harvested for silage. Within each cover crop treatment (radish or no cover), low and high fertilizer application was also compared. The variables measured included above ground plant C and dry matter production and the distribution of TOC, C:N ratio and POXC in the upper 105 cm of the soil profile. Thus, the objectives of this study were (1) to evaluate the effect of forage radish on soil organic carbon distribution in profile, (2) to determine effects of radish cover cropping on POXC in the soil profile, (3) to measure the effect of band-applied V5 stage corn side-dress nitrogen solution on the soil TOC and POXC in the soil profile, and (4) to determine the relationship between the soil organic carbon and permanganate oxidizable carbon at different soil depths.

2. Materials and methods

2.1. Field site description and experimental design

The study was conducted on two fields of the USDA Dairy Farm (39°01′N, 76°89′W) at Beltsville Agriculture Research Center, Beltsville, Maryland. A completely randomized split-plot design experiment with four replicates was conducted in field BARC1-18 from May 2011 through August 2012 and in field BARC1-21 from May 2012 through August 2013.

The dominant soils types at BARC1-18 are Christiana soils (Fine, kaolinitic, mesic Aquic Hapludults) with silt loam A horizons and clay loam Bt and C horizons. The BARC1-21 site is approximately 1 km away from BARC1-18, the soil is a complex of Russett soil (Fine-loamy, mixed, semi-active, mesic Aquic Hapludults) with silt loam A horizons mainly in Blocks 3 and 4 and Christiana soils mainly in Blocks 1 and 2. The general soil properties of the Ap horizon (0–20 cm) and the management histories for the study period and the previous 3 years) of two fields are presented in Tables 1 and 2, respectively. Fig. 1 shows the monthly mean air temperature and cumulative rainfall values for the sites during the study period.

During this study, no-till management was used in all fields. Sites BARC1-18 and 1-21 were divided into four blocks in May 2011 and 2012 respectively. Each block contained two main plots each randomly assigned one of two winter cover crop treatments: forage radish cover crop and no cover crop (crop residue and winter weeds only) (Fig. 2). The block dimension was 9.5 m × 110 m. The main plot size was 4.6 m × 110 m. In mid-June preceding the cover crop planting, 112 kg N ha⁻¹ as urea ammonium nitrate (UAN) solution was side-dressed in every other corn row middle in bands of liquid fertilizer applied 150 cm apart such that each row of corn had access to nearby N on one side or the other, but not both sides. This allowed us to track this fertilizer N by differential growth and N uptake over the side-dress bands by cover crop plants drilled in rows just 15 cm apart. The N side-dressed and non-sidedressed strips were considered to be sub-plots characterized as high N (side-dressed row) and low N (non- side-dressed row) (Fig. 2). The sub plot size was 0.75 m × 110 m. In order to determine the effects of different N fertilizer rates on silage corn yield and examine whether corn silage, with or without a radish cover crop, benefits from side-dressed nitrogen fertilizer on these periodically manured soils, four N fertilization rates (no nitrogen, N0; 56 kg ha⁻¹, N1; 112 kg ha⁻¹, N2; and 168 kg ha⁻¹, N3) were applied in June 2012 (BARC1-18) and 2013 (BARC1-21). These N rates were factorially combined with previous fall cover crop treatment as sub-subplots in a split-split plot design with four replications giving a total of 32 sub plots of 20 m length and 4.6 m width each.

