Short-Term Fates of Carbon-13-Depleted Cowpea Shoots in No-Till and Standard Tillage Soils

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The fate of C from ^{13}C-depleted cowpea [*Vigna unguiculata* (L.) Walp. ssp. *unguiculata*] shoots was examined to assess factors affecting residue C dynamics in soil cores taken from plots under no-till (NT) vs. standard tillage (ST). Intact soil cores were incubated in the greenhouse after removing them from field plots, and then ^{13}C-depleted residue was incorporated at 0- to 15-cm depth in ST cores, or laid on the surface of NT cores. Cores were taken from both the planted and unplanted zones in NT, and from an intermediate position in the ST system, where residues had always been evenly incorporated. Cores without soil were used as a control. After 31 d, more C from the residue was retained as microbial biomass C (MBC) and more nematodes were found in the 0- to 15-cm layer in ST than in NT cores. For NT treatments, after 31 d, higher C from residue was observed in microbial biomass in the 0- to 5-cm layer in cores from planted than in those from the unplanted zones. Despite the low assimilation of newly added residue C in NT soil, similar amounts of CO_2 were emitted from ST and NT, probably due to high activity of microbes in the phyllosphere of the residue in NT. In the short term, ST more effectively assimilated shoot residue into soil microbial and nematode C than NT. These results were unexpected given the typical increase in soil C that is observed in the surface layer of NT systems, and suggest that lack of disturbance, changes in microenvironment, or the composition of soil biota in NT must enhance C storage from shoot residue during longer time periods than measured here.

Abbreviations: +Res, residue added treatment; –Res, no residue added treatment; Ctr, cores taken from bed center; Int = cores taken from an intermediate position between the plant row and the bed center; MBC, microbial biomass carbon; NBC, nematode biomass carbon; NT, no-till; Row, cores taken from plant row; ST, standard tillage.

The placement of plant residue in no-till (NT) and standard tillage (ST) systems affects decomposition rates. Residues mixed with soil in ST can decompose faster than those on the soil surface in NT (Beare et al., 1992; Burgess et al., 2002). Decomposition of residue in the two types of tillage systems may be complicated, however, by differences in decomposers, their activity, and the spatial heterogeneity of litter deposition. Consequently, rates of CO_2 production and the fate of C from decomposing residues may show temporal patterns that reflect differing decomposition processes in NT vs. ST.

Specific groups of soil biota may be associated with the increase in soil C, particularly in the surface soil, where C accumulation occurs most rapidly in NT systems (Six et al., 2006). Along with soil fungi that extend hyphae into the decomposing litter layer (Holland and Coleman, 1987; Wardle, 1995), microbes on plant residue surfaces, the phyllosphere, can be important for the release of C from residue in NT (Osono, 2003; Potthoff et al., 2005). Decomposition of litter by aboveground microbes may thus affect C sequestration in soil. Another factor is that the microfauna, e.g., protozoa and some nematodes, are sensitive to disturbance (Ferris et al., 2004; Berkelmans et al., 2003) and may be more important in NT soils, thus increasing the potential sequestration of soil C.

Residue decomposition in NT systems may be affected by increased spatial variability in biological activity vs. that in ST soils. In NT systems, soil organic matter becomes stratified in the surface layer and decreases with depth (Dick and Durkalski, 1998; Kay and VandenBygaart, 2002; Jarecki and Lal, 2005). Heterogeneity may also occur horizontally in NT if crops are repeatedly planted in the same zone. Soil zones in plant rows may be more active biologically than those in the bed center due to the accumulation of aboveground litter and rhizodeposition of exudates, exfoliates (sloughed cells and root hairs), and debris (Ingham et al., 1985; Cheng, 1996; Luo et al., 1996; Kuzyakov, 2002). Microbial biomass and its grazers, nematodes and protozoa, would probably be higher in the zones with higher deposition of root and shoot litter (Ingham et al., 1985; Cheng, 1996; Ferris et al., 2004). High biological activity in these undisturbed planted zones in NT may increase the rate of decomposition of plant residue and alter C fates in soil compared with unplanted zones.

Residues labeled with stable isotopes have provided insights into the dynamics of litter decomposition and C fates, e.g., the strong contribution of root C to soil C (Denef et al., 2001; Puget and Drinkwater, 2001). Of particular interest is the partitioning of C from residue to microbes and nematodes, for which recent

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methods have improved the resolution of ^{13}C recovery (Potthoff et al., 2003). In this study, we conducted a greenhouse incubation experiment to test the fates of ^{13}C-depleted aboveground residue of cowpea in NT vs. ST soils from a legume–vegetable rotation in California's Mediterranean-type climate (Minoshima et al., 2007). The objectives were to: (i) monitor C dynamics after adding cowpea shoots under simulated ST and NT incubations; and (ii) better understand the factors that affect the assimilation of C from decomposing residues in the different tillage systems.

