

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

Yi Wang^{a,b}, Cong Tu^b, Lei Cheng^b, Chunyue Li^c, Laura F. Gentry^e, Greg D. Hoyt^d, Xingchang Zhang^a, Shuijin Hu^{b,*}

^a College of Resources and Environment, Institute of Soil and Water Conservation, Northwest A&F University, Yangling, Shaanxi 712100, China

^b Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695, USA

^c Northwest Land and Resources Research Center, Shaanxi Normal University, Xi'an, Shaanxi 710062, China

^d Department of Soil Science, North Carolina State University, Mountain Horticultural Crops Research and Extension Center, 455 Research Drive, Mills River, NC 28759, USA

^e Department of Crop Sciences, University of Illinois, Urbana, IL 61801, USA

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 $d < 1.0 \text{ g cm}^{-3}$ (from 1.53–3.39% to 0.80–1.09%), and increased the very heavy fraction with $d > 1.6 \text{ g cm}^{-3}$ (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 fractionations 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.

© 2011 Elsevier B.V. All rights reserved.

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 $0.47 \mu\text{mol mol}^{-1}$ 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

* Corresponding author at: 2510 Thomas Hall, Raleigh, NC 27695-7616, USA.
Tel.: +1 919 515 2097; fax: +1 919 515 7716.

E-mail address: shuijin_hu@ncsu.edu (S. Hu).

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 US dollars per ton of C. Currently, the Chicago Climate Exchange (CCX) will pay land managers about \$2 per ton of CO₂ reduction for adopting management practices for sequestering CO₂ (<http://www.chicagoclimatex.com>). Soil C can be enhanced 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 CO₂ 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 (Triplett 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; Pascault 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; Triplett 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 crops 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

The monthly rainfall and maximum and minimum air temperatures for the experimental and long-term periods.

Month	Experiment (June, 2009–May, 2010)			Long-term (1995–2010)		
	Rainfall (mm)	Maximum temperature (°C)	Minimum temperature (°C)	Rainfall (mm)	Maximum temperature (°C)	Minimum temperature (°C)
January	138	5.4	−5.5	101	8.9	−3.1
February	80	4.6	−4.0	79	10.3	−1.9
March	52	12.5	1.2	87	14.9	1.4
April	52	22.4	5.6	83	20.0	5.4
May	135	24.7	12.7	86	24.1	10.3
June	125	28.2	15.4	120	27.3	14.8
July	59	27.0	15.8	109	28.9	16.9
August	99	27.8	16.8	105	28.9	16.7
September	170	24.1	14.6	108	25.1	12.7
October	90	18.4	7.0	65	20.4	6.4
November	138	15.9	2.7	91	15.0	1.0
December	193	7.9	−2.8	89	9.7	−2.4

Table 2a

Description of the six production practice systems in this study.

Management	Tillage	Fertilizers	Pesticides
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 P ₂ O ₅ (triple super phosphate) K: Every two years with 112 kg ha ⁻¹ of K ₂ O (potassium chloride) CaCO ₃ : 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) Herbicides: napropamide 1.12 kg (AI) ha ⁻¹ (Devrinol 50 WDG, Zeneca) metribuzin at 0.28 kg (AI) ha ⁻¹ (Sencor 75 WDG, Bayer, KS, City, MO) paraquat at 0.67 kg (AI) ha ⁻¹ (Gramoxone Extra, Zeneca) ethalfluralin at 1.23 kg (AI) ha ⁻¹ (Curbit 3 EC, UAP, Greeley, CO) metolachlor at 1.07 kg (AI) ha ⁻¹ (Dual 7.64 EC, Novartis, Greensboro, NC) atrazine 2.25 kg (AI) ha ⁻¹ (Bicep 6 F, Novartis)
Tillage with organic inputs, TO	Plow and disk	N: Each crop with 168 kg ha ⁻¹ N (soybean meal) P: Every three years with 112 kg ha ⁻¹ of P ₂ O ₅ (rock phosphate, 3% available P) K: Every two years with 112 kg ha ⁻¹ of K ₂ O (potassium–magnesium sulfate) CaCO ₃ : Every four years with 2240 kg ha ⁻¹ of dolomitic limestone	Insect, disease, and weed management as approved for organic farming Insecticides: <i>bacillus thuringiensis</i> (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
No-tillage with chemical inputs, NC	No-tillage	Same as TC	Same as TC
No-tillage with organic inputs, NO	No-tillage	Same as TO	Same as TO
Tillage with no inputs, TN	Plow and disk	No inputs	No inputs
Fescue grasses, FG	No-tillage	No inputs	No inputs

Hummel et al. (2002a,b).

