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Preferential Accumulation of Microbial Carbon in Aggregate Structures of No-Tillage Soils

Rodney T. Simpson, Serita D. Frey,* Johan Six, and Rachel K. Thiet

ABSTRACT

We examined the effect of reduced tillage on the accumulation of fungal- versus bacterial-derived organic matter within the soil matrix by quantifying the amino sugars glucosamine (Glc), galactosamine (Gal), and muramic acid (MurA) in aggregate-size fractions isolated from no-tillage (NT) and conventional-tillage (CT) soil. Intact soil cores (0- to 5- and 5- to 20-cm depth) were collected from the long-term tillage experiment at Horseshoe Bend in Athens, GA. Four water-stable aggregate-size fractions were isolated: large macroaggregates (>2000 μm), small macroaggregates (250–2000 μm), microaggregates (53–250 μm), and the silt + clay fraction (<53 μm). Small macroaggregates were further separated into coarse particulate organic matter (POM) (>250 μm), microaggregates contained within macroaggregates, and the silt + clay fraction. Amino sugars were extracted from all fractions, purified, and analyzed by gas chromatography. Fungal-derived amino sugar C (FAS-C) comprised 63%, while bacterial-derived amino sugar C (BAS-C) accounted for 37% of the total amino sugar C pool under both tillage treatments. No-tillage soil contained 21% more amino sugar C than the CT soil across the entire plow layer. Both, the percentage of total organic C as FAS-C and BAS-C were significantly higher in the silt + clay fraction of NT versus CT soil. The percentage of total organic C as FAS-C was significantly higher in small macroaggregates of NT versus CT soil due to a preferential accumulation of FAS-C in the microaggregates contained within these macroaggregates. These results indicate that microbial-derived C is stabilized in NT soils, due primarily to a greater fungal-mediated improvement of soil structural stability and concurrent deposition of fungal-derived C in microaggregates contained within macroaggregates.

Differences in soil organic matter (SOM) content between NT and CT management systems have been attributed to loss upon cultivation of SOM with an intermediate turnover rate (Metherell, 1992). Particulate organic matter, which is a plant-derived intermediate SOM pool, has been widely studied and shown to account for part of the difference in soil C between NT and CT soils (Beare et al., 1994; Six et al., 1999; Wander and Bidart, 2000). Changes in the intermediate pool not accounted for by POM are hypothesized to be due to microbial byproducts (Cambardella and Elliott, 1994), including cell wall residues and extracellular polysaccharides. Carbohydrates and amino sugars of microbial origin are often significantly higher under NT compared with CT (Arshad et al., 1990; Ball et al., 1996; Beare et al., 1997; Guggenberger et al., 1999), and Cambardella and Elliott (1994) hypothesized that these materials are sequestered as intermicroaggregate SOM within the macroaggregate structure where they serve as binding agents for aggregate formation. They further predicted that the intermediate microbial-derived SOM pool, particularly in NT soils, is mostly of fungal origin because fungi play an important role in macroaggregate formation (Tisdall and Oades, 1982; Gupta and Germida, 1988) and comprise a relatively greater proportion of the total microbial biomass in NT compared with CT (Beare et al., 1997; Frey et al., 1999). Thus, there is considerable interest in isolating a microbial-derived C pool from soil and determining its composition and location within the soil aggregate structure.

Soil amino sugars, which are components of microbial cell walls, have been used to assess the microbial contribution to SOM accumulation and turnover in soils (Zhang and Amelung, 1996; Chantigny et al., 1997; Six et al., 2001). In particular, the ratio of fungal-derived Glc to bacterial-derived MurA is considered an indicator of the relative contribution of fungi and bacteria to the total amino sugar pool (Zhang et al., 1998; Amelung et al., 1999; Guggenberger et al., 1999). Guggenberger et al. (1999) measured amino sugars in different water-stable aggregate-size fractions isolated from the surface (0–5 cm) of NT and CT soils and found higher concentrations in aggregate-size classes >53 μm compared with silt and clay particles. The Glc/MurA ratio was significantly higher in NT compared with CT aggregates, indicating a greater accumulation of fungal relative to bacterial cell wall residues in NT soils.