MATERIALS AND METHODS

Experimental Design and Treatments

Obtaining Intact Cores from the Field

The cowpea field plot was located at the Long Term Research in Agricultural Systems (LTRAS) site at the University of California at Davis. The soil is classified as Rincon silty clay loam (fine, montmorillonitic, thermic Mollic Haploxeralfs). Soil biological, chemical, and physical properties are described in detail in Minoshima et al. (2007). After many decades of oat (*Avena sativa* L.) hay crops, the legume–vegetable rotation with NT and ST treatments was established in the spring of 2003. Briefly, NT consisted of permanent beds that were first prepared in May 2003, with no subsequent tillage operations except for the clearing of furrows in midsummer. All plant residues were flail-mowed and distributed on the bed tops. For ST, plant residues were incorporated to 20-cm depth and beds were shaped between each crop with the Sundance system (Sundance Farms, Coolidge, AZ), which consists of disks and lister bottoms, and afterward, a ring roller was used to smooth the beds before planting. Total soil C was 11 and 10 g C kg^{-1} soil at 0- to 5- and 5- to 15-cm depths, respectively, in samples taken across the entire bed top in both ST and NT.

Cowpea ('California Blackeye CB 46') was planted on 12 July 2004 in two rows, 80 cm apart, on the surface of the shaped 120-cm beds. At 78 d after planting, four locations (100 by 120 cm) were chosen where aboveground cowpea biomass was of similar height in both ST and NT treatments. Cowpea shoots were clipped at the soil surface. Intact cores (diameter = 14.5 cm, height = 30 cm) were obtained by driving polyvinylchloride (PVC) cylinders, sharpened at one end, into the soil. Five intact cores were taken from plant rows (20 cm from the shoulder's edge of the bed tops) and a second set of five cores from the bed center (60 cm from the shoulder's edge) in each of the four locations of the NT plots, representing zones of anticipated higher and lower root activity, respectively. In each of the four ST plots, five cores were taken from an intermediate position at 40 cm from the bed shoulder. The single position was used because tillage distributed residues more evenly across the bed top in ST than in NT plots. Thus, 40 intact cores from NT and 20 intact cores from ST were collected. Four cores from each of the three zones (12 in total) were immediately destructively harvested in the field, representing the first sampling time. The 0- to 5- and 5- to 15-cm layers were separated and were stored at 4°C until processing. The remaining 48 cores (32 from NT and 16 from ST plots) were transferred to a greenhouse at the University of California at Davis. The tops of the cylinders were covered with cheesecloth to reduce moisture loss.

Preparation of Carbon-13-Depleted Cowpea Litter

Cowpea seeds (same cultivar as used in the field) were planted in 4-L pots filled with U.C. Soil Mix (composed of equal volumes of composted redwood, washed sand and peat moss, and white pumice) on 12 Aug. 2004. Four seeds were planted in each of 40 pots, which were transferred to a greenhouse at the University of California at Santa Cruz after 5 d, where ^{13}C-depleted CO_2 air was continuously supplied. The atmospheric CO_2 concentration was maintained at 31 mmol m^{-3} in the greenhouse, provided from tanks of CO_2 with a $\delta^{13}C$ signature of −30.7‰. The $\delta^{13}C$ is expressed relative to Pee Dee Belemnite.

Plants harvested on 27 Sept. 2004 from three replicate pots had a $\delta^{13}C$ signature of −39.03 ± 0.50‰. This was based on separate measurements of leaves and stems at plant heights of 0 to 15, 15 to 30, 30 to 45, and >45 cm above the soil surface.

Carbon-13-Depleted Cowpea Litter Decomposition in Intact Cores from the Field

Cowpea plants were stored at room temperature in the dark for 4 d, then the shoots were clipped just above the soil surface, chopped into 2-cm pieces in the lab, stored in cooler boxes, and transferred to the greenhouse. The ^{13}C-depleted cowpea residue was added to the intact cores in the greenhouse on 1 Oct. 2004. The added shoot residue (100 g fresh weight, 9 g dry weight per core) represents about 20% more than the amount of residue that would have been added by the plants at our field site but is within the typical range of incorporated crop residues. The percentage of C and N in residue was 37 and 4%, respectively, (C/N = 9.1) and thus 3.3 g of C and 0.37 g of N were added to each cylinder as cowpea residue.

For NT, the ^{13}C-depleted cowpea residue was placed on the soil surface. For ST, the 0- to 15-cm layer of soil was removed, mixed with residue, and returned to the cylinders. There were two zones in NT, plant rows and the bed center (Row and Ctr). The single zone in ST was designated intermediate (Int). Residue was added to half of the cores from each zone; thus there were paired cores with and without residue (+Res or −Res). Each of the treatments had four replicates, and thus 24 cores were used for each of the second and third soil sampling times (Table 1).

The intact cores were incubated in a greenhouse at 25°C for 10 d (second sampling) and 31 d (third sampling) after residue was added. During the incubation, 100 g of water was evenly distributed in each core, using a syringe, at six and 19 d after adding the residue. In addition, a treatment of 100 g of fresh cowpea residue, without soil, was established in a similar set of three PVC cylinders (diameter = 14.5 cm, height = 7 cm) sealed at the bottom with plastic wrap. Only CO_2 emission was measured in this treatment.