<table>
<thead>
<tr>
<th>Site</th>
<th>Clay (g kg⁻¹)</th>
<th>Silt (g kg⁻¹)</th>
<th>Sand (g kg⁻¹)</th>
<th>Organic Carbon (g kg⁻¹)</th>
<th>pH</th>
<th>Mehlich 3 P (mg kg⁻¹)</th>
<th>Available K (mg kg⁻¹)</th>
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</thead>
<tbody>
<tr>
<td>BARC1-18</td>
<td>138</td>
<td>597</td>
<td>265</td>
<td>11.4</td>
<td>6.8</td>
<td>58</td>
<td>147</td>
</tr>
<tr>
<td>BARC1-21</td>
<td>152</td>
<td>620</td>
<td>228</td>
<td>15.2</td>
<td>6.3</td>
<td>73</td>
<td>105</td>
</tr>
</tbody>
</table>
Table 2
Management history at two research sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>Years</th>
<th>Main Crop</th>
<th>Cover Crop</th>
<th>Dairy Manure Slurry</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-18</td>
<td>2008</td>
<td>Soybean (Glycine max L.)</td>
<td>Cereal Rye (Secale cereal L.)</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>2009</td>
<td>Silage Corn (Zea mays L.)</td>
<td>None</td>
<td>47,000 L ha⁻¹</td>
</tr>
<tr>
<td></td>
<td>2010</td>
<td>Soybean</td>
<td>Cereal Rye</td>
<td>47,000 L ha⁻¹</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>Silage corn</td>
<td>Forage Radish</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>2012</td>
<td>Silage corn</td>
<td>Triticale (Triticum aestivum L.)</td>
<td>None</td>
</tr>
<tr>
<td>1-21</td>
<td>2008</td>
<td>Silage Corn</td>
<td>Barley (Hordeum vulgare L.)</td>
<td>47,000 L ha⁻¹</td>
</tr>
<tr>
<td></td>
<td>2009</td>
<td>Soybean</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>2010</td>
<td>Silage corn</td>
<td>Wheat (Triticum aestivum L.)</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>Soybean</td>
<td>Wheat cover</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>2012</td>
<td>Soy Silage</td>
<td>Forage Radish</td>
<td>47,000 L ha⁻¹</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>Corn Silage</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Mean monthly temperature and cumulative monthly precipitation. This figure shows mean monthly temperature and cumulative monthly precipitation at USDA Dairy Farm of Beltsville Agriculture Research Center, Beltsville, Maryland (BARC1-18 and 1-21) from 1 Jan.2011 through 31 Aug. 2013.

The soil at BARC1-18 was cropped in a no-till soybean/cereal rye cover crop/corn rotation prior to this study (Table 2). During this experiment, a silage corn/radishes cover crop/silage corn rotation was used. No-till management was also practiced at BARC 1-11 for this experiment and during the previous 4 yr with a corn/barley/soybean or corn/wheat/soybean rotation (Table 2). After corn silage harvest in mid-August of each year, 47,000 L ha⁻¹ dairy cow manure slurry was spread and turbo tilled (Houle model, 5300) into the upper 5 cm of soil. In mid to late August, forage radish (Cover Crop Solutions) was no-till drilled (John Deere, 1590; 9 kg seed ha⁻¹) into silage corn stubble for cover crop plots 100 m long in BARC 1-18 and 110 m long in BARC1-21 by 4.6 m wide. A same sized plot on one side or the other (randomly chosen) was not planted to a cover crop and designated the control plot. Forage radishes grew rapidly in fall and early winter until they were freeze-killed beginning in December or January. In May the field was again planted to silage corn (Pioneer, 34B62) with no planting time fertilizer. At side-dress time in June (V5 stage of corn growth), the previous fall cover crop plots were sub-divided into N rate plots as described above. Whole corn plants were harvested (CLAAS, JAGUAR 940) for silage in mid to late August.

2.2. Field sampling

2.2.1. Plants

Cover crop plants were taken from random locations at least 1 m from the ends of the plot. Plants were collected by block in January of 2011 and December of 2013 (Fig. 3). Two 0.25 m² quadrats (centered in the high N side-dress or low N no side-dress subplot) of plant material (radishes in cover treatment and weeds in no cover treatment) were harvested in each subplot, and the dry weight of these samples was determined after drying. Subsamples were removed for analysis of carbon contents. Plant shoots were

Fig. 2. Diagram of cover crop main and N sub-plot layout and Photo of main and subplots in the field. (Left) Diagram of cover crop main and N sub-plot layout. Black dashed lines are corn rows. Dotted lines are side-dress fertilizer locations. Shaded areas are subplots: high N side-dressed (dark gray) and low N non side-dressed (light gray) corn row middles. (Right) Photo of main and subplots in the field 8 weeks after radish planting which shows the visibly taller radish growth in the high N strips.
harvested for all treatments. For the forage radish, the large fleshy tap-roots, but not the fine roots, were sampled to approximately 15 cm depth by pulling up the whole plant, separating the fleshy root from shoot material and washing the roots free of soil. After collection, plants were dried at 65 °C and ground to pass a 1-mm sieve. Plant dry matter measurements were used in conjunction with C analysis to determine plant C content:

\[ C \text{ content (kg ha}^{-1}\text{)} = \text{DM}^* C \]

where DM is plant dry matter (kg ha\(^{-1}\)), and C is total C concentration (kg kg\(^{-1}\)).

