Long-term impact of farming practices on soil organic carbon and nitrogen pools and microbial biomass and activity

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ARTICLE INFO

Article history:
Received 2 March 2011
Received in revised form 28 July 2011
Accepted 1 August 2011
Available online 31 August 2011

Keywords:
Microbial C and N
Microbial respiration
Reduced tillage
Soil C fractions
Soil C and N pools
Sustainable farming practice

ABSTRACT

Conventional agriculture with intensive tillage and high inputs of synthetic chemicals has critically depleted the soil C pools. Alternative practices such as no-tillage and organic inputs have been shown to increase soil C content. However, the long-term impact of these practices on soil C pools was not fully understood under humid and warm climate conditions such as the southeast USA. We hypothesized that a combination of sustainable production practices will result in greater microbial biomass and activity and soil organic C than any individual practice. To test this hypothesis, we conducted a long-term experiment examining how different farming practices affect soil C and N pools and microbial biomass and activities in a fine-sandy loam (FAO: Acrisol) in the southern Appalachian mountains of North Carolina, USA. The experiment was a randomized complete design with four replications. Six management treatments, i.e., tillage with no chemical or organic inputs (Control, TN), tillage with chemical inputs (TC), tillage with organic inputs (TO), no-tillage with chemical inputs (NC), no-tillage with organic inputs (NO), and fescue grasses (FG), were designed. Organic C and N pools and microbial properties in 0–15 cm soils were markedly different after 15 years of continuous treatments. Both no-tillage and organic inputs significantly promoted soil microbial biomass by 63–139% and 54–126%; also microbial activity increased by 88–158% and 52–117%, respectively. Corresponding increases of soil organic C by 83–104% and 19–32%, and soil organic N by 77–94% and 20–32% were measured. The combination of no tillage and organic management increased soil organic C by 140% over the conventional tillage control, leading to a soil C content comparable to an un-disturbed grassland control. No tillage reduced the proportion of organic C in the light fraction with d < 1.0 g cm⁻³ (from 1.53–3.39% to 0.80–1.09%), and increased the very heavy fraction with d > 1.6 g cm⁻³ (from 95% to 98%). Organic inputs, however, had little impact on C distribution among different density fractions of the soil except light fraction in tillage treatment. Over all, no-tillage practices exerted greater influence on microbial biomass levels and activity and soil organic C levels and fractions than organic inputs. Our results support the hypothesis and indicate that management decisions including reducing tillage and increasing organic C inputs can enhance transformation of soil organic C from the labile into stable pools, promote soil C accumulation, improve soil fertility and while mitigate atmospheric CO₂ rise.

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1. Introduction

Soils represent the largest global carbon (C) stock (1550 Pg), containing nearly three times as much C as vegetation and twice that of the atmospheric pool (Lal, 2003; Schlesinger and Andrews, 2000). It is estimated that the C sink capacity of the earth’s soil is about 1 Pg C year⁻¹, which could offset 0.47 μmol mol⁻¹ of atmospheric CO₂ annually (Jagadamma and Lal, 2010). Enhanced C sequestration in agricultural soils not only has the potential to help reduce atmospheric CO₂ concentrations (Sperow et al., 2003), but also promotes the productivity and sustainability of agricultural systems (Lal, 2004). It has been well documented that increasing soil C enhances soil fertility, reduces erosion and
nutrient runoff, and improves water quality (Kurkalova et al., 2004; Lubowski et al., 2006; Feng et al., 2007). For example, Kurkalova et al. (2004) estimated that increasing C sequestration by 100 kg C ha$^{-1}$ year$^{-1}$ in Iowa agricultural soils would reduce soil erosion by water by 350 kg ha$^{-1}$ year$^{-1}$, soil erosion by wind by 0.4 kg ha$^{-1}$ year$^{-1}$, and soil N runoff by 0.14 kg ha$^{-1}$ year$^{-1}$. Increased C stocks in agricultural soils also enhance soils’ ability to support sustainable crop growth while bringing farmers or landowners additional incomes. Nordhaus and Yang (1996) estimated that C sequestration would yield economic benefit of $6–21 per ton of C. Currently, the Chicago Climate Exchange (CCX) will pay land managers about $2 per ton of CO$_2$ reduction for adopting management practices for sequestering CO$_2$ (http://www.chicagoclimatex.com). Soil C can be minimized through increasing organic C inputs and reducing organic C decomposition via agricultural management decisions (Lal, 2004; Paustian et al., 2000).