Table 1. The location of core removal from the no-till (NT) and standard tillage (ST) field plots and their treatment codes.

Treatment code	Tillage regime	Residue location	Original core position
NT+Res/Row	NT	surface	plant row
NT−Res/Row	NT	none	plant row
NT+Res/Ctr	NT	surface	bed center
NT−Res/Ctr	NT	none	bed center
ST+Res/Int	ST	incorporated	intermediate
ST−Res/Int	ST	none	intermediate

Sampling for Carbon Dioxide Emission and Soil Properties

Carbon Dioxide Measurements

Carbon dioxide emission data were obtained at six times using a closed-chamber method (Rolston, 1986): 1 d before and 2, 4, 7, 11, and 18 d after adding residue. Sampling of CO_2 was terminated after 18 d due to reduction of activity. Headspace CO_2 was sampled immediately after caps were placed on the top of cylinders, and again 24 min later, and then 3 mL of the gas sample was taken and injected into 12-mL Exetainers (Labco Ltd., High Wycombe, UK) containing12 mL of He. The total

amount and the ^{13}C signature of CO_2 were measured by PDZ Europa TGII trace gas analyzer interfaced to a PDZ Europa 20–20 stable isotope ratio mass spectrometer (PDZ Europa Ltd., Crewe, UK) at the Stable Isotope Facility at the University of California at Davis.

To determine $\delta^{13}C$ values of CO_2 in each treatment, the Keeling plot method was used (Keeling, 1961). By plotting the $\delta^{13}C$ of sampled CO_2 on the *y* axis and 1/41 μmol m^{-3}–CO_2 on the *x* axis, a linear relationship is established, with the intercept of this plot being the isotopic ratio of the CO_2 produced in each treatment. For the sampling at 18 d, these calculations were not made because the relationship was not significant for the –Res treatments.

Soil Sampling

Soil was sampled 3 d before adding residue, when intact cores were taken from the field, and 10 and 31 d after adding residue. Residue was removed with forceps from all treatments before analysis. Soil from the 0- to 5- and 5- to 15-cm layers was stored at 4°C before processing within 3 d for microbial biomass C (MBC) and 7 d for nematodes. Soil moisture was determined gravimetrically after drying at 105°C for at least 48 h.

Duplicate subsamples from each soil sample (25 g moist soil) were analyzed for MBC by the fumigation extraction method (Vance et al., 1987). Soluble C in the K_2SO_4 extracts and its ^{13}C signature were measured by a total organic C analyzer (OI Analytical, College Station, TX) interfaced to the PDZ Europa 20–20 stable isotope ratio mass spectrometer.

The $\delta^{13}C$ of microbial biomass was calculated as the difference between extractable C in the fumigated and unfumigated soils as follows:

$$\delta^{13}C_f C_f = \delta^{13}C_n C_n + \delta^{13}C_b C_b$$

where $\delta^{13}C_f$ is the $\delta^{13}C$ of fumigated extracts, $\delta^{13}C_n$ is the $\delta^{13}C$ of unfumigated extracts, $\delta^{13}C_b$ is the $\delta^{13}C$ of MBC, C_f is the quantity of C in fumigated extracts, C_n is the quantity of C in unfumigated extracts, and C_b is the quantity of C in the microbial biomass ($C_f - C_n$).

Nematodes were extracted from 300 g of soil using a combination of decanting and sieving and Baermann funnel methods (Barker, 1985) and counted under a dissecting microscope. At the 31-d sampling, nematodes were identified. Four replicates per treatment at each depth were combined into one sample and at least 200 nematodes were identified to the genus or family level at higher magnification and classified by trophic habit (Yeates et al., 1993). Since trophic groups other than bacterial and fungal feeders were almost absent at this field site (Minoshima et al., 2007), only microbial-feeding nematodes were used to evaluate the assimilation of C from cowpea by nematodes. Nematode biomass C (NBC) and the ^{13}C signature of nematodes at 31 d were measured as follows. Nematode samples were transferred to centrifuge tubes in 14 mL of double deionized water. Nematodes were uniformly suspended, 7 mL of the suspension was centrifuged at 2000 rpm for 3 min, and the supernatant was removed. The suspension and centrifugation steps were repeated three times to eliminate bacteria. The final pellet was dried at 60°C for 12 h. Total C and ^{13}C were determined as above.

The proportion of cowpea-derived C (*f*) in the total CO_2, MBC, and NBC was calculated as

$$f = (\delta_t - \delta_s)/(\delta_c - \delta_s)$$

where δ_t is the ^{13}C of CO_2, MBC, or NBC in a residue-added treatment, δ_s is the ^{13}C as CO_2, MBC, or NBC in the paired treatments with no added residue, and δ_c is the ^{13}C of the cowpea (–39.03‰).

The quantity of C derived from the cowpea (C_c) was determined as

$$C_c = Cf$$

where *C* is the total amount of CO_2, MBC, or NBC in residue-added treatments.