Our objective was to determine how tillage influences the distribution of fungal- versus bacterial-derived cell wall residues within the soil aggregate structure of these two tillage regimes. Determining the location of fungal- and bacterial-derived cell wall residues in soil aggregates of NT and CT soils will improve our mechanistic understanding of the impacts of tillage on soil C storage and turnover. We hypothesized that the greater amounts of amino sugars previously observed in NT compared with CT is due to an enrichment of amino sugars in microaggregates located within macroaggregates in NT soil. We predicted that this enrichment would be the result of a relatively greater accumulation of fungal compared with bacterial-derived amino sugars in NT compared with CT, given the important role of fungal hyphae in macroaggregate formation.

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Abbreviations: BAS-C, bacterial-derived amino sugar carbon; CT, conventional tillage; FAS-C, fungal-derived amino sugar carbon; Gal, galactosamine; Glc, glucosamine; MurA, muramic acid; NT, no-tillage; POM, particulate organic matter; SOM, soil organic matter.

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MATERIALS AND METHODS

Sample Collection

Intact soil cores were collected during fall 2000 from two depths (0–5 and 5–20 cm) in adjacent NT and CT plots at the Horseshoe Bend long-term tillage comparison experiment located near Athens, GA (33° 54′ N, 83° 24′ W). This experiment was established in 1978 on a bottomland terrace within a meandering loop of the North Oconee River (Hendrix, 1997). The site was divided into eight 0.1-ha plots and placed under either NT or CT management in a completely randomized design with four replicates per treatment. These tillage regimes have been maintained to the present, with the cropping system consisting of a summer grain crop followed by a winter cover crop. The soil is a fine loamy siliceous thermic Rhodic Kanhapludult in the Hiwassee series with 66% sand, 13% silt, and 21% clay. There is no significant difference in texture between the two tillage treatments in the top 0 to 20 cm (Frey, unpublished data, 1993). Mean annual precipitation (MAP) and temperature (MAT) are 1270 mm and 16.5°C, respectively. Further details about the Horseshoe Bend site can be found in Hendrix (1997). For our study, six cores (5.6-cm diam.) were collected from each of the four replicate plots per tillage treatment and composited by depth increment. Field moist soil was crumbled to pass an 8-mm sieve and air-dried.

Aggregate Separation

Four water-stable aggregate-size classes were isolated using the wet sieving method of Elliott (1986): large macroaggregates (2000–8000 μm), small macroaggregates (250–2000 μm), microaggregates (53–250 μm), and the silt plus clay fraction (<53 μm) (Fig. 1). Subsamples (100 g) were spread over a 2000-μm sieve and submersed in water for 5 min in room temperature deionized water. Aggregates were separated by manually moving the sieve up and down 50 times in 2 min. The stable aggregates remaining on the sieve were backwashed into an aluminum pan. Water and soil that passed through the sieve was poured onto the next sieve (250 μm) and the sieving was repeated. This process was repeated a third time with a 53-μm sieve. The aggregate fractions were oven-dried (65°C), weighed, and stored at room temperature.