Soil microbes are the living part of soil organic matter and play critical roles in soil C and N cycling and ecosystem functioning (Doran, 1987). They serve as both source and sink of plant nutrients (Dalal, 1998). The activity of soil microbes greatly influences short-term dynamics and long-term stability of organic matter in soil. Microbes are usually C-limited in agricultural soils (Smith and Paul, 1990), and microbial biomass and activities are thus closely related to labile organic C in soil. It is well-known that soil microbial biomass and activity respond sensitively to changes in organic C levels or quality resulting from agronomic practices and other disturbances (Powlson et al., 1987; Lundquist et al., 1999; Tu et al., 2006). High microbial activities are inherently coupled to high C turnover and CO$_2$ release; thus management practices that reduce microbial access to organic matter should promote soil C accumulation.

Public concerns over high-energy inputs or use of synthetic chemicals (pesticides and fertilizers) have led to an increasing interest in alternative farming practices (also known as sustainable production practices) that are less dependent on energy-intensive technology in agriculture (Lichtfouse et al., 2009). In the past decades, a multitude of sustainable crop production practices have been developed and adopted at varying spatial scales; they commonly include no-tillage or reduced tillage practices (Tripplett and Dick, 2008), integrated pest management, crop rotations/intercropping and/or cover cropping (Lichtfouse et al., 2009). These practices can directly or indirectly affect soil microbes and soil C dynamics by increasing C inputs and reducing C loss (Chen et al., 2009; Alvarez et al., 1995; Pascualt et al., 2010; Jacobs et al., 2010). Many studies have shown that production systems that minimize soil disturbance (reduced tillage, minimum tillage, and no tillage) generally increase soil organic C, and microbial biomass and activity compared to conventional tillage in various soil types and climatic regions (Paustian et al., 2000; Kushwaha et al., 2001; Tripplett and Dick, 2008; Jacobs et al., 2010). However, there is a concern that reducing tillage may only have limited short-term effects through facilitating organic C redistribution to the top layer and therefore the long-term potential for C sequestration is still debatable (Baker et al., 2007; Luo et al., 2010).

In humid and warm regions of the world, such as the southeast USA, decomposition rates of organic C are increased because of high soil microbial activity levels during much of the year. The effectiveness of various agricultural practices in facilitating soil C accumulation is less studied in these regions. We hypothesized that a combination of sustainable production practices will result in greater microbial biomass and activity and soil organic C than any individual practice. To test this hypothesis, we investigated the cumulative effects of various production practices on soil microbial biomass and activity, and soil organic C and N after continuous treatments for 15 years in a long-term field experiment established in fall 1994 with aims at examining long-term effects of different sustainable production practices on yield and pest and disease pressures in vegetable crops in the mountains of western North Carolina.

2. Materials and methods

2.1. The field experiment

The field experiment was established in fall 1994 at the Mountain Horticultural Crops Research Station (N 35°25’39", W 82°33’21", elevation 624 m) in Mills River, NC. The monthly rainfall and air temperature of the site are provided in Table 1. Prior to the beginning of this experiment, this site was continuously cultivated with moldboard plow tillage and fumigated annually for over 30 years. The soil parent material was alluvial deposits. The soil pH was 6.2 (0–15 cm depth). The soil contained 560 g sand kg$^{-1}$ soil, 260 g silt kg$^{-1}$ soil, and 180 g clay kg$^{-1}$ soil. The soil type is a Delanco fine sandy loam (fine-loamy, mixed, mesic, Aquic Hapludult), equivalent to Acrisol in FAO (Overstreet et al., 2010).

Six production practice systems were established in fall 1994, i.e., tillage with no chemical or organic inputs (Control, TN), tillage with chemical inputs (TC), tillage with organic inputs (TO), no-tillage with chemical inputs (NC), no-tillage with organic inputs (NO), and fescue grasses (FG). A detailed description for the six production practices and the sequence of vegetables grown is given in Tables 2a and 2b, respectively. Winter cover crows of wheat (Triticum aestivum L.) or rye (Secale cereale, M.Bieb) and crimson clover (Trifolium incarnatum L.) or hairy vetch (Vicia villosa Roth.) were fall planted in the synthetic treatments and the organic treatments, respectively.