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 2b

Sequence of vegetables grown on the field plots during the experiment.

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

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

(Hu et al., 1997). 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 NO₃⁻ and NH₄⁺ 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 K₂SO₄ 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 MBC was calculated as follows:

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

where $k_{\text{ec}} = 0.33$, 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:

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

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 CO₂ trapped in NaOH was titrated with 0.1 mol L⁻¹ HCl. Microbial respiration was estimated as mg CO₂ 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 NH₄⁺ and NO₃⁻ 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 CHNS/O 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 practice 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

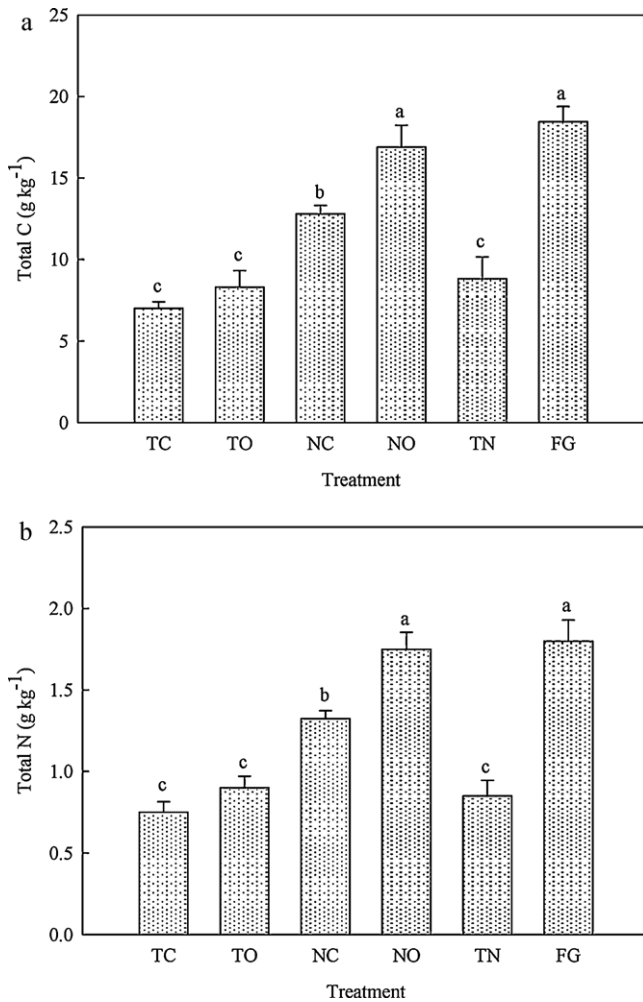


Fig. 1. Soil total organic C (a) and organic N (b) under different production systems in Mills River, NC in fall 2009. TC, tillage plus chemical inputs; TO, tillage plus organic inputs; NC, no-till plus chemical inputs; NO, no-till plus organic inputs; TN, tillage but no inputs; FG, tall fescue grass.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

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: $FG \geq NO > NC > TO > TN > TC$ (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