2.2.2. Soils

Soil was sampled to 105 cm depth where allowed by site conditions and equipment capabilities using Giddings hydraulic probe. The soil cores were 3.81 cm in diameter. Cores were placed in 4-cm-diameter PVC troughs for examination and divided into 15-cm sections. Six soil cores were taken in each cover crop plot. With respect to the rows of corn plant stubble, three cores were taken in the side-dress middle (high-N, subplot) and three in non-side dress middle (low-N subplot). The three cores were homogenized within the subplot to represent the particular depth increment and subplot. Soil sampling dates are given in Fig. 3.

2.3. Laboratory analyses

After collection, the wet weight of total soil was determined in the lab. Then subsamples (~15 g) were dried at 105 °C for gravimetric soil moisture determination. The remaining soil in each sample was immediately force-air dried at 55 °C for 24 h and passed through a 1-mm sieve for other analysis. Soil bulk density was calculated for each 15-cm depth increment.

Soil and plant total nitrogen and carbon was analyzed by the dry combustion method at 900 °C using a C elemental analyzer (CHN 2000; LECO, St. Joseph, MI) for samples (ground to pass 0.25 mm sieve). Permanganate oxidizable carbon in soil was estimated by reaction with dilute permanganate solution (Weil et al., 2003). Briefly, portions (2.5 g) of air-dried soil were dispersed in 20 mL of permanganate solution, with shaking on a reciprocating shaker (120 strokes minute \(^{-1}\)) for exactly 120 s. The permanganate solution contained 0.02 M potassium permanganate (KMnO\(_4\)) and 0.1 M calcium chloride (CaCl\(_2\)), and was adjusted to approximately pH 7.2 using 0.1 M sodium hydroxide (NaOH). After shaking the soil permanganate slurry was allowed to settle for 10 min, then 1 mL of the upper supernatant was transferred into a 50 mL centrifuge tube and mixed with 49 mL deionized water. This diluted supernatant was read on a DU720 spectrophotometer, and the reduction of permanganate was quantified as the decline compared to a no-soil control in light absorbance at 550 nm. The change in the concentration of KMnO\(_4\) was used to estimate the amount of oxidized C. Sample POXC was calculated as in Weil et al. (2003) as follows:

\[ \text{POXC (mg kg}^{-1}\text{)} = (0.02 \text{ mol L}^{-1} - (a + bz)) \]

\[ \times (9000 \text{ mg C mol}^{-1}) \times \left( \frac{0.02 \text{ L solution}}{0.0025 \text{ kg soil}} \right) \]

where 0.02 mol L\(^{-1}\) is the initial concentration of the KMnO\(_4\) reactant, a and b are the intercept and slope of the standard curve, respectively, z is the sample absorbance, 9000 mg C mol\(^{-1}\) is the amount of C (0.75 mol) oxidized by 1 mol of MnO\(_4^2-\) to Mn\(^{7+}\), and 0.0025 kg soil is the amount of soil reacted with KMnO\(_4\).

2.4. Statistical analyses

The experiments at both sites used a randomized complete block split-split-plot design with four replications, where the two pre-cover crop corn side-dressed N levels (yes or no) comprised the sub-plots and the four post-cover crop corn side dress levels comprised the sub-sub-plots. The sub-sub plots were incorporated into the analysis only for variables measured after the side-dressing N was applied in the year following the cover crop.
treatments. Analysis of variance was calculated by DPS 7.05 statistical software (Ruifeng Information Technology Ltd. Co, Hangzhou, China) for C (TOC, POXC) in a given 15 cm increment of the soil profile, for the sum of all C in the sampled (0–105 cm) profile, for plant dry matter, for plant C:N concentration. Block effects were treated as random variables, whereas cover crop treatments and N levels were considered fixed effects. Linear regression analyses were used to examine the relationship between TOC and POXC for samples grouped as surface soil (0–30 cm), shallow subsoil (30–60 cm) and deep soil (60–105 cm). Mean comparisons were performed using Fisher’s protected least significant difference (LSD) when the ANOVA indicated a statistically significant effect ($P < 0.05$).