The amount of CO_2 emitted from soil in residue-added treatments was calculated as

$$Q_s = Q_t - Q_c$$

where Q_s is the CO_2 derived from soil, Q_t is the total amount of CO_2 emitted, and Q_c is the CO_2 derived from cowpea residue as described above.

This set of calculations was done because the soil contribution to CO_2 emissions in the –Res treatments might be different than that in the +Res treatments, e.g., due to priming of decomposition rates or to differences in moisture content (see below).

Statistical Analysis

The data were subjected to analysis of variance using the GLM procedures of SAS, Version 9.1 (SAS Institute, Cary, NC). Means and interactions were separated by Tukey's multiple-range test and significant effects were expressed at $P < 0.05$.

RESULTS

Carbon Dioxide Emissions

Rates of CO_2 emission were similar from all residue-containing cylinders, including the control with no soil, across sampling times regardless of the presence of soil (Fig. 1A). Thus, CO_2 emissions from residue-added treatments were little affected by tillage treatment or soil processes.

Adding residue increased CO_2 emissions compared with no-residue treatments only for the first 11 d (Fig. 1A). More specifically, this was true for ST and NT plant row treatments on Days 2, 4, and 7, and for the NT unplanted zone on Days 2, 4, 7, and 11 (statistical comparisons not shown). Without residue added, there were few differences between treatments, except at the onset (NT–Res/Row > ST–Res/Int) and after watering on Day 7 (both NT treatments > ST–Res/Int).

The $\delta^{13}C$ values of CO_2 among all treatments before adding residue was –21.5 ± 0.20‰. Mean $\delta^{13}C$ values of CO_2 across residue-added treatments including the control were –35.3 ± 0.8, –34.3 ± 1.0, –33.0 ± 0.3, –27.7 ± 1.1, and –25.0 ± 0.6‰ at 2, 4, 7, 11, and 18 d, respectively. Across the three treatments without residue, means were –23.8 ± 1.2, –24.5 ± 1.6, –22.9 ± 1.2, –24.3 ± 0.9, and –21.5 ± 0.8‰, respectively. In all treatments, there was a significant linear relationship between $\delta^{13}C$ of CO_2 and 1/41 μmol m^{-3}–CO_2 ($P < 0.05$) on each sampling date except for the –Res treatments at 18 d.

Based on the $\delta^{13}C$ values and the calculation of residue-derived CO_2 emissions (Fig. 1B), the trends in the rate of residue-derived CO_2 emission were similar to the overall rates of CO_2 emission from residue-added treatments except for ST+Res/Int on Day 4 (compare patterns in Fig. 1A and 1B). The CO_2 emission dramatically decreased between Days 2 and 4 in ST+Res/Int, and was significantly lower in ST+Res/Int than in NT+Res/Row and the control at Day 4 and in NT+Res/Ctr at Day 7 (Fig. 1B). Note that an instrument problem occurred so that no $\delta^{13}C$ signature of CO_2 was obtained for the NT+Res/Ctr on Day 4, and the no-soil treatment on Day 2.

Microbial Biomass Carbon

At the 0- to 5-cm depth, total MBC was similar among treatments except at the end of the experiment, 31 d after adding

residue, when it was highest in the NT Row treatment and lowest in ST–Res/Int (Fig. 2A). At 5 to 15 cm, however, there were no differences between treatments on Day 31 (Fig. 2B). In ST+Res, MBC at 5 to 15 cm was much higher on Day 10, followed by a steep decline 21 d later.

Higher assimilation of C from cowpea residue into MBC was observed at the 0- to 15-cm depth in ST than in NT throughout the duration of the study (Fig. 3). In ST, C from residue was quickly assimilated into MBC but decreased by 31 d ($P < 0.05$). In contrast, in both NT treatments, higher amounts of the residue C were assimilated into MBC at 31 d than at 10 d