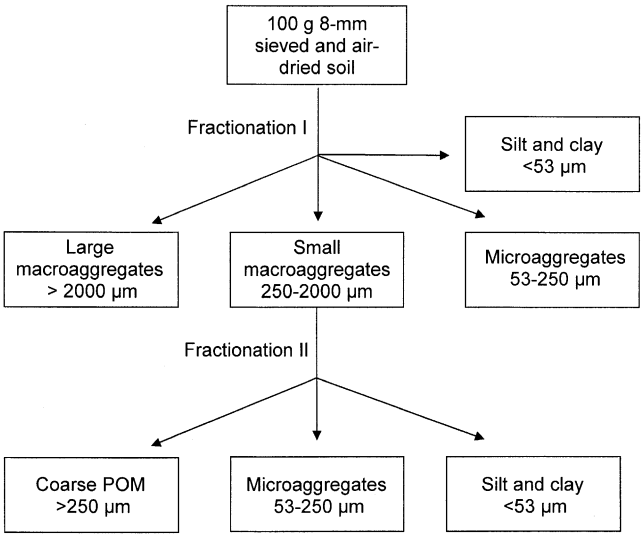

Fig. 1. Aggregate separation scheme. Fractionation I involved wet sieving air-dry soil into four aggregate-size classes. In fractionation II, small macroaggregates (250–2000 μm) were further separated into coarse sand and POM (>250 μm), microaggregates (53–250 μm) and the silt and clay fraction (<53 μm).

Microaggregates contained within small macroaggregates (250–2000 μm) were isolated using the method of Six et al. (2000a). A 10-g subsample of macroaggregates was placed on top of a 250-μm sieve along with 50 glass beads (4-mm diam). The sieve was shaken so that the macroaggregates were broken up with the aid of the glass beads. Continuous water flow carried microaggregates through the 250-μm sieve and washed them onto a 53-μm sieve so that they were not broken up by the shaking. Once all macroaggregates were broken up, the 53-μm sieve was moved up and down in water in the same manner as the first aggregate separation procedure. This separation yielded three fractions: the material remaining on the 250-μm sieve consisted of coarse POM; aggregates passing through the 250-μm sieve but retained on the 53-μm sieve were stable microaggregates isolated from macroaggregates; and material passing through the 53-μm sieve was clay and silt particles not associated with stable microaggregates. All aggregate and POM fractions were dried (65°C), weighed, and finely ground. Total organic C and N of all aggregate fractions were quantified by dry combustion using a Leco CHN-1000 analyzer (Leco, St. Joseph, MI).

Amino Sugar Extraction and Quantification

The amino sugars Glc, Gal, and MurA were extracted from whole soil, aggregate fractions, and POM according to the method of Zhang and Amelung (1996). Subsamples containing approximately 0.3 mg N, along with an internal standard (myo-inositol), were hydrolyzed with 6 *M* HCl at 105°C for 8 h. The hydrolysate was washed through a fiberglass filter (Schleicher & Schuell #30; Schleicher & Schuell, Dassel, Germany), evaporated to dryness using a rotary evaporator, and redissolved in deionized water. After neutralizing with KOH to precipitate Fe, the samples were centrifuged for 15 min at 3600 g in 50-mL polypropylene copolymer (PPCO) centrifuge tubes (Nalgene, Rochester, NY). Supernatant containing the amino sugar fraction was decanted, flash-frozen in liquid N_2, and freeze-dried. Methanol was used to redissolve the freeze-dried supernatant to precipitate salts. The sample was centrifuged for 10 min at 8400 × g in a microcentrifuge (Beckman Coulter), which concentrated the salts as a pellet at the bottom of the tube. The supernatant, containing the amino sugars, was drawn off with a pipette and transferred to a 5-mL vial. Methylglucamine was added as a second internal standard and the solution dried under compressed air. The samples were redissolved in deionized water, flash frozen in liquid N_2 and freeze-dried.

The freeze-dried samples were derivatized to convert nonvolatile compounds to a more volatile form necessary for gas chromatographic analysis (Iqbal et al., 1996). For preparation of aldononitrile derivatives, the procedure was as follows: freeze-dried samples were redissolved in a derivatization reagent containing hydroxylamine hydrochloride (32 mg mL^{-1}) and 4-(dimethylamino)pyridine (40 mg mL^{-1}) in pyridine–methanol (4:1 v/v) and heated to 75 to 80°C for 30 min. After cooling, acetic anhydride was added and the solution was reheated for 30 min. Dichloromethane was added to end the derivatization reaction and 1 *M* HCl was added, forming an organic/aqueous separation with the amino sugars contained in the organic layer. The aqueous layer was removed, followed by three washing steps with deionized water to remove the derivatization reagent and acetic acid from the organic phase. The organic phase was dried and resuspended in ethyl acetate–hexane (1:1). Amino sugars were quantified by capillary gas chromatography (Varian Model CP3800; Varian, Palo Alto, CA) equipped with an autoinjector, flame ionization detector,

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and a 30 m by 0.25 mm ID (0.25 μm) fused silica column (Alltech).