3. Results

3.1. Cover crop dry matter, nitrogen and carbon concentrations and C: N ratio

Forage radish dry matter production ranged from 1965 to more than 3361 kg ha$^{-1}$ for shoots and 1532 to more than 3740 kg ha$^{-1}$ for the fleshy tap roots (Table 3). Forage values for radish shoot and root dry matter were significantly higher in the high N than in the low N subplots for any individual site-year. Weeds dry matter was not collected at BARC 1-18 in winter. Forage radish produced less root dry matter than shoot regardless of N level and both radish shoot and root dry matter was increased markedly by the high N level at BARC 1-18 and 1-21. Weeds produce much less shoot dry matter than did the radish and but did respond significantly to N level at BARC 1-21. Root dry matter was not determined for weeds.

Nitrogen uptake by cover crops is influenced by both the total dry matter accumulation and the tissue N concentration. Under high N level, N uptake by radish shoots at BARC1-21 was significantly greater than under low N level (110.7 kg N ha$^{-1}$ compared with 59.5 kg N ha$^{-1}$) (Table 3). The amounts of N and C captured in the radish shoots at BARC1-21 were nearly 4 times greater than in weeds under both N levels (Table 3). The forage radish roots had a significantly greater average C: N ratio than the shoots (23 versus 15). The C: N ratio was lower in forage radish shoots than in winter weeds in the high N plots.

In BARC 1-18, the whole plant (shoot + fleshy root) N uptake by radish was 154 kg N ha$^{-1}$ in the high N subplots compared to 77 kg N ha$^{-1}$ in the low N subplots. In BARC 1-21, the whole plant (shoot + fleshy root) N uptake by radish was 145 kg N ha$^{-1}$ in the high N subplots compared to 80 kg N ha$^{-1}$ in the low N subplots.

3.2. Soil C: N ratio

The soil C: N ratio in surface soil (0–15 cm) ranged from 10:1 to 12:1 in RAD plots and from 11:1 to 12:1 in NC plot at both sites (Fig. 4). There was no apparent overall trend in C:N ratio with depth, but there was a significant effect of fall cover crop treatment at several depths in the subsoil. At the 60–75 cm depth the C:N ratio was significantly higher in the NC than in the RAD plots. In contrast, at the 90–105 cm depth the C:N ratio was significantly higher in the RAD than in the NC plots. These effects were consistent across both sites and N treatments (Fig. 4).

3.3. Soil organic carbon and permanganate oxidizable carbon

The depth distribution of TOC was similar at each site (BARC1-18 and 1-21) for different seasons (Feb 2012 and May 2013), and was not significantly impacted by cover treatment (Figs. 5 and 6). A non-linear decrease in TOC occurred (Fig. 7) from the 0–15 to 90–105 cm depth intervals for high and low N conditions, from an average high of 11.0 g kg$^{-1}$ at 0–15 cm to 1.6 g kg$^{-1}$ at 60–105 cm at BARC1-18 (Fig. 5). At BARC1-21, TOC decreased from 11.82 g kg$^{-1}$ in the uppermost layer to 2.0 g kg$^{-1}$ below 60 cm (Fig. 6). While there was no significant difference in TOC between radish (RAD) and no cover crop (NC) treatments for each depth interval at each site, the TOC in RAD was higher (10.3 g C/kg) compared with NC (9.3 g C/kg) in surface soil depth (0–30 cm) when analyzed across both site years.

The POXC levels declined dramatically from the 0–15 to the 15–30 cm layers, and below. However, the decline with depth for POXC at both sites was more variable than that for TOC and did not fit well to a similar non-linear equation (Fig. 8). Forage radish impacts on POXC were observed both in the surface soil layer as well as in subsoil layers, under both high and low N conditions (Figs. 5 and 6). POXC increased more in cover treatment than no cover with soil depth increase, especially in deeper soil. The RAD cover crop treatment significantly increased POXC in the 0–15 cm layer averaged across high and low N conditions (649 mg kg$^{-1}$ compared with 624 mg kg$^{-1}$ at BARC 1-18; 569 mg kg$^{-1}$ compared with 492 mg kg$^{-1}$ at BARC 1-21) (Figs. 5 and 6). In addition, POXC also was significantly higher following RAD than NC at 90–105 cm (431 mg kg$^{-1}$ compared with 295 mg kg$^{-1}$ at BARC 1-18; 143 mg kg$^{-1}$ compared with 123 mg kg$^{-1}$ at BARC 1-21). Overall, N fertilizer significantly increased POXC levels for both surface and deep soil layers in 2012 and 2013 ($P < 0.05$) (Figs. 5 and 6). When all the nitrogen fertilized plots from both site years are considered, soil POXC in 0–30 cm was significantly greater following radish