Table 1

<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td>Rainfall (mm)</td>
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</tr>
<tr>
<td>January</td>
<td>138</td>
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<td>February</td>
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<td>September</td>
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<td>November</td>
<td>138</td>
<td>15.9</td>
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<tr>
<td>December</td>
<td>193</td>
<td>7.9</td>
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</table>
Tillage with chemical inputs, TC  Plow and disk  N: Each crop with 168 kg ha⁻¹ N (ammonium nitrate)  P: Every three years with 112 kg ha⁻¹ of P₂O₅ (triple super phosphate)  K: Every two years with 112 kg ha⁻¹ of K₂O (potassium chloride)  CaCO₃: Every four years with 2240 kg ha⁻¹ of dolomitic limestone  Herbicides, fungicides and insecticides were used as recommended  Insecticides:  esfenvalerate at 0.05 kg AI ha⁻¹ (Asana XL, DuPont, Wilmington, DE)  endosulfan at 1.12 kg AI ha⁻¹ (Thiodan 3 EC, FMC, Philadelphia, PA)  imidacloprid at 0.05 kg AI ha⁻¹ (Provado 1.6 F, Bayer, Kansas City, MO)  Fungicides:  chlorothalonil at 1.68 kg AI ha⁻¹, (Bravo 6 F, Zeneca, Wilmington, DE)  copper hydroxide at 2.24 kg AI ha⁻¹ (Kocide 101, Grifﬁn, Valdosta, GA)  No-tillage with chemical inputs, NC  Plow and disk  N: Each crop with 168 kg ha⁻¹ N (soybean meal)  P: Every three years with 112 kg ha⁻¹ of P₂O₅ (rock phosphate, 3% available P)  K: Every two years with 112 kg ha⁻¹ of K₂O (potassium–magnesium sulfate)  CaCO₃: Every four years with 2240 kg ha⁻¹ of dolomitic limestone  Insecticides, disease, and weed management as approved for organic farming  Fungi:  bacillus thuringiensis (Dipel and Xentari, Abbott, North Chicago, IL)  insecticidal soap (M-Pede, Mycogen, San Diego, CA)  Disease:  copper hydroxide (Kocide 101)  Weed:  hand weeding or mowing Insecticide and copper hydroxide were used on the same dates as in TC  The experiment was a randomized complete design with four replications. Each plot (12.2 m × 22.4 m) was separated by a 12.2 m alleyway of grass (*Festuca elatior* L.) in order to minimize fertilizer and pesticide drift.

### Table 2a

<table>
<thead>
<tr>
<th>Management</th>
<th>Tillage</th>
<th>Fertilizers</th>
<th>Pesticides</th>
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<tr>
<td>Tillage with chemical inputs, TC</td>
<td>Plow and disk</td>
<td>N: Each crop with 168 kg ha⁻¹ N (ammonium nitrate)  P: Every three years with 112 kg ha⁻¹ of P₂O₅ (triple super phosphate)  K: Every two years with 112 kg ha⁻¹ of K₂O (potassium chloride)  CaCO₃: Every four years with 2240 kg ha⁻¹ of dolomitic limestone</td>
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</tr>
<tr>
<td>Tillage with organic inputs, TO</td>
<td>Plow and disk</td>
<td>N: Each crop with 168 kg ha⁻¹ N (soybean meal)  P: Every three years with 112 kg ha⁻¹ of P₂O₅ (rock phosphate, 3% available P)  K: Every two years with 112 kg ha⁻¹ of K₂O (potassium–magnesium sulfate)  CaCO₃: Every four years with 2240 kg ha⁻¹ of dolomitic limestone</td>
<td>Insecticides, disease, and weed management as approved for organic farming  Fungi:  bacillus thuringiensis (Dipel and Xentari, Abbott, North Chicago, IL)  insecticidal soap (M-Pede, Mycogen, San Diego, CA)  Disease:  copper hydroxide (Kocide 101)  Weed:  hand weeding or mowing Insecticide and copper hydroxide were used on the same dates as in TC</td>
</tr>
</tbody>
</table>