We assume in our calculations that MurA derives exclusively from bacterial cell walls, while Glc is present in fungal cell walls, bacterial cell walls, and microarthropod exoskeletons. We corrected the Glc data for the Glc present in bacterial cell walls by assuming that the ratio of Glc to MurA in bacteria is 1:1 (Brock and Madigan, 1988). Thus the glucosamine concentrations reported here represent fungal-derived Glc plus an unknown contribution from microarthropods. Guggenberger et al. (1999) noted that the contribution of microarthropods to the total soil Glc concentration is likely minimal because microarthropod biomass is typically <0.5% of fungal biomass (Beare, 1997). Thus, we assume that the Glc concentrations reported here derive primarily from fungi. The source of Gal is less well defined than for Glc and MurA, but available evidence indicates a predominantly bacterial origin (W. Amelung, personal communication, 2001).

To convert amino sugar concentrations to amino sugar C concentrations, we assumed a 40% C content for Glc and Gal and a 43% C content for MurA. To determine the contribution of amino sugar C to total organic C (TOC), we calculated the percentage of the TOC (mg C g^{-1} soil) that was amino sugar C (mg amino sugar C g^{-1} soil).

Data Analysis

Comparisons of amino sugar concentrations and ratios in the aggregate fractions and across tillage treatments were performed using rank analysis of variance (PROC RANK, PROC GLM; SAS Institute, 1999). A nonparametric procedure was selected because the data violated the assumptions of normality and homogeneity of variance for standard analysis of variance. The Ryan-Einot-Gabriel-Welsch (REGW) multiple range test was used to determine significant differences among means at $P < 0.05$.

RESULTS AND DISCUSSION

Both NT and CT soils contained approximately 40 g amino sugar C m^{-2} in the 5- to 20-cm depth increment, but differed significantly in the 0- to 5-cm depth increment, with NT having higher amounts of C associated with Glc, Gal, and MurA, compared with CT (Fig. 2). When calculated for the entire plow layer (0–20 cm), the NT soil contained 21% more amino sugar C than the CT soil (Fig. 2 inset). Amino sugar concentrations ranged from 23 to 494, 12 to 226, and 2 to 33 mg kg^{-1} soil for Glc, Gal, and MurA, respectively. These concentrations are lower than those found in other soils (Zhang et al., 1998; Guggenberger et al., 1999); however, sampling depth likely explains this difference. We sampled the entire plow layer, while most published amino sugar data are from surface (<10 cm) samples. We observed similar plow-layer concentrations for 13 other long-term tillage comparison experiments (Frey et al., unpublished data, 2001). Fungal-derived Glc was the most abundant amino sugar, representing 66% of the total amino sugar C pool for both CT and NT. Muramic acid accounted for <5% of total amino sugar C for both tillage treatments.