<table>
<thead>
<tr>
<th>Items</th>
<th>Cover Crop</th>
<th>BARC 1-18 (2011,12)</th>
<th></th>
<th></th>
<th>BARC 1-21 (2012,12)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>Shoot</td>
<td>Root</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>High N</td>
<td>Low N</td>
<td>High N</td>
<td>Low N</td>
<td>High N</td>
</tr>
<tr>
<td>Dry matter, kg ha$^{-1}$</td>
<td>Forage radish</td>
<td>3031a</td>
<td>1965b</td>
<td>3748a</td>
<td>2172b</td>
<td>3361a</td>
</tr>
<tr>
<td>N content, kg ha$^{-1}$</td>
<td>Forage radish</td>
<td>78.5a</td>
<td>43.0b</td>
<td>75.3a</td>
<td>34.0b</td>
<td>110.7a</td>
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<td>C content, kg ha$^{-1}$</td>
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<td>17.3a</td>
<td>20.1a</td>
<td>25.1a</td>
<td>11.0b</td>
</tr>
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</table>

$^1$ Lowercase letters indicate significantly different means for each site and year ($P < 0.05$).
(535.7 mg POXC/kg) than following no cover crop (418.2 mg POXC/kg).

The proportion of TOC present as POXC generally increased with soil depth. The POXC/TOC ratio was not influenced by cover treatments in the 0–45 cm soil layer (Figs. 5 and 6). However, after RAD at both sites, the soil TOC generally had a higher of POXC at depths from 75 to 105 cm for both high and low N conditions.

Soil POXC decreased dramatically with soil depth below A horizon in June and August 2012. Where N fertilizer was applied (all plots except N0, June 2012), soil POXC was significantly greater following radish than following no cover crop (0–30 cm) (Fig. 9). At 0–15 cm, POXC was 5, 33, and 30% higher for RAD than NC in June for N0, N100, and N150, respectively. At 0–30 cm, POXC in RAD soils was 23% higher in N50, 20% in N100, and 26% in N150 compared with that in NC soils in August. Meanwhile, soil POXC exhibited a logarithmic decrease with depth for all treatments in August, although RAD showed a more pronounced decrease than NC. Forage radish impacts on POXC were observed not only for surface soils, but for subsurface soils in June 2012. The RAD soils in N0 in the 45–60 cm and 60–75 cm depth both had 37% higher POXC, and 32% higher in N150 in the 30–45 cm in June compared with NC. On the contrary, the NC soils in N50 in the 30–60 cm had 13% higher POXC, and 37% higher in N100 in the 60–75 cm in June compared with RAD.

Soil POXC from all sampling points and soil depth intervals at two fields (BARC1-18 and 1-21), were regressed with their total organic C contents and were found to be highly correlated overall, but not for 30–60 cm (Fig. 10). However, for POXC in deep soil (60–105 cm), the slope of the regression against TOC (POXC/TOC ratio = 0.22) was much steeper than for the surface soil (0–30 cm) with POXC/TOC ratio = 0.05.

4. Discussion

4.1. Cover crop dry matter, nitrogen and carbon concentrations and C: N ratio

Forage radishes were highly variable in dry matter partitioning, with the fleshy root accounting for 36–55% of total plant dry matter. Dean and Weil (2009) reported similar variability in root/
shoot ratio and ascribed this in part to the highly variable size of the fleshy taproot of the radishes, often inversely related to localized plant density. Forage radish displayed the potential to take up large quantities of soil N in fall following silage corn. The N uptake capabilities were especially apparent in the N-rich soil environment (Table 3). Thorup-Kristensen (1993) also reported that N uptake by cover crops increased with increasing amounts of soil mineral N. For the two fields (BARC1-18 and 1-21) sampled in Dec. 2011 and 2012, the forage radishes roots contained on average, 36–55% of the total C and 23–50% of the total N with the remainder in the above ground biomass (Table 3). This result is also consistent with the data reported for similar conditions in New York State, where 45–57% of total C and 29–52% of total N were present in the roots (Ketterings et al., 2011).