### Table 2b

<table>
<thead>
<tr>
<th>Year</th>
<th>Plants</th>
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<tbody>
<tr>
<td>1995</td>
<td>Sweet corn (Zea mays L)</td>
</tr>
<tr>
<td>1996</td>
<td>Cucumber (Cucumis sativus L)  Fall Cabbage [Brassica oleracea L. (Capitata Group)]</td>
</tr>
<tr>
<td>1997</td>
<td>Tomato (Solanum lycopersicum L)</td>
</tr>
<tr>
<td>1998</td>
<td>Sweet corn (Zea mays L)</td>
</tr>
<tr>
<td>1999</td>
<td>Cucumber (Cucumis sativus L)  Fall Cabbage [Brassica oleracea L. (Capitata Group)]</td>
</tr>
<tr>
<td>2000</td>
<td>Tomato (Solanum lycopersicum L)  Peppers (Capsicum annuum L)</td>
</tr>
<tr>
<td>2001</td>
<td>Summer Squash (Cucurbita pepo L)  Fall Broccoli [B. oleracea L. (Italica Group)]</td>
</tr>
<tr>
<td>2002</td>
<td>Tomato (Solanum lycopersicum L)  Peppers (Capsicum annuum L)</td>
</tr>
<tr>
<td>2003</td>
<td>Tomato (Solanum lycopersicum L)  Peppers (Capsicum annuum L)</td>
</tr>
<tr>
<td>2004</td>
<td>Summer Squash (Cucurbita pepo L)  Fall Broccoli [B. oleracea L. (Italica Group)]</td>
</tr>
<tr>
<td>2005</td>
<td>Tomato (Solanum lycopersicum L)  Peppers (Capsicum annuum L)</td>
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<tr>
<td>2006</td>
<td>Tomato (Solanum lycopersicum L)  Summer Squash (Cucurbita pepo L)</td>
</tr>
<tr>
<td>2007</td>
<td>Sweet corn (Zea mays L)</td>
</tr>
<tr>
<td>2008</td>
<td>Sweet corn (Zea mays L)</td>
</tr>
<tr>
<td>2009</td>
<td>Sweet corn (Zea mays L)</td>
</tr>
<tr>
<td>2010</td>
<td>Sweet corn (Zea mays L)</td>
</tr>
</tbody>
</table>

2.2. Soil sampling

Soil samples were collected in each plot both in fall (late October), 2009 (immediately after harvest) and in spring (early May), 2010 (immediately before planting). Five sampling points within each plot were randomly selected. At each point, approximately 20 soil cores of 1.9 cm diameter by 15 cm depth were taken within a 1 m radius of the point. All soil cores from each plot were bulked, crumbled, and thoroughly mixed within a plastic bag. There were a total of 48 bulked samples for the study. All samples were stored in sealed plastic bags in a cooler and transported to the laboratory. The composite samples were passed through a 3 mm diameter sieve and any visible living plant material, stones and visible organism were manually removed. The sieved samples were kept in the refrigerator at 4°C. All biological determinations were performed within one week of sampling.

2.3. Sample measurements

2.3.1. Total soil organic C and N

Total soil organic C (SOC) and N (SON) were determined using a Perkin-Elmer 2400 CHNS/O elemental analyser (Norwalk, CT, USA) after air-drying and grinding to fine powder.

2.3.2. Soil extractable C and N

Soil extractable organic C was estimated by equilibrating 20.0 g dry weight equivalent soil with 50 ml of 0.5 mol L⁻¹ K₂SO₄ solution.
The concentrations of C in the solutions were then determined using a total organic C (TOC) analyzer (TOC-5050A, Shimadzu Corporation, Kyoto, Japan). To estimate extractable N in soil, 10.0 g dry weight equivalent soil samples were shaken with 100 mL of 1 mol L\(^{-1}\) KCl solution (Hart et al., 1994). The concentrations of NO\(_3^–\) and NH\(_4^+\) in the extracts were, respectively, determined with QuikChem methods 10-107-04-1-A and 10-107-06-2-A on a Lachat flow injection analyzer (Lachat Instruments, Milwaukee, WI, USA).

### 2.3.3. Soil microbial biomass C and N

Microbial biomass C (MBC) and microbial biomass N (MBN) were determined by the chloroform-fumigation–extraction method (Ross, 1992; Vance et al., 1987). Soil of 20.0 g (dry weight equivalent) was fumigated with ethanol–free chloroform for 48 h. Both fumigated and non-fumigated soils were extracted with 50 mL of 0.5 mol L\(^{-1}\) K\(_2\)SO\(_4\) by shaking for 30 min on an end-to-end shaker. The TOC analyzer was used to determine the organic C (C\(_{org}\)) in the extracts. The MB was calculated as follows:

\[
MBC = \frac{C_{org} \text{in fumigated soil} - C_{org} \text{in non-fumigated soil}}{k_{ec}}
\]

where \(k_{ec} = 0.33\), the factor used here to convert the extracted organic C to MBC (Sparling and West, 1988).

The concentration of N in the extractant was determined on the Lachat flow injection analyzer after digestion using alkaline persulfate oxidation (Cabrera and Beare, 1993). The MBN was calculated using the equation:

\[
MBN = \frac{-\text{total N extracted from fumigated soil}}{k_{en}} + \frac{-\text{total N extracted from non-fumigated soil}}{k_{en}}
\]

where \(k_{en}\) is 0.45, the factor used to convert the extracted organic N to MBN (Jenkinson, 1988).