Amino sugar concentrations represent an accumulation of microbial cell wall residues in soil and are not necessarily correlated with living microbial biomass (Amelung et al., 2001; Turrion et al., 2002). Amino sugars are presumed to be more resistant to decomposition than the biomass from which they are derived, because total soil amino sugar concentrations are one to two orders of magnitude higher than amino sugar concentrations estimated to be present in intact microbial cells (Guggenberger et al., 1999). However, relative turnover times for Glc, Gal, and MurA have not been

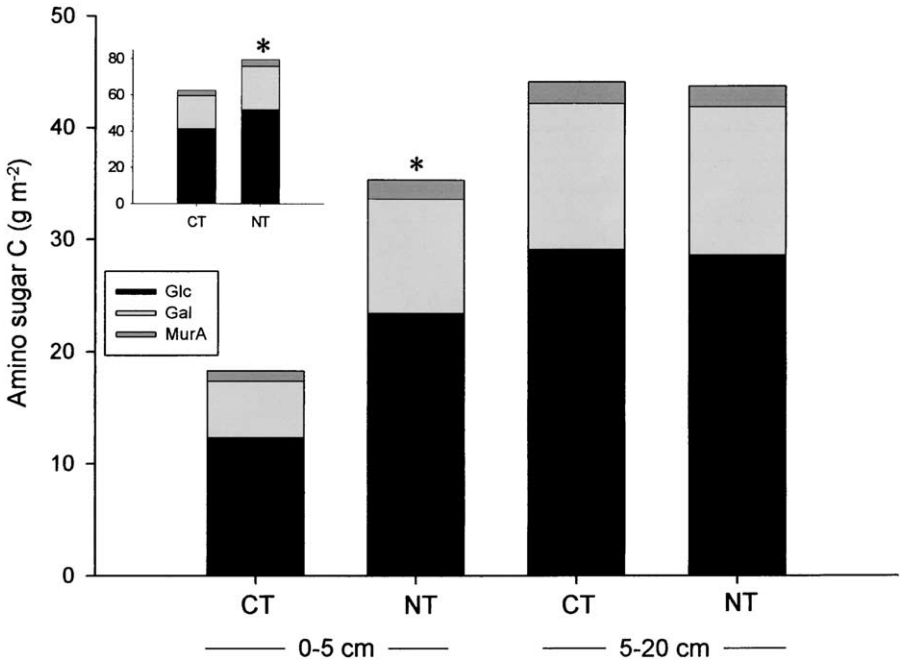

Fig. 2. Total amino sugar C (g m^{-2}) at 0 to 5 and 5 to 20 cm in conventional tillage (CT) and no-tillage (NT) soils at Horseshoe Bend, GA. There was a significant difference ($P < 0.05$) between NT and CT at the 0- to 5-cm depth for all three amino sugars. Inset: Total amino sugar C in the plow layer (0–20 cm).

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Table 1. Distribution of water-stable aggregates isolated from NT and CT soils at Horseshoe Bend, GA.

Tillage treatment	Depth	Aggregate-size fraction			
		>2000 μm	250–2000 μm	53–250 μm	<53 μm
	cm	% of whole soil			
CT	0–5	14.00 b*†	43.74 a*	38.23 a*	4.02 b*
	5–20	15.11 c	47.80 a	33.80 b	3.29 d
NT	0–5	51.29 a*	31.04 b*	16.18 c*	1.49 d*
	5–20	24.29 b	46.97 a	26.07 b	2.68 c

† Values followed by a different letter indicate a significant difference ($P < 0.05$) across aggregate fractions within a given tillage treatment and depth increment. An * indicates a significant difference between tillage treatments within the same depth increment and aggregate-size class.

conclusively documented (Parsons, 1981). Guggenberger et al. (1999) found that the accumulation of amino sugars was significantly higher in NT compared with CT at sites that also showed a tillage effect on aggregation and POM-C. Accumulation of amino sugars also depended on silt + clay content and previous management. The potential to physically stabilize amino sugars under NT was lower in soils with a lower silt + clay content. Sites that had been under CT but were later switched to NT tended to have lower amino sugar content than sites under NT management for a longer time.