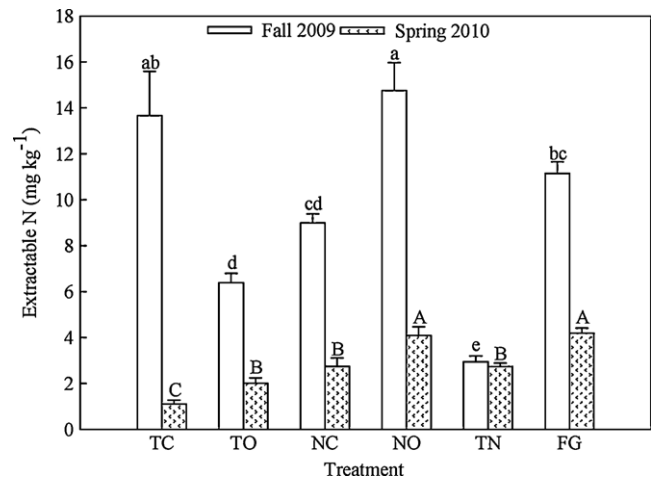


Fig. 2. Soil extractable N under different production systems in Mills River, NC. TC, tillage plus chemical inputs; TO, tillage plus organic inputs; NC, no-till plus chemical inputs; NO, no-till plus organic inputs; TN, tillage but no inputs; FG, tall fescue grass. Bars represent means and the lines 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.

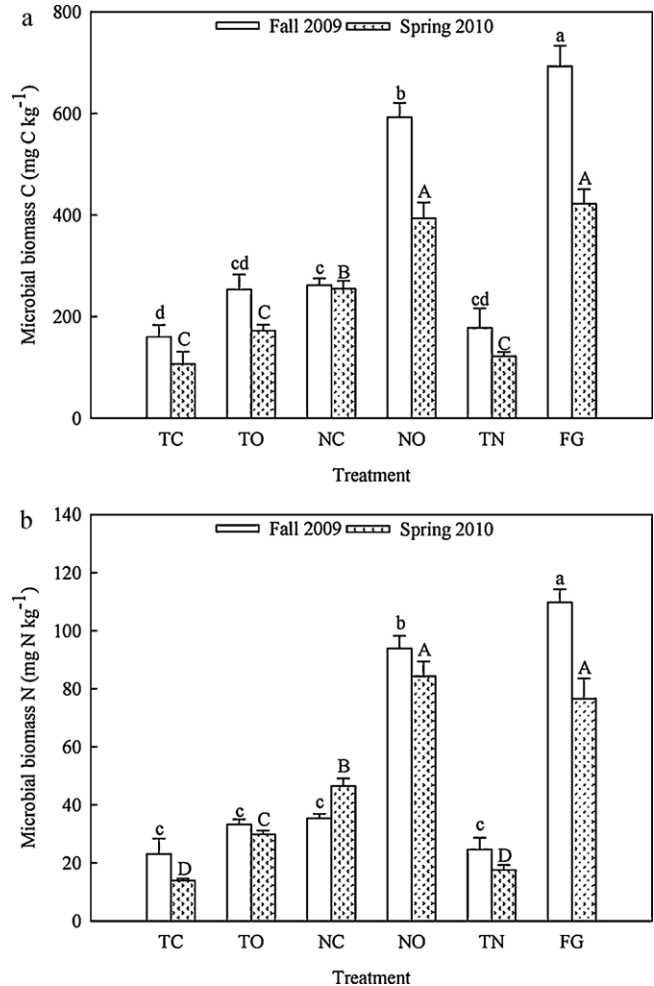


Fig. 3. Soil microbial biomass C (a) and microbial biomass N (b) under different production systems in Mills River, NC. TC, tillage plus chemical inputs; TO, tillage plus organic inputs; NC, no-till plus chemical inputs; NO, no-till plus organic inputs; TN, tillage but no inputs; FG, tall fescue grass. Bars represent means 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.