### 2.3.4. Soil microbial respiration

Heterotrophic microbial respiration was measured in the absence of plant roots using an incubation–alkaline absorption method (Coleman et al., 1978). Soil equivalent to 20.0 g dry weight was weighed and the water content of the soil was adjusted to about 60% water holding capacity (Alef, 1995), which was measured according to the method described by Forster (1995), and placed in a 1-L Mason jar with a suspended beaker containing 5 mL of 0.5 mol L\(^{-1}\) NaOH. The jars were incubated at 25.0 °C in the dark immediately after sealing. On day 7 after incubation, beakers were replaced with one containing fresh NaOH solution, and the jars were incubated for an additional 7 d. The CO\(_2\) trapped in NaOH was titrated with 0.1 mol L\(^{-1}\) HCl. Microbial respiration was estimated as mg CO\(_2\) kg\(^{-1}\) soil d\(^{-1}\) by averaging the data.

### 2.3.5. Net N mineralization

Net nitrogen mineralization was determined using the method described by Hart et al. (1994). Briefly, soils of 10.0 g dry weight equivalent were weighed into Erlenmeyer flasks. The flasks were covered with plastic wrap pierced with a small hole to minimize water loss yet maintain gas exchange, and the soils were incubated for 28 d in the dark at room temperature (22 ± 1 °C). Soil water content was maintained at approximately 60% water holding capacity by monitoring the weight change and adding water weekly during the incubation period. Soil NH\(_4^+\) and NO\(_3^–\) were extracted with 1 mol L\(^{-1}\) KCl at a 1:10 soil to solution ratio, and their concentrations were determined with the Lachat flow injection analyzer. Net mineralized N in soil was the difference between KCl-extractable inorganic N contents before and after incubation.

### 2.3.6. Soil organic matter fractionation

Density fractionation of soil organic matter was performed using a procedure modified from Baisden et al. (2002). Briefly, 50.0 g soil samples were first extracted with 50 mL of distilled water in 100 mL flasks. After gentle dispersal by hand, the flasks were left standing overnight at room temperature. The first fraction (F1, light OM with \(d < 1.0 \text{ g cm}^{-3}\)) was collected by filtering the supernatant through Whatman No. 1 filter paper. The sediment in the flasks was resuspended in 50 mL of potassium iodide solution (\(d = 1.6 \text{ g cm}^{-3}\)) by hand-stirring. The suspension was allowed to stand at room temperature for at least 1 h. The supernatant containing OM with \(d < 1.6 \text{ g cm}^{-3}\) was collected by filtration as the second fraction (F2, heavy OM), and the remaining sediment was regarded as the third fraction (F3, very heavy OM with \(d > 1.6 \text{ g cm}^{-3}\)). All fractions were oven-dried at 65 °C and ground to a fine powder before C determination on a Perkin-Elmer 2400 CHN/S/0 elemental analyser (Norwalk, CT, USA).

### 2.4. Statistical analysis

All results were expressed as an average of four replicates with standard error, based on oven-dry soil weight. The effect of the different production systems and the effect of input and tillage practices were separately subjected to ANOVA using the general linear model (GLM) procedure of the SAS (SAS Systems, Cary, NC, USA). Differences among the means were separated by least significant difference, at the 0.05 probability level.

### 3. Results

#### 3.1. Total and extractable C and N in soil

Different practices have significantly affected both soil organic C (SOC) and organic N (SON) (Fig. 1a and b). Total organic C and N contents were highest in FG system with 18.5 mg kg\(^{-1}\) and 1.80 mg kg\(^{-1}\), whereas lowest in the TC system, being only 7.0 mg kg\(^{-1}\) and 0.75 mg kg\(^{-1}\), respectively. No significant differences in SOC and SON were found between NO and FG systems, and between TO and TC. However, the SOC and SON were significantly higher (77–83%) in NC than in TC systems. In addition, SOC and SON in NO system were 44% and 35% higher compared to NC system, respectively.