There was a significant difference in aggregate-size distribution between CT and NT soils, as observed in previous studies (Beare et al., 1994; Bossuyt et al., 2002). Surface soil (0–5 cm) in CT was dominated by small macroaggregates (250–2000 μm) and microaggregates (53–250 μm), with 82% of the soil contained in these two fractions (Table 1). In contrast, greater than 80% of NT surface soil was in large plus small macroaggregates. In both tillage treatments, <5% of the whole soil was comprised of silt and clay particles unassociated with any aggregate fraction. Differences were not as apparent between tillage practices at 5 to 20 cm, and the aggregate distributions for both treatments were similar to the CT soil at 0 to 5 cm. Thus, the primary difference between tillage practices was the shift toward larger aggregate-size classes at the surface of NT. Due to a lack of mechanical homogenization, the surface soil of NT systems is often associated with an accumulation of SOM (Beare et al., 1994), a fungal-dominated microbial community (Beare et al., 1997; Frey et al., 1999), increased macroaggregation (Gupta and Germida, 1988; Beare et al., 1994; Six et al., 2000b), and reduced macroaggregate turnover (Six et al., 1999).

Amino sugar C concentrations measured in the aggregate fractions isolated from surface soil (0–5 cm) are reported on a whole-soil basis (Fig. 3). Under CT management, there was a similar amount of amino sugar C in the small macroaggregates and microaggregates, accounting for approximately two thirds of the total (Fig. 3A). In NT surface soil, microaggregates contained <15% of total amino sugar C, and there was a significant accumulation of all three amino sugars in the large and small macroaggregates (Fig. 3B). Large macroaggregates showed a greater increase in amino sugar C (400–500%) than small macroaggregates (100–150%) under NT management. Although amino sugars are primarily associated with silt and clay particles (Zhang et al., 1998) and we observed a relatively high amino sugar concentration in the silt + clay fraction unassociated with aggregates (<53 μm), this fraction made up a small percentage of the whole soil (1.5–4.0%). Thus, silt and clay particles unassociated with aggregates do not play a significant role in the storage of amino sugar C on a whole soil basis. Our results indicate that amino sugars are primarily associated with silt- and clay-sized particles contained within water-stable macroaggregates, especially in NT soils. There were no significant differences between CT and NT soils at 5 to 20 cm with respect to amino sugar concentrations (data not shown).

To determine where microbial-derived amino sugars are located within macroaggregates, only small macroaggregates were further fractionated because there were not enough large macroaggregates in the CT soil to warrant further analysis. In addition, previous studies have shown that C distributions across aggregate fractions do not typically differ between large and small macroaggregates (Six et al., 1999; J. Six, unpublished data, 2003). The distribution of size fractions isolated from macroaggregates differed little between depths or tillage practice (Table 2). Small macroaggregates from both tillage treatments were comprised of roughly equal amounts (40–50%) of coarse sand plus POM (>250 μm) and water-stable microaggregates (53–250 μm). Less than 10% of the small macroaggregate fraction consisted of silt and clay particles not associated with water-stable microaggregates. The only significant difference observed between NT and CT was a lower proportion of microaggregates within macroaggregates in the CT versus NT surface soil, confirming similar results reported by Six et al. (2000a) for a long-term tillage experiment at Sidney, NE.

The concentration of amino sugars in macroaggregate-derived fractions was highest in the microaggregate fraction, followed by the silt + clay and coarse-POM fractions (Table 3). Glucosamine, as observed previously for whole soil and aggregate fractions, was the most abundant amino sugar measured in the small macroaggregate fractions. While both the microaggregate and the silt + clay fractions were enriched in Glc in NT surface soil relative to CT, the microaggregate fraction was significantly more enriched in Glc (i.e., more than five times) and Gal in NT surface soil compared with CT. The coarse POM-associated amino sugars were not significantly different between tillage treatments and there was no effect of tillage on amino sugar concentrations at the 5- to 20-cm depth for any of the macroaggregate-derived fractions. These data indicate that the significantly higher concentrations of microbial-derived amino sugars in macroaggregates of surface NT soil (Fig. 3) are