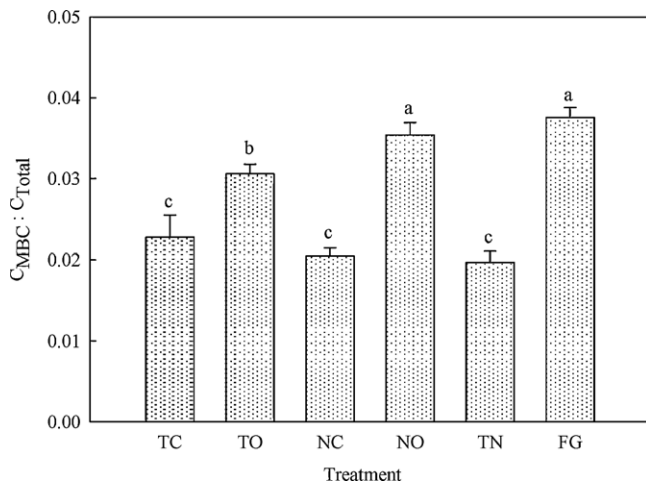


Fig. 4. Ratios of microbial biomass C (MBC) to soil total C under different production systems in Mills River, NC in fall 2009. TC, tillage plus chemical inputs; TO, tillage plus organic inputs; NC, no-till plus chemical inputs; NO, no-till plus organic inputs; TN, tillage but no inputs; FG, tall fescue grass. Bars represent means and the lines represent the standard error. Bars with the same letters are not significantly different at $P < 0.05$ (LSD).

tillage practice was not significant ($P = 0.07$ in 2009, $P = 0.11$ in 2010).

Net nitrogen mineralization (NNM) was significantly different among production systems (Fig. 5b). The highest NNM occurred in the NO and FG treatments, whereas the lowest NNM was found in TN. The systems NC, TO and TC were not significantly different regarding their NNM values, except at the 2010 spring sampling, when soils under NC presented a NNM value close to that of the NO system.

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

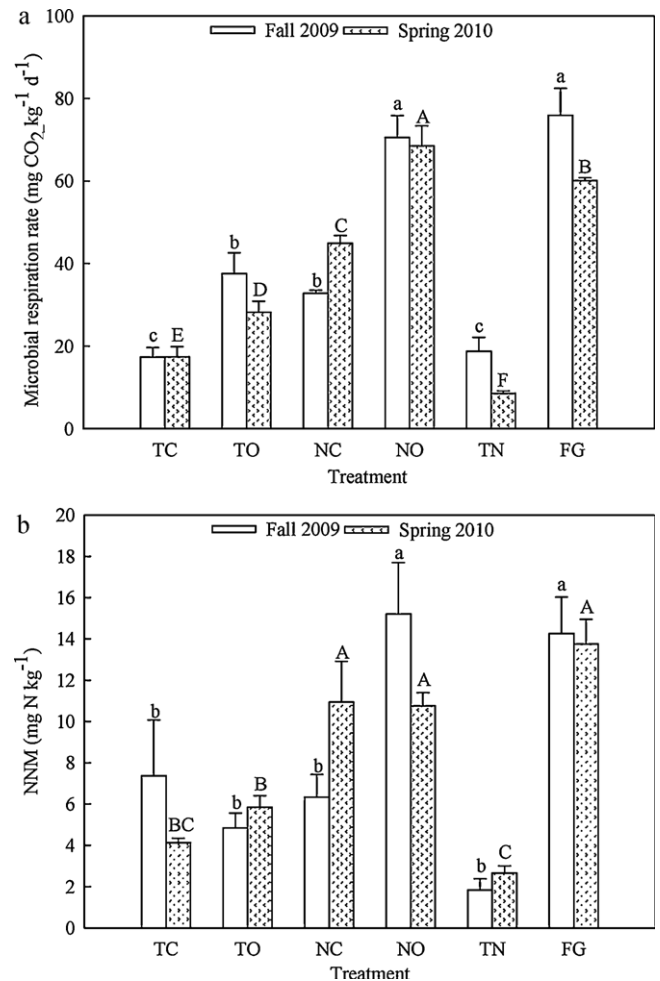


Fig. 5. Soil microbial respiration (a) and net nitrogen mineralization (NNM) (b) under different production systems in Mills River, NC. TC, tillage plus chemical inputs; TO, tillage plus organic inputs; NC, no-till plus chemical inputs; NO, no-till plus organic inputs; TN, tillage but no inputs; FG, tall fescue grass. Bars represent means 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 $1000 \text{ kg C ha}^{-1}$ 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

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.