Soil extractable C was not statistically different among systems at either sampling date, with mean 82 ± 3.0 mg kg\(^{-1}\) in the fall and 72 ± 2.5 in the spring (data not shown). However, extractable N was significantly affected by production systems (Fig. 2). In fall 2009, the greatest soil extractable N was determined for NO (15 mg N kg\(^{-1}\)), followed by TC (14 mg N kg\(^{-1}\)), FG (11 mg N kg\(^{-1}\)), NC (9.0 mg N kg\(^{-1}\)), TO (6.0 mg N kg\(^{-1}\)) and TN (3.0 mg N kg\(^{-1}\)). In spring 2011, soil extractable N was relatively lower compared to the fall samples, but both NO and FG systems had the highest extractable N. The lowest extractable N (about 1 mg kg\(^{-1}\)) was found in soils from TC system. Soil extractable N was 49–81% higher in organic than chemical plots with the exception of TC in fall 2009.

#### 3.2. Microbial biomass C and N

Soil microbial biomass C (MBC) was significantly different among production systems, and similar trends occurred at both sampling dates (Fig. 3a). The greatest MBC was found in FG system (692 and 422 mg C kg\(^{-1}\) in fall and spring, respectively), and the lowest in TC systems (160 and 106 mg C kg\(^{-1}\) in fall and spring samples, respectively). The MBC was slightly lower in the NO system than in the FG system, but significantly higher than in the TO and NC systems. Compared to tillage, no-tillage practices significantly increased MBC by 63–139% and 128–134% in both
chemical (NC vs. TC) and organic inputs (NO vs. TO) systems. Likewise, organic inputs resulted in 58–62%, 54–126% higher MBC than chemical inputs in both tillage (TO vs. TC) and no-tillage (NO vs. NC) systems.

Ratios of MBC to total organic C were significantly greater in organic inputs and continuous grass systems than in conventional chemical inputs systems, with the highest in organic plus no-tillage systems (Fig. 4).

Similarly to MBC, soil MBN contents followed this rank order: FG > NO > NC > TO > TN > TC (Fig. 3b). Both no-tillage and organic inputs led to higher MBN contents in soil.

3.3. Soil microbial respiration and net N mineralization

In samples collected in fall 2009, the highest soil respirations were observed in both NO and FG systems, followed by TO and NC systems, and the lowest in the TC and TN systems (Fig. 5a). A similar trend was observed in the 2010 spring samples except that soil respiration was higher in the TC than TN system. Both no-tillage practice and organic inputs significantly enhanced microbial respiration, with microbial respiration being 88–158% higher in no-tillage than tillage soils and 52–117% higher in organic than chemical inputs soils. The interaction between organic inputs and

Fig. 1. Soil total organic C (a) and organic N (b) under different production systems in Mills River, NC in fall 2009. TC, tillage plus chemical inputs; TO, tillage plus organic inputs; NC, no-till plus chemical inputs; NO, no-till plus organic inputs; TN, tillage but no inputs; FG, tall fescue grass. Bars represent mean and the lines represent the standard error. Bars with the same letters are not significantly different at $P < 0.05$ (LSD).

Fig. 2. Soil extractable N under different production systems in Mills River, NC, TC, tillage plus chemical inputs; TO, tillage plus organic inputs; NC, no-till plus chemical inputs; NO, no-till plus organic inputs; TN, tillage but no inputs; FG, tall fescue grass. Bars represent mean and the lines represent the standard error. Bars with the same lowercase and uppercase letters are not significantly different at $P < 0.05$ (LSD) in fall 2009 and spring 2010, respectively.

Fig. 3. Soil microbial biomass C (a) and microbial biomass N (b) under different production systems in Mills River, NC, TC, tillage plus chemical inputs; TO, tillage plus organic inputs; NC, no-till plus chemical inputs; NO, no-till plus organic inputs; TN, tillage but no inputs; FG, tall fescue grass. Bars represent mean and the lines represent the standard error. Bars with the same lowercase and uppercase letters are not significantly different at $P < 0.05$ (LSD) in fall 2009 and spring 2010, respectively.
organic matter to microbial decomposition, thus promoting the protection of soil organic C (Balesdent et al., 2000; Bronick and Lal, 2005; Jacobs et al., 2009). In our experiment, annual external C inputs in organic systems were estimated at 1000 kg C ha⁻¹ during the past 15 years without considering organic C from plant roots and root exudates and cover crops (Overstreet et al., 2010). Apart from a direct contribution to soil organic C and N, organic materials applied to soil may also improve C and N accumulation indirectly through enhancing microbial biomass and activity. High microbial biomass and activities facilitate the transformation and stabilization of organic C in soil, as indicated by increased C in the very heavy fraction in our experiment (Table 3 and Figs. 3 and 5). Recent studies have shown that microbial biomass-derived C constitutes up to 50% of soil organic C (Simpson et al., 2007; Kindler et al., 2009; Liang et al., 2010; Liang and Balser, 2011). Also, many microbial secondary compounds function as binding agents for the formation of microaggregates (Tisdall, 1994; Rillig and Mummey, 2006), increasing the protection of soil organic C against decomposition. Greater contents of total organic C and N in no-till chemical inputs (NC) than in tillage plus organic inputs (TO) systems (Fig. 1) indicated that no-till practice may play a dominant role in promoting soil C and N accumulation over organic input practice. This is also