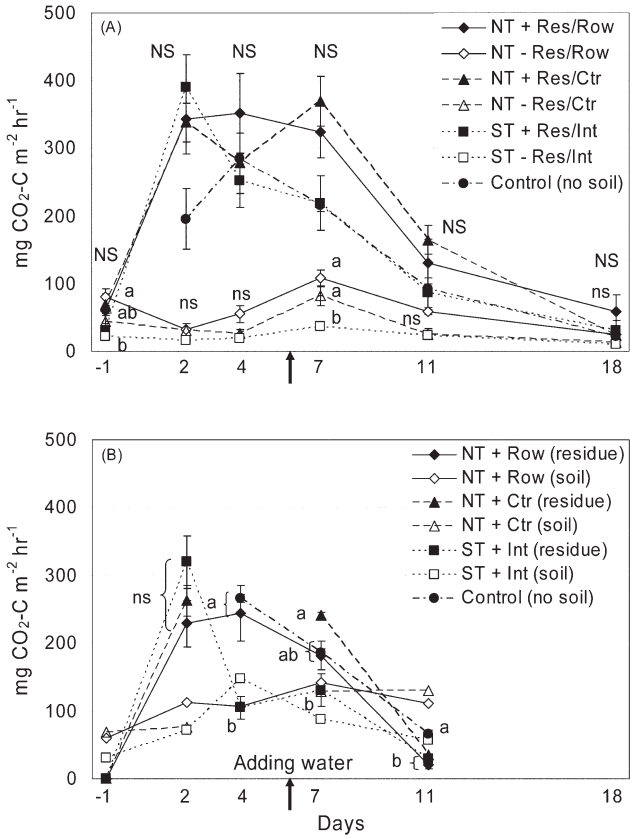

Fig. 1. (A) Total CO_2 emissions for about 3 wk after adding cowpea residue, and (B) residue-derived and soil-derived CO_2 emissions from residue-added cylinders; NT = no-till, ST = standard tillage, +Res = residue added, –Res = no residue added, Row = cores taken from plant row, Ctr = cores taken from the bed center, Int = cores taken from intermediate position between the plant row and the bed center, C = control (residue without soil). In (A), on each date, NS indicates no statistical differences for residue-added treatments, including control, while ns refers to comparison of treatments with no added residue. (Note that for the latter, emissions were significantly higher in NT –Res/Row than in ST –Res/Int on Day 1, and in both NT treatments than ST on Day 7 after watering.) In (B), statistical differences were compared with residue-added CO_2 emissions including the control, and are expressed by a or b at each date separately; ns indicates no statistical differences among treatments. There are no data at Day 18 because there was no significant relationship between $\delta^{13}C$ of CO_2 and 1/41 μmol m^{-3}–CO_2. The error bar shows the standard error. Estimates of soil-derived CO_2 were calculated using the equation $Q_s = Q_t - Q_c$, where where Q_s = CO_2 derived from soil, Q_t = total amount of CO_2 emitted, and Q_c = CO_2 derived from residue. Thus, statistical differences are not shown.

in the 0- to 5-cm layer ($P < 0.05$). Less residue C was recovered as MBC in NT soil at 0 to 5 cm from bed centers than from the plant row on Day 31. Almost no residue C was assimilated into MBC in the 5- to 15-cm layer in NT soils.

A greater proportion of MBC was derived from residue in ST than in NT, 20 to 30% vs. 1 to 10%, respectively, at the 0- to 15-cm depth on both dates (data not shown but see Fig. 2 and 3). In both NT treatments, both total MBC and residue-derived C, pooled across sampling times, were higher in the 0- to 5- than in the 5- to 15-cm layer ($P < 0.05$). In contrast, MBC derived from plant residue was more evenly distributed with depth in ST treatments except when it markedly increased in the 5- to 15-cm layer between 10 and 31 d after adding residue.

Horizontal spatial heterogeneity also occurred between the two zones in the 0- to 5-cm layer in NT, but only at 31 d after adding residue (Fig. 3). The MBC derived from residue was higher in the planted zone (NT+Res/Row) than in the unplanted zone in the center of the bed (NT+Res/Ctr).

Water content in the soil samples differed between treatments (data not shown). On Day 3, it was 0.24 kg H_2O kg^{-1} soil in the NT soils, and lower (0.21 kg H_2O kg^{-1} soil) in the ST soil. For the subsequent two sampling times, the highest water content was in the NT treatments with residue (0.24 kg H_2O kg^{-1} soil). In contrast, water content in NT without residue treatments and both ST treatments regardless of whether they were with or without residue decreased to 0.20 and 0.13 kg H_2O kg^{-1} soil at 10 and 31 d, respectively.

Nematodes

Like MBC at 31 d after residue incorporation, C from residue was clearly assimilated into nematode biomass in both soil layers in ST, but very little was found as NBC in the NT soil (Fig. 4). In fact, nematodes only contained residue-derived C in NT soils from the planted zone at 0 to 5 cm. Any residue-derived C assimilated by nematodes in the 0- to 5-cm layer from the unplanted zone or in the 5- to 15-cm depth in either location in NT was below detection levels. This does not appear as significant in the mean comparison tests due to the high SE in NT+Res/Row.

Total NBC at 0 to 5 cm was not significantly different among the treatments at 31 d after residue incorporation (Fig. 5). The high variation, as indicated by the SE, obscured a trend toward higher values in ST with residue and in the planted NT zone. At 5 to 15 cm, NBC was highest in the ST+Res/Int treatment, and lowest in the unplanted NT zone.

At 31 d, the most abundant nematode taxa were bacterial feeders of the genus *Acrobeloides* and in the family *Rhabditidae*, and the fungal feeder *Aphelenchus*. Adding residue increased the numbers of bacterial-feeding nematodes compared with the –Res treatment in both the 0- to 5- and 5- to 15-cm layers in ST (29070 ± 11650 vs. 3970 ± 670 and 20010 ± 3060 vs. 5050 ± 1600 kg^{-1} soil [mean ± SE], respectively). For NT, residue addition had no effect on bacterial-feeding nematodes from the planted row or unplanted bed center cores at either 0- to 5- or 5- to 15-cm depths, yet the high coefficient of variation (23% on average for the eight NT treatments) masked a trend of higher numbers in the treatments with added residue. Fungal-feeding nematodes were similar in both layers across all treatments and depths (3570 ± 280 nematodes kg^{-1} soil [mean ± SE]). In the residue-added treatments, bacterial feeders were approximately

four times higher, on average, than fungal feeders (13410 ± 4080 vs. 3500 ± 250 nematodes kg^{-1} soil [mean ± SE]).