System ^a	Light fraction $d \leq 1.0 \text{ g cm}^{-3}$	Heavy fraction $1.0 \text{ g cm}^{-3} < d \leq 1.6 \text{ g cm}^{-3}$	Very heavy fraction $d > 1.6 \text{ g cm}^{-3}$
TC	3.39 ± 0.63 a ^b	1.35 ± 0.27 b	95.3 ± 0.68 b
TO	1.53 ± 0.35 b	2.88 ± 0.84 a	95.6 ± 1.03 b
NC	1.09 ± 0.22 b	1.20 ± 0.14 b	97.7 ± 0.33 a
NO	0.80 ± 0.17 b	1.02 ± 0.14 b	98.2 ± 0.22 a
TN	0.77 ± 0.16 b	1.15 ± 0.59 b	98.1 ± 0.54 a
FG	0.84 ± 0.17 b	1.34 ± 0.07 b	97.8 ± 0.19 a

^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).

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 (Beare 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 (Foeroid 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 (Babujia 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 terrestris* the dominant species (Overstreet et al., 2010). *Lumbricus terrestris* 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 (Pulleman 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 2×10^6 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 Shuijin Hu (2007-34381-18625; 2009-35101-05351) and China Scholarship Council: Postgraduate Scholarship Program (2007102999) to Yi Wang. We

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.