3.4. Organic carbon in soil fractions

The percentage of C in the soil light fractions decreased in the following rank order: TC > TO > NC > FG > NO > TN, whereas in the very heavy fractions increased almost in the same order (Table 3). The content of C in the heavy fractions of the soil was significantly higher in no-tillage than in tillage systems, but the organic C content in the light fractions was exactly the opposite. Similarly, the percentage of light fraction C to the total C was lower and that of the very heavy fraction higher in organic than in chemical inputs systems.

4. Discussion

Results of the present study clearly support our hypothesis that sustainable production practices used in combination with one another have greater positive impact soil microbial properties and organic C and N than when applied individually. Significant increases in total soil C and N, and organic C in the heavy SOM fraction of the no-till organic input system (Table 2a and Fig. 1) indicated that combinations of reduced tillage and organic inputs can not only increase total pool size of soil organic C and N, but also increases the stability of soil organic matter. Long-term C sequestration in soil is dependent on both organic C inputs and its stability in soil. The stability of soil organic C and N is controlled by various mechanisms, including (1) physico-chemical associations with silt and clay particles, (2) physical aggregate protection, and (3) biochemical formation of recalcitrant soil organic compounds (Six et al., 2002; Blanco-Canqui and Lal, 2004). It is broadly accepted that no-till and/or reduced tillage can alleviate disruption of soil macroaggregates and lower exposures of microaggregates and free
demonstrated by no significant differences in organic C and N contents between tillage plus organic inputs and tillage plus chemical inputs.

One important aspect for examining the long-term impact of management practices is to assess whether and how these practices affect the distribution of organic C in different C pools with different turnover times and alter the C-protection mechanisms in soil. Heavy fraction C is associated with soil clays and shows decadal stability (Baisden et al., 2002; Leifeld and Fuhrer, 2009). Previous experiments have shown that management practices critically affected C distribution into long-term and short-term pools (Bear et al., 1995; Hendrix et al., 1998; Jacobs et al., 2009; Hai et al., 2010). For example, Jacobs et al. (2009) showed that 40 years minimum tillage has resulted in more organic C associated with the very heavy fraction than the conventional tillage. In a wheat–corn conventional tillage system, Hai et al. (2010) found that 26-years application of animal manure significantly increased mineral–associated organic C (very heavy fraction) in comparison to chemical fertilizer. This has also been evidenced by our results of the high proportion of C in the heavy fraction in the no till plus organic inputs treatment (Table 3). The results of significant increases in the percentage of organic C in the very heavy fraction, although small in the absolute values, did suggest the increased stability of soil organic C in the no-till organic system.

One issue still under debate is the long-term potential of soil C accumulation in no-tillage systems. Some studies suggest that the net C sequestration is limited under no-tillage systems mainly due to the redistribution of organic C among different soil layers (West and Post, 2002; Baker et al., 2007; Gal et al., 2007; Angers and Eriksen-Hamel, 2008; Luo et al., 2010). However, it has been shown that the ability of the crop species or cultivars to develop deep roots exerts major controls in C accumulation in deep soil (Adviento-Borbe et al., 2007; Wright et al., 2007; Boddey et al., 2010). Soil C accumulation in deeper soil layers may also have occurred in our systems because deep rooting crop (sweet corn) was grown. Carbon accumulation in soil is a long time process (Foereid and Høgh-Jensen, 2004), due to fast turnover of newly added organic materials (Richter et al., 1999). In our study, both total C and C distribution patterns were similar between no-till organic treatment and the continuous grass system (Table 3 and Fig. 1). These results indicate that it is possible to maximize soil C and N accumulation in agricultural production systems in humid and warm climates through effective production management practices.