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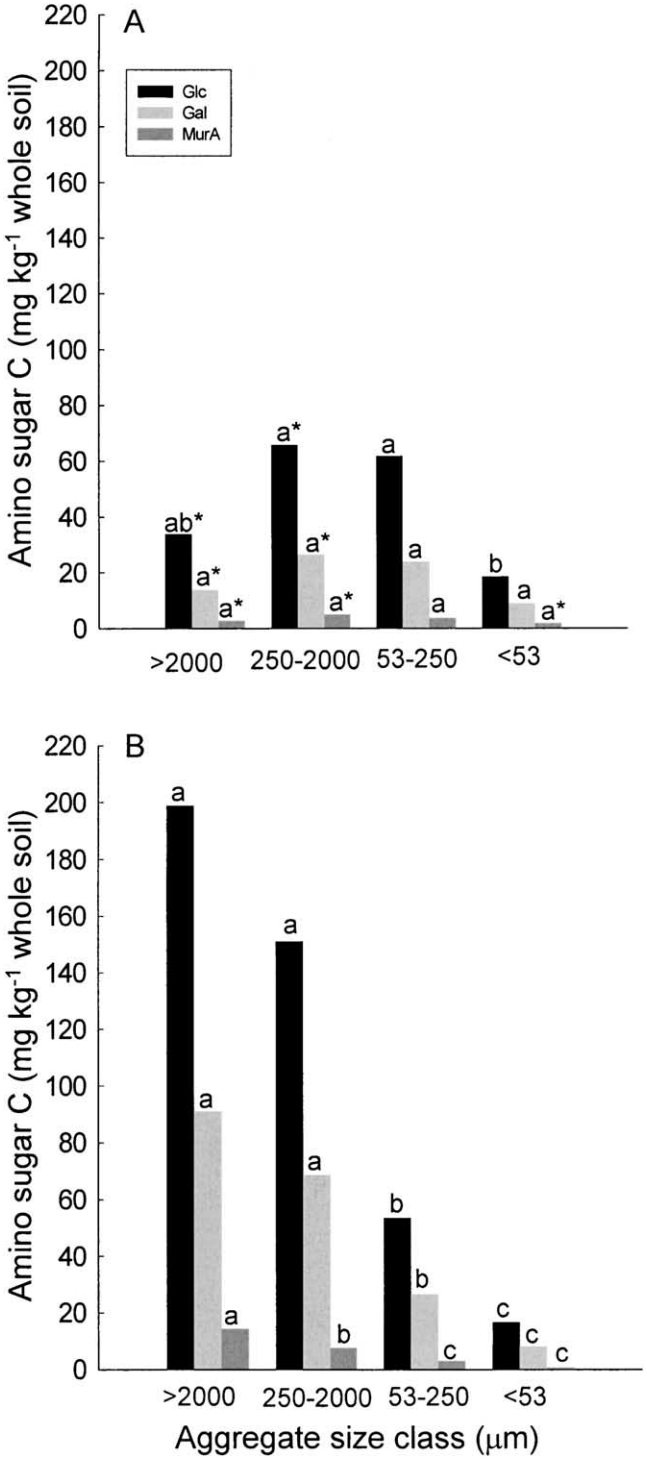

Fig. 3. Contribution of aggregate-size classes to whole soil amino sugar C (mg kg^{-1} whole soil) in surface soil (0–5 cm) of (A) conventional tillage (CT) and (B) no-tillage (NT) soils at Horseshoe Bend, GA. Bars with different letters are significantly different ($P < 0.05$) across aggregate size classes for a given amino sugar. An * indicates a significant difference between CT and NT within an aggregate size class and amino sugar.

due to accumulation of amino sugar C in water-stable microaggregates contained within the macroaggregates. The ability of NT macroaggregates to store C is dependent on slower aggregate turnover in NT compared with CT, which enhances the formation of stable microaggregates within macroaggregates (Six et al., 1999, 2000a).

Table 2. Coarse POM (>250 μm), microaggregate (53–250 μm), and silt plus clay (<53 μm) fractions isolated from small