References

- Adviento-Borbe, M.A.A., Haddix, M.L., Binder, D.L., Walters, D.T., Dobermann, A., 2007. Soil greenhouse gas fluxes and global warming potential in four high-yielding maize systems. *Glob. Change Biol.* 13, 1972–1988.
- Alef, K., 1995. Soil respiration. In: Alef, K., Nannipieri, P. (Eds.), *Methods in Applied Soil Microbiology and Biochemistry*. Academic Press, San Diego, CA, USA, pp. 214–219.
- Alvarez, R., Diaz, R.A., Barbero, N., Santanatoglia, O.J., Blotta, L., 1995. Soil organic carbon, microbial biomass and CO₂-C production from three tillage systems. *Soil Till. Res.* 33, 17–28.
- Angers, D.A., Eriksen-Hamel, N.S., 2008. Full-inversion tillage and organic carbon distribution in soil profiles: a meta-analysis. *Soil Sci. Soc. Am. J.* 72, 1370–1374.
- Babujia, L.C., Hungria, M., Franchini, J.C., Brookes, P.C., 2010. Microbial biomass and activity at various soil depths in a Brazilian oxisol after two decades of no-tillage and conventional tillage. *Soil Biol. Biochem.* 42, 2174–2181.
- Baisden, W.T., Amundson, R., Cook, A.C., Brenner, D.L., 2002. Turnover and storage of C and N in five density fractions from California annual grassland surface soils. *Global Biogeochem. Cyc.* 16, 64/01–64/16.
- Baker, J.M., Ochsner, T.E., Venterea, R.T., Griffis, T.J., 2007. Tillage and soil carbon sequestration – what do we really know? *Agric. Ecosyst. Environ.* 118, 1–5.
- Balesdent, J., Chenu, C., Balabane, M., 2000. Relationship of soil organic matter dynamics to physical protection and tillage. *Soil Till. Res.* 53, 215–230.
- Beare, M.H., Coleman, D.C., Crossley, D.A., Hendrix, P.F., Odum, E.P., 1995. A Hierarchical Approach to Evaluating the Significance of Soil Biodiversity to Biogeochemical Cycling. *Kluwer Academic Publ.*, pp. 5–22.
- Blanco-Canqui, H., Lal, R., 2004. Mechanisms of carbon sequestration in soil aggregates. *Crit. Rev. Plant Sci.* 23, 481–504.
- Boddey, R.M., Jantalia, C.P., Conceição, P.C., Zanatta, J.A., Bayer, C., Mielniczuk, J., Dieckow, J., Dos Santos, H.P., Denardin, J.E., Aita, C., Giacomini, S.J., Alves, B.J.R., Urquiaga, S., 2010. Carbon accumulation at depth in Ferralsols under zero-till subtropical agriculture. *Glob. Change Biol.* 16, 784–795.
- Bronick, C.J., Lal, R., 2005. Soil structure and management: a review. *Geoderma* 124, 3–22.
- Cabrera, M.L., Beare, M.H., 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. *Soil Sci. Soc. Am. J.* 57, 1007–1012.
- Chen, H.Q., Fan, M.S., Billen, N., Stahr, K., Kuzyakov, Y., 2009. Effect of land use types on decomposition of C-14-labelled maize residue (*Zea mays* L.). *Eur. J. Soil Biol.* 45, 123–130.
- Coleman, D.C., Anderson, R.V., Cole, C.V., Elliott, E.T., Woods, L., Campion, M.K., 1978. Trophic interactions in soils as they affect energy and nutrient dynamics. IV. Flows of metabolic and biomass carbon. *Microb. Ecol.* 4, 373–380.
- Dalal, R.C., 1998. Soil microbial biomass – what do the numbers really mean? *Aust. J. Exp. Agric.* 38, 649–665.
- Don, A., Steinberg, B., Schöning, I., Pritsch, K., Joschko, M., Gleixner, G., Schulze, E.D., 2008. Organic carbon sequestration in earthworm burrows. *Soil Biol. Biochem.* 40, 1803–1812.
- Doran, J.W., 1987. Microbial biomass and mineralizable nitrogen distributions in no-tillage and plowed soils. *Biol. Fert. Soils* 5, 68–75.
- Edwards, C.A., Bohlen, P.J., 1996. *Biology and Ecology of Earthworms*, 3rd ed. Chapman & Hall, London, UK.
- Feng, H., Kurkalova, L.A., Kling, C.L., Gassman, P.W., 2007. Transfers and environmental co-benefits of carbon sequestration in agricultural soils: retiring agricultural land in the Upper Mississippi River Basin. *Clim. Change* 80, 91–107.
- Foeroid, B., Høgh-Jensen, H., 2004. Carbon sequestration potential of organic agriculture in northern Europe – a modelling approach. *Nutr. Cycl. Agroecosyst.* 68, 13–24.
- Fonte, S.J., Six, J., 2010. Earthworms and litter management contributions to ecosystem services in a tropical agroforestry system. *Ecol. Appl.* 20, 1061–1073.
- Forster, J.C., 1995. Soil physical analysis. In: Alef, K., Nannipieri, P. (Eds.), *Methods in Applied Soil Microbiology and Biochemistry*. Academic Press, San Diego, CA, USA, pp. 105–122.
- Gal, A., Vyn, T.J., Micheli, E., Klavdivo, E.J., McFee, W.W., 2007. Soil carbon and nitrogen accumulation with long-term no-till versus moldboard plowing over-estimated with tilled-zone sampling depths. *Soil Till. Res.* 96, 42–51.
- Hai, L., Li, X.G., Li, F.M., Suo, D.R., Guggenberger, G., 2010. Long-term fertilization and manuring effects on physically-separated soil organic matter pools under a wheat-wheat-maize cropping system in an arid region of China. *Soil Biol. Biochem.* 42, 253–259.
- Hart, S.C., Stark, J.M., Davidson, E.A., Firestone, M.K., 1994. Nitrogen mineralization, immobilization, and nitrification. In: Weaver, R.W., Bottomley, S., Bezdicek, P., Smith, D., Tabatabai, S., Wollum, A. (Eds.), *Methods of Soil Analysis. Part 2: Microbiology and Biochemical Properties*. Soil Science Society of America, Inc., Madison, WI, pp. 985–1018.
- Hendrix, P.F., Franzluebbers, A.J., McCracken, D.V., 1998. Management effects on C accumulation and loss in soils of the southern Appalachian Piedmont of Georgia. *Soil Till. Res.* 47, 245–251.
- Hu, S., Coleman, D.C., Carroll, C.R., Hendrix, P.F., Beare, M.H., 1997. Labile soil carbon pools in subtropical forest and agricultural ecosystems as influenced by management practices and vegetation types. *Agric. Ecosyst. Environ.* 65, 69–78.
- Hummel, R.L., Walgenbach, J.F., Barbercheck, M.E., Kennedy, G.G., Hoyt, G.D., Arellano, C., 2002a. Effects of production practices on soil-borne entomopathogens in western North Carolina vegetable systems. *Environ. Entomol.* 31, 84–91.