DISCUSSION

Overall, this month-long decomposition study showed that ST soil retained more shoot residue C in the microbial biomass and nematodes than NT soils, yet CO_2 emissions were similar among all residue-added treatments. Soil–residue contact appears to enhance the assimilation of residue C by soil organisms and the C recovery in active C pools. Phyllosphere organisms may play a role in CO_2 release in NT soils, since CO_2 emissions were similar with and without soil contact. This may be one factor limiting the short-term C assimilation by soil organisms in NT. Another factor may be spatial heterogeneity within NT fields. The planted zone retained more residue-derived C in the microbial biomass and nematodes than the unplanted zone, yet the unplanted zone occupies >75% of the NT field area, suggesting low C recovery from shoot residue in the field as a whole during this initial phase of decomposition. The higher C assimilation by biota in ST was unexpected, given the typical accumulation of soil C in the surface layer of NT systems (Dick and Durkalski, 1998; Kay and VandenBygaart, 2002; Jarecki and Lal, 2005). Longer term C dynamics were beyond the scope of this study, but these results indicate that there must be factors in NT that subsequently override these short-term dynamics and lead to higher retention of residue C in NT with time. Likely possibilities are infrequent disturbance, altered microenvironments, and changes in microbial communities (Jackson et al., 2003).

Residue incorporation in ST caused greater microbial assimilation of cowpea shoot C compared with NT. When shoot residue of low C/N ratio (<15–20) is incorporated into California soils under intensive management, it is rapidly utilized by soil microbes, and the MBC decreases to preincorporation levels within about a month (Lundquist et al., 1999b; Jackson, 2000). This is consistent with the current study, even though samples were only taken twice after residue incorporation. Even though most plants allocate more C to shoots than roots, root-derived C may be preferentially retained in the soil because of the continuous deposition of root exudates and fine roots, protection of this C within aggregates, and greater recalcitrance of root than shoot litter (Gale and Cambardella, 2000; Puget and Drinkwater, 2001). A greater proportion of root-derived C may be stabilized within aggregates that physically protect the C from microbial decomposition compared with shoot material, which requires contact, incorporation, or fungal transfer to combine with soil (Gale and Cambardella, 2000). This study supports the idea that shoot C is not effectively retained in soil when laid on the soil surface.

Spatial hetereogeneity in residue C recovery in the different zones of the NT system may have been largely due to the microbial activity conferred from decomposing roots left from the prior cowpea crop in the field, especially since the treatments were in place for only 1 yr and there was probably not time for substantial changes in C storage and activity in the planted row compared with the unplanted zone. Due to time constraints, we were not able to measure root biomass. If C from older root and shoot litter had been most important, MBC at the onset of the experiment (–3 d) and respiration (–1 d) might have been higher in the NT planting row cores before adding residue, compared with the cores from the unplanted zone. Subsequent differences between the two NT treatments were most pronounced at 31 d; at 0- to 5-cm depth, the planting zone had higher MBC, and no NBC derived from residue was detectable in the unplanted zone. The surface layer in

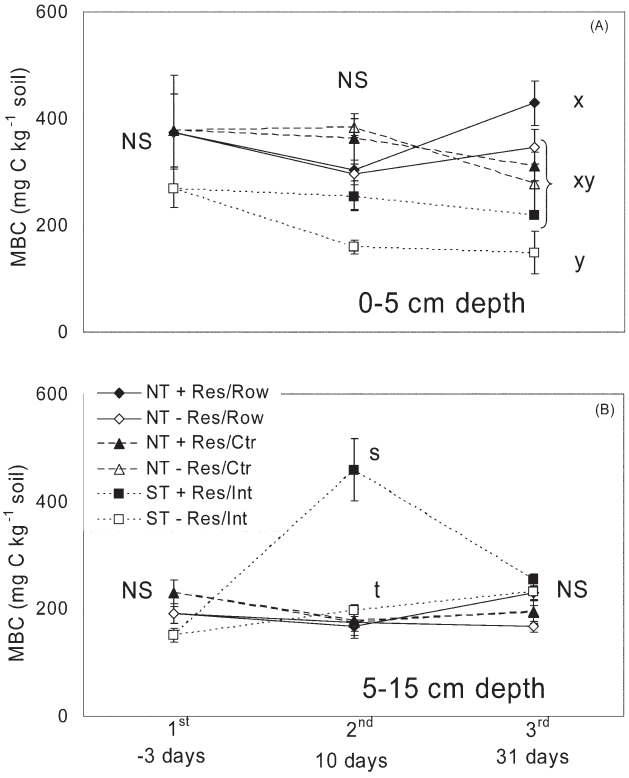

Fig. 2. Microbial biomass C (MBC) at three sampling times at (A) 0- to 5-cm depth and (B) 5- to 15-cm depth; NT = no-till, ST = standard tillage, +Res = residue added, –Res = no residue added, Row = cores taken from plant row, Ctr = cores taken from the bed center, Int = cores taken from an intermediate position between the plant row and the bed center. Statistical differences were expressed with *s* and *t* at 10 d, and *x* and *y* at 31 d; NS means no statistical difference among treatments at a sampling time. The error bars show the standard error.