The 63–80 kg N ha⁻¹ increase (143-80 to 157-77) in N uptake by radish plant growing in the subplots that had received N side-dress solution in the previous June (Table 3), suggests that a large proportion of the 112 kg N ha⁻¹ applied as a side-dressing to the corn in June was not used by the corn crop and remained within the root zone of the radish during the fall in both years of the study. By contrast, the weeds in the no cover plots did not respond significantly to the residual side-dressed N, suggesting the N was deeper than the weed root systems could reach in fall.

The C:N ratio of forage radishes in the two sites (19:1-27:1 for roots and 11:1-17:1 for shoots) was consistent with the findings (22:1-32:1 for roots and 12:1-16:1 for shoots as reported in Ketterings et al., 2011).

4.2. Soil C:N ratio

The C:N ratio in the organic matter of arable (Ap) horizons commonly ranges from 8:1 to 15:1 (Weil and Brady, 2016), this is in accordance with our results (Fig. 4). The soil C:N ratio at subsoil depth (60–75 cm) in NC was significantly higher than in RAD at both sites (Fig. 4). This may be associated with the high clay content in the argillic (Bt) horizon at this depth in combination with decomposed radish root materials in the RAD plots. Higher clay content is often associated with more decomposed organic matter with lower C:N ratio (Diekow et al., 2005a; Ouédraogo et al., 2006; Yamashita et al., 2006). The C:N ratio in RAD tended to decline while increase in NC with depth (45–75 cm) at both sites (Fig. 4). Diekow et al. (2005b) found that soil C:N ratio declined by
Fig. 6. Soil total carbon, permanganate oxidizable carbon and POXC/TOC (%) at BARC1-21.
Soil total carbon, permanganate oxidizable carbon and POXC/TOC (%) under (a) high N and (b) low N conditions for each 15 cm depth increment sampled at Beltsville Agriculture Research Center 1-21 in May 2013. (The horizontal bar represents the standard error. High N and Low N represent side-dressed row and non-side-dressed row separately. The legend RAD and NC represent cover treatments at Forage Radish and No Cover). *Significantly greater means than smallest mean within a depth increment (P < 0.05). High N treatment had a significant effect on POXC in the same depth at P < 0.05.

Fig. 7. Relationship between total soil organic C (TOC) and soil depth.
Relationship between total soil organic C (TOC) and soil depth for means of all sampling points for each 15 cm depth increment sampled at USDA Dairy Farm of Beltsville Agriculture Research Center, Beltsville, Maryland (BARC1-18 and 1-21) experimental sites in February 2012 and May 2013 separately.

Fig. 8. Relationship between permanganate-oxidizable C (POXC) and soil depth.
Relationship between permanganate-oxidizable C (POXC) and soil depth for means of all sampling points for each 15 cm depth increment sampled at USDA Dairy Farm of Beltsville Agriculture Research Center, Beltsville, Maryland (BARC1-18 and 1-21) experimental sites in February 2012 and May 2013 separately.
Soil permanganate oxidizable carbon for each 15 cm depth increment sampled at Beltsville Agriculture Research Center 1-18 in June and August 2012. (The horizontal bar represents the standard error; the legend RAD and NC represent cover treatments at Forage Radish and No Cover; the legend N0, N56, N112, and N168 represent N fertilizer application rates at 0, 56, 112, and 168 kg ha⁻¹ yr⁻¹). Significantly greater means than smallest mean within a depth increment (P < 0.05).

![Fig. 9.](image)

Relationship between total soil organic C (TOC) and permanganate-oxidizable C (POXC) for all sampling points for three soil depth intervals at USDA Dairy Farm of Beltsville Agriculture Research Center, Beltsville, Maryland (BARC1-18 and 1-21) experimental sites. Significant correlation at P < 0.05.

![Fig. 10.](image)

Relationship between total soil organic C (TOC) and permanganate-oxidizable C (POXC) for all sampling points for three soil depth intervals at USDA Dairy Farm of Beltsville Agriculture Research Center, Beltsville, Maryland (BARC1-18 and 1-21) experimental sites. Significant correlation at P < 0.05.

depth between 47.5 and 107.5 cm along with an increase in clay content. However, they observed an opposite trend between 7.5 and 47.5 cm depths. Sa’ et al. (2001) also reported that a trend of increased soil C:N ratio with depth. This may be attributed to some high C:N soluble organic compounds (e.g., organic acids) leaching into deeper layers (Diekow et al., 2005a,b). It remains unclear why the use of a fall RAD cover crop either increased the total N or reduced the total C to result in a significantly lower C/N ratio in the Bt horizon.