Several mechanisms may be attributed to the observed increases in soil C and N in our systems. The gradual release of N from organic fertilizer sources (soybean meal) and cover crops may have facilitated deep rooting of corn, thus promoting soil C and N accumulation (Babuia et al., 2010; Boddey et al., 2010). Also, alteration in soil organisms may have promoted soil C movement downwards. In our systems, earthworm population and activities were significantly higher in no-till plus organic input systems than in conventional systems, with the earthworm Lumbricus terrestres the dominant species (Overstreet et al., 2010). Lumbricus terrestres is an anecic species that builds permanent, vertical burrows extending from the soil surface down to the B horizon and are well known for their ability to move organic matter downwards (Edwards and Bohlen, 1996). It has been documented that earthworm activity directly contributes to the transport of soil C into deep layers and earthworm casts contribute to the physical protection of organic C against microbial decay via formation of soil microaggregates (Pullman et al., 2005; Don et al., 2008; Fonte and Six, 2010).

It is also worth mentioning that the treatment effects on all microbial parameters exhibited similar trends between two sampling dates, although the absolute values were slightly different. Significantly lower extractable N was observed in the spring than the fall samples, probably due to N uptake by the winter cover crop and weeds. The proportion of C in microbial biomass was significantly higher in organic than other treatments (Fig. 4), suggesting that the relative activities or turnover may be higher. Promotion of soil C and N accumulation under no-till organic input treatments has implications for waste managements in this region. In the North Carolina, USA, the swine and chicken industry are huge, producing approximately 2 × 10⁶ t of dry solid manures yearly (Bull, unpublished). Therefore, long-term organic inputs would not only be possible, but may also provide an effective means for organic waste disposal under the strict guidelines of the environment and health regulations.

5. Conclusions

In accordance with our hypothesis, this study confirmed that continuous implementation of sustainable production practices (especially in combination of no-tillage and organic inputs) can increase total soil organic C and facilitate the transformation of organic C to the more stable C pools, leading to a soil C content and distribution comparable to the undisturbed grassland control. No-tillage plus organic management enhanced soil microbial biomass and activities, whereas conventional tillage with chemical inputs had deleterious impact on soil microorganisms and reduced soil organic C. No-till practice may play a dominant role in soil organic C and N accumulation over organic inputs. These findings indicate that under our warm, humid climate in the southeast USA, sustainable production practices still can promote soil C accumulation and increase soil fertility, potentially contributing to alleviate atmospheric CO₂ rise.

Acknowledgements

This research was supported in part by USDA to Shuqin Hu (2007-34381-18625; 2009-35101-05351) and China Scholarship Council: Postgraduate Scholarship Program (2007102999) to Yi Wang. We

Table 3

Mean relative proportions (%) of organic C in the density fractions to the total organic C in the soil under the different production systems, Mills River, NC.

<table>
<thead>
<tr>
<th>Systema</th>
<th>Light fraction d ≤ 1.0 g cm⁻³</th>
<th>Heavy fraction 1.0 g cm⁻³ &lt; d ≤ 1.6 g cm⁻³</th>
<th>Very heavy fraction d &gt; 1.6 g cm⁻³</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>3.9 ± 0.63 a</td>
<td>1.35 ± 0.27 b</td>
<td>95.3 ± 0.68 b</td>
</tr>
<tr>
<td>TO</td>
<td>1.53 ± 0.35 b</td>
<td>2.88 ± 0.84 a</td>
<td>95.6 ± 1.03 b</td>
</tr>
<tr>
<td>NC</td>
<td>1.09 ± 0.22 b</td>
<td>1.20 ± 0.14 b</td>
<td>97.2 ± 0.33 a</td>
</tr>
<tr>
<td>NO</td>
<td>0.80 ± 0.17 b</td>
<td>1.02 ± 0.14 b</td>
<td>98.2 ± 0.22 a</td>
</tr>
<tr>
<td>TN</td>
<td>0.77 ± 0.16 b</td>
<td>1.15 ± 0.59 b</td>
<td>98.1 ± 0.54 a</td>
</tr>
<tr>
<td>FG</td>
<td>0.84 ± 0.17 b</td>
<td>1.34 ± 0.07 b</td>
<td>97.8 ± 0.19 a</td>
</tr>
</tbody>
</table>

a TC, tillage plus chemical inputs; TO, tillage plus organic inputs; NC, no-till plus chemical inputs; NO, no-till plus organic inputs; TN, tillage but no inputs; FG, tall fescue grass.

b Mean ± S.E. (number of observations n = 4). The values with the same letters within one column are not significantly different at P < 0.05, least significant difference (LSD).
would like to thank Lisa Lentz for total C and N determination, Guillermo Ramirez for his technical help in nitrogen analyses at the Soil Analytical Lab, NCSU. We also thank the staff at the Mountain Horticultural Crops Research Station, NCSU. Sincere thanks are extended to the anonymous reviewers for their comments on earlier versions of the manuscript.

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