- Hummel, R.L., Walgenbach, J.F., Hoyt, G.D., Kennedy, G.G., 2002b. Effects of production system on vegetable arthropods and their natural enemies. *Agric. Ecosyst. Environ.* 93, 165–176.
- Jacobs, A., Helfrich, M., Hanisch, S., Quendt, U., Rauber, R., Ludwig, B., 2010. Effect of conventional and minimum tillage on physical and biochemical stabilization of soil organic matter. *Biol. Fert. Soils* 46, 671–680.
- Jacobs, A., Rauber, R., Ludwig, B., 2009. Impact of reduced tillage on carbon and nitrogen storage of two Haplic Luvisols after 40 years. *Soil Till. Res.* 102, 158–164.
- Jagadamma, S., Lal, R., 2010. Distribution of organic carbon in physical fractions of soils as affected by agricultural management. *Biol. Fert. Soil* 46, 543–554.
- Jenkinson, D.S., 1988. Determination of microbial biomass carbon and nitrogen in soil. In: Wilson, J.R. (Ed.), *Advances in Nitrogen Cycling in Agricultural Ecosystems*. CAB International, Wallingford, UK, pp. 368–386.
- Kindler, R., Miltner, A., Thullner, M., Richnow, H.-H., Kästner, M., 2009. Fate of bacterial biomass derived fatty acids in soil and their contribution to soil organic matter. *Org. Geochem.* 40, 29–37.
- Kurkalova, L., Kling, C.L., Zhao, J.H., 2004. Multiple benefits of carbon-friendly agricultural practices: empirical assessment of conservation tillage. *Environ. Manag.* 33, 519–527.
- Kushwaha, C.P., Tripathi, S.K., Singh, K.P., 2001. Soil organic matter and water-stable aggregates under different tillage and residue conditions in a tropical dryland agroecosystem. *Appl. Soil Ecol.* 16, 229–241.
- Lal, R., 2003. Global potential of soil carbon sequestration to mitigate the greenhouse effect. *Crit. Rev. Plant Sci.* 22, 151–184.
- Lal, R., 2004. Soil carbon sequestration impacts on global climate change and food security. *Science* 304, 1623–1627.
- Leifeld, J., Fuhrer, J., 2009. Long-term management effects on soil organic matter in two cold, high-elevation grasslands: clues from fractionation and radiocarbon dating. *Eur. J. Soil Sci.* 60, 230–239.
- Liang, C., Balsler, T.C., 2011. Microbial production of recalcitrant organic matter in global soils: implications for productivity and climate policy. *Nat. Rev. Microbiol.* 9, doi:10.1038/nrmicro2386-c1031.
- Liang, C., Cheng, G., Wixon, D.L., Balsler, T.C., 2010. An absorbing Markov Chain approach to understanding the microbial role in soil carbon stabilization. *Biogeochemistry* (September 17), doi:10.1007/s10533-10010-19525-10533.
- Lichtfouse, E., Navarrete, M., Debaeke, P., Souchère, V., Alberola, C., Ménassieu, J., 2009. Agronomy for sustainable agriculture: a review. In: Lichtfouse, E., Navarrete, M., Debaeke, P., Souchère, V., Alberola, C. (Eds.), *Sustainable Agriculture*. Springer, New York, NY, pp. 1–7.
- Lubowski, R.N., Plantinga, A.J., Stavins, R.N., 2006. Land-use change and carbon sinks: econometric estimation of the carbon sequestration supply function. *J. Environ. Econ. Manag.* 51, 135–152.
- Lundquist, E.J., Scow, K.M., Jackson, L.E., Uesugi, S.L., Johnson, C.R., 1999. Rapid response of soil microbial communities from conventional, low input, and organic farming systems to a wet/dry cycle. *Soil Biol. Biochem.* 31, 1661–1675.
- Luo, Z., Wang, E., Sun, O.J., 2010. Can no-tillage stimulate carbon sequestration in agricultural soils? A meta-analysis of paired experiments. *Agric. Ecosyst. Environ.* 139, 224–231.
- Nordhaus, W.D., Yang, Z.L., 1996. A regional dynamic general-equilibrium model of alternative climate-change strategies. *Am. Econ. Rev.* 86, 741–765.
- Overstreet, L.F., Hoyt, G.D., Imbriani, J., 2010. Comparing nematode and earthworm communities under combinations of conventional and conservation vegetable production practices. *Soil Till. Res.* 110, 42–50.
- Pascault, N., Nicolardot, B., Bastian, F., Thiebaud, P., Ranjard, L., Maron, P.A., 2010. In situ dynamics and spatial heterogeneity of soil bacterial communities under different crop residue management. *Microb. Ecol.* 60, 291–303.
- Paustian, K., Six, J., Elliott, E.T., Hunt, H.W., 2000. Management options for reducing CO₂ emissions from agricultural soils. *Biogeochemistry* 48, 147–163.
- Powlson, D.S., Brookes, P.C., Christensen, B.T., 1987. Measurement of soil microbial biomass provides an early indication of changes in total soil organic-matter due to straw incorporation. *Soil Biol. Biochem.* 19, 159–164.
- Pulleman, M.M., Six, J., Uyl, A., Marinissen, J.C.Y., Jongmans, A.G., 2005. Earthworms and management affect organic matter incorporation and microaggregate formation in agricultural soils. *Appl. Soil Ecol.* 29, 1–15.
- Richter, D.D., Markewitz, D., Trumbore, S.E., Wells, C.G., 1999. Rapid accumulation and turnover of soil carbon in a re-establishing forest. *Nature* 400, 56–58.
- Rillig, M.C., Mummey, D.L., 2006. Mycorrhizas and soil structure. *New Phytol.* 171, 41–53.
- Ross, D.J., 1992. Influence of sieve mesh size on estimates of microbial carbon and nitrogen by fumigation extraction procedures in soils under pasture. *Soil Biol. Biochem.* 24, 343–350.
- Schlesinger, W.H., Andrews, J.A., 2000. Soil respiration and the global carbon cycle. *Biogeochemistry* 48, 7–20.
- Simpson, A.J., Simpson, M.J., Smith, E., Kelleher, B.P., 2007. Microbially derived inputs to soil organic matter: are current estimates too low? *Environ. Sci. Technol.* 41, 8070–8076.
- Six, J., Callewaert, P., Lenders, S., De Gryze, S., Morris, S.J., Gregorich, E.G., Paul, E.A., Paustian, K., 2002. Measuring and understanding carbon storage in afforested soils by physical fractionation. *Soil Sci. Soc. Am. J.* 66, 1981–1987.