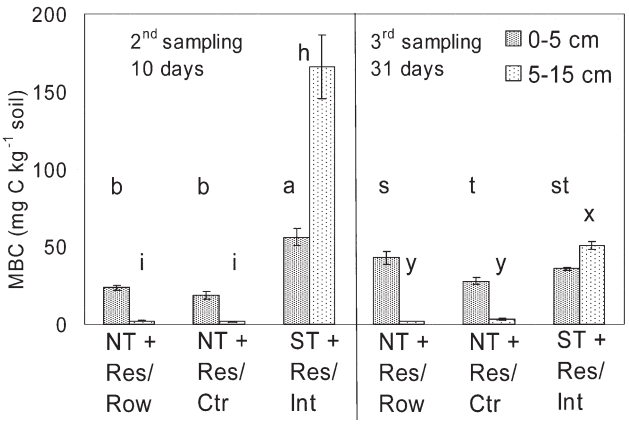

Fig. 3. Microbial biomass C (MBC) derived from cowpea residue at 10 and 31 d; NT = no-till, ST = standard tillage, +Res = residue added, Row = cores taken from plant row, Ctr = cores taken from the bed center, Int = cores taken from an intermediate position between the plant row and the bed center. Statistical differences are expressed with a or b and h or i at 0- to 5- and 5- to 15-cm depths, respectively, at 10 d, and with *s* or *t* and *x* or *y*, respectively, at 31 d. The error bars show the standard error.

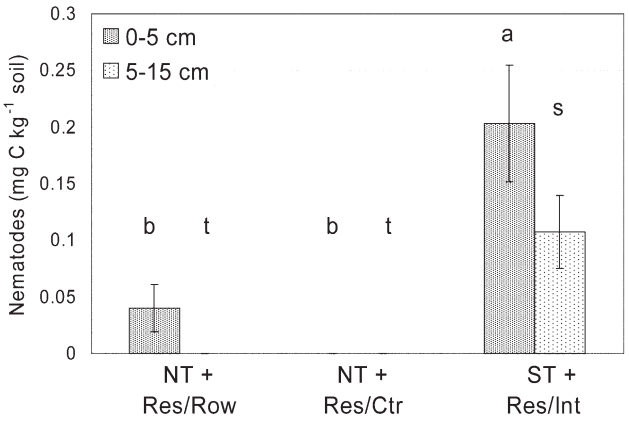

Fig. 4. Amount of C derived from cowpea residue in nematode biomass C at 31 d; NT = no-till, ST = standard tillage, +Res = residue added, Row = cores taken from plant row, Ctr = cores taken from the bed center, Int = cores taken from an intermediate position between the plant row and the bed center. Statistical differences are expressed with *a* or *b* and *s* or *t* at 0- to 5- and 5- to 15-cm depths, respectively. The error bars show the standard error.

NT can be well occupied with roots (Coleman et al., 2002; Qin et al., 2005), and thus decomposing root and rhizodeposits may have supplied the C that stimulated these processes (O'Leary and Wilkinson, 1988; Steer and Harris, 2000).

The assimilation of C into microbial biomass depends on community structure as well as environmental factors such as moisture (Six et al., 2006; Feng et al., 2003). In the accompanying field experiment, soil at 0 to 5 cm in the NT treatment had higher MBC and fungal biomass, as indicated by ergosterol and phospholipid fatty acid fungal biomarkers, than the ST treatment (Minoshima et al., 2007). Fungi may retain C in their biomass longer than bacteria due to their higher microbial growth efficiency, i.e., the amount of new biomass C produced per unit substrate C metabolized (Sakamoto and Oba, 1994; Blagodatskaya and Anderson, 1998). Greater abundance of fungi in NT may thus partially explain the greater stability in MBC in the 0- to 5-cm layer after 31 d compared with ST.

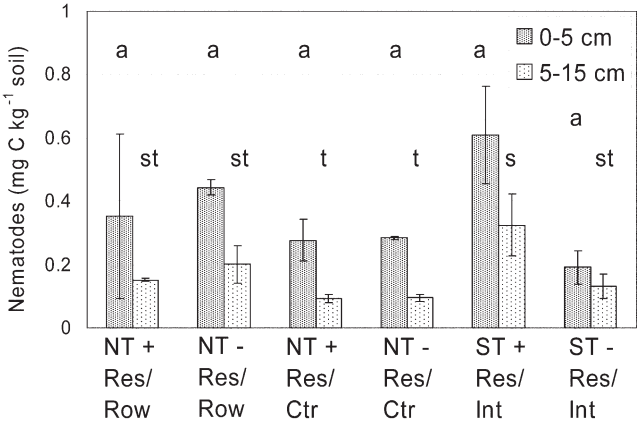

Fig. 5. Nematode biomass C at 31 d; NT = no-till, ST = standard tillage, +Res = residue added, –Res = no residue added, Row = cores taken from plant row, Ctr = cores taken from the bed center, Int = cores taken from an intermediate position between the plant row and the bed center. There were no statistical differences at 0- to 5-cm depth. Statistical differences are expressed with *s* and *t* at 5- to 15-cm depth. The error bars show the standard error.