4.3. Soil organic carbon and permanganate oxidizable carbon

Although forage radish is an increasingly popular cover crop in both Europe (Allison et al., 1998; Kristensen and Thorup-Kristensen, 2004) and North America (Bryant et al., 2013), very little, if any, work has been carried out on its effects on carbon distribution in profile. The TOC decline with soil depth in this study is typical of arable soils and reflects decreased root density with depth (Mutebi et al., 2011). This pattern of decreased root density with depth is thought to be associated with a parallel decline in rhizodeposition with depth (Petersen et al., 2005). Mutebi et al. (2013) also reported that ¹⁴C mainly concentrated across the 0–45 cm soil depth for different tillage treatments after forage radish biomass incorporation. While there were no significant differences in TOC between RAD and NC for any depth interval at either site, the TOC in RAD tended (P < 0.05) to be higher compared with NC in surface soil depth (0–30 cm) (Figs. 5 and 6). High above- and below-ground biomass forage radish yields 2–4 t ha⁻¹ (Kristensen and Thorup-Kristensen, 2004) could significantly boost soil carbon input, thus reducing the potential depletion of soil carbon resulting from a prolonged fallow period from late summer to late spring.
Forage radish impacts on active carbon (POX) were observed to be significant in both surface soil and subsoil layers (Figs. 5 and 6). As a low C:N ratio of forage radish biomass (Table 3), high and fast initial mineralization occurred (Mafongoya et al., 1998). This high and fast initial mineralization is the result of a fast turnover of the most easily decomposable components of radish material, such as free amino acids, amino sugars, carbohydrates and other cell constituents (Watkins and Barralough, 1996; Mutegi et al., 2013). On the basis of the seven-pool SOM model (CN-SIM) results, researchers estimated that about 8–10% of original forage radish C remains in the soil for up to 30 yr after biomass incorporation (Mutegi et al., 2013). Combining these CN-SIM model forecast results with Jenkinson and Rayner (1977) findings, Mutegi et al. (2013) estimate that over a 30-yr period of continuous autumn forage radish establishment as cover crop, at least 4.9 tCha⁻¹ forage radish C with a residence time of more than 20 yr in the soil could be stored in the soil.

Weil et al. (2003) stated that compared to TOC, POX measured by their method estimates a C pool more closely associated with soil biological functions. In this study, the POX was significantly higher in the high N soil at several depths, especially in the lower subsoil, suggesting that the more N-rich soil stimulated more root growth and rhizodeposition by both the corn crop that was fertilized and following radish cover crop.

A strong positive relationship between POX and SOC has been widely reported previously (Weil et al., 2003; Wuest et al., 2006; Jokela et al., 2009; Culman et al., 2012). The SOC is determined by complete oxidation of all soil C, while POX relies on partial oxidation of the only the more easily oxidized C pool. Thus Culman et al. (2012) suggests that POX can also serve as a rapid and field-adaptable method to estimate TOC.

In our study, TOC and POX were significantly related at both experiment sites (Fig. 10). However, the overall correlation between the two carbon measures was not as close as in other studies where all samples came from the surface soil. In fact, our data suggest that the relationship between TOC and POX may be different in subsoil than in surface soils. The significantly higher ratio of POX/TOC in the subsoil layers could be due to recent root exudates and rhizodeposition making up a larger fraction of the much small total amount of organic matter present in the subsoil. Also, with higher microbial populations and respiration activity nearer the soil surface, compounds comprising POX may be mineralization more quickly than in deeper soil layer, thus causing POX in surface soil to be more rapidly oxidized or converted into more protected TOC than in the deeper soil in this experiment.

5. Conclusions

There has been very little, if any, previous investigations about the carbon distribution in profile and sequestration potential of autumn-winter established forage radish. Our data indicate that forage radish impacted soil carbon quantities and distribution in surface and deep depth compared with fallow treatments, especially for POX. Additionally, a strong positive relationship between POX and SOC has also been displayed in this study. We concluded that although forage radish is grown for a short period, and at a time characterized by low temperature (autumn-winter), forage radish cover crops contribute a substantial amount of C during growth and after biomass decomposed. Forage radish has a potential for mitigating against soil C depletion through enhancement of soil organic matter.

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References