- Smith, J.L., Paul, E.A., 1990. The significance of soil microbial biomass estimations. In: Bollag, J.M., Stotzky, G. (Eds.), *Soil Biochemistry*, vol. 6. Marcel Dekker, Inc., New York, NY, USA, pp. 357–396.
- Sparling, G.P., West, A.W., 1988. A direct extraction method to estimate soil microbial C: calibration in situ using microbial respiration and ^{14}C labeled cells. *Soil Biol. Biochem.* 20, 337–343.
- Sperow, M., Eve, M., Paustian, K., 2003. Potential soil C sequestration on US agricultural soils. *Clim. Change* 57, 319–339.
- Tisdall, J.M., 1994. Possible role of soil-microorganisms in aggregation in soils. *Plant Soil* 159, 115–121.
- Triplett Jr., G.B., Dick, W.A., 2008. No-tillage crop production: a revolution in agriculture! *Agron J.* 100, S153–S165.
- Tu, C., Louws, F.J., Creamer, N.G., Mueller, J.P., Brownie, C., Fager, K., Bell, M., Hu, S.J., 2006. Responses of soil microbial biomass and N availability to transition strategies from conventional to organic farming systems. *Agric. Ecosyst. Environ.* 113, 206–215.
- Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* 19, 703–707.
- West, T.O., Post, W.M., 2002. Soil organic carbon sequestration rates by tillage and crop rotation: a global data analysis. *Soil Sci. Soc. Am. J.* 66, 1930–1946.
- Wright, A.L., Dou, F.G., Hons, F.M., 2007. Crop species and tillage effects on carbon sequestration in subsurface soil. *Soil Sci.* 172, 124–131.