The initial burst of activity in the NT treatments, however, may have been due to the activity of microbes in the residue's phyllosphere, rather than due to colonization of soil fungi, since the soil-free control demonstrated similar CO_2 emissions to the NT soil treatment. Leaf respiration and active leaf mitochondria also undoubtedly contributed directly to CO_2 emissions, but these would have been minor amounts and probably occurred within the first 6 h after adding residue (Park, 2004; Kelly and Wiskich, 1998; Papadopoulou et al., 2001). Phyllosphere microbes function as saprophytes after shoot materials are placed on the soil surface (Okane et al., 1998; Marcell and Beattie, 2002). Total culturable epiphytic phyllosphere bacterial populations are often as high as 10^{10} cells kg^{-1} of leaf (Anderson et al., 1991; Jacques et al., 1995) and fungi population densities are 10^7 to 10^9 colony-forming units kg^{-1} leaf (Behrendt et al., 2004). Saprophytic fungi in the phyllosphere derive soluble carbohydrates from freshly fallen leaves before their colonization by soil-borne fungi (Stone, 1987). The detritusphere (e.g., plant residue and tightly adhering soil) can be dominated at the initial stages of decomposition by residual phyllosphere bacteria, with fungal populations increasing with time (McMahon et al., 2005). The rapid response of phyllosphere organisms to C availability in leaf residue in the NT treatment could explain why the assimilation of residue C into MBC in the 0- to 5-cm layer was only about half that in ST at 10 d after adding residue.

The NBC that was derived from residue by the end of the experiment is an integrative indicator of microbial dynamics, since the microbial-grazing nematodes have longer life spans (1–3 wk) than their prey. The effects of the residue inputs persist longer in the nematode fauna than in the microbes, which provides the benefit of allowing detection of differences between treatments with less frequent sampling than is necessary for detection of peak microbial activity. Higher nematode populations may serve a role in capturing C that can be lost during turnover of MBC. In soil from the ST plots, there was greater assimilation of residue C into nematode biomass and less stratification than in NT soil.

Our observations with nematodes are consistent with other studies that point out that a month-long time frame is necessary to capture population dynamics (Bouwman and Zwart, 1994; Lenz and Eisenbeis, 2000; Fu et al., 2000). Differentiating between the residue-derived C of bacterial- and fungal-feeding nematodes in this study was difficult due to the high variability in the counts of bacterial feeders. The variability is probably associated with aggregations of nematodes in and around bacterial colonies, which are in turn aggregated around accessible residue fragments. Bacterial-feeding nematodes probably played an important role in assimilation of residue C since they were approximately four times more abundant than fungal feeders in the residue-added treatments at 31 d. Nematodes responded quickly to residue incorporation by ST, increasing their abundance seven times compared with no-residue treatments, consistent with the general response of these nematodes to organic enrichment (Ferris and Bongers, 2006). Higher abundances of fast-growing bacterial-feeding nematodes in ST than NT were observed in the accompanying field sampling component of this project (Sánchez-Moreno et al., 2006).

Since nematode biomass C was approximately 600 times lower than MBC for all treatments at 31 d, MBC was probably

not negatively impacted by nematode grazing. Provided that nutrient resources are available for bacteria, their productivity should outstrip the grazing pressure of their nematode predators. Nematodes also did not seem to regulate MBC in the field (Minoshima et al., 2007). Microbial biomass C is known to be more strongly regulated by resource availability (Mikola and Setälä, 1998; Coleman et al., 2002), and in this study, this appears to be mainly the supply of C substrates and possibly water content (Lundquist et al., 1999a), which was greater in the NT treatment with residue.

In summary, this short-term study demonstrates that NT management favored lower assimilation of shoot residue into active C pools compared with ST, apparently due to less contact with soil and soil organisms. During the longer term, however, NT has been demonstrated to be an effective way to store soil C and increase the diversity and activity of the soil food web, especially if it is combined with rotations that provide continuous C inputs via litter and root activity (Halvorson et al., 2002; Sherrod et al., 2005; Minoshima et al., 2007). These data suggest that higher C retention in NT may not be due to higher recovery of C from shoot residues compared with ST, but rather to belowground processes such as root C transformations, biophysical stabilization mediated in part by fungi, and the lack of disruption of hyphae and aggregates that act to further physically stabilize C (Six et al., 2006). Projecting these results to implications for field management suggests that minimizing soil disturbance may be a key factor for C sequestration in agricultural soils, but that increasing shoot–soil contact in NT, e.g., by finely mulching crop residues so that they touch the soil surface, may also improve the capture of shoot residue C in the soil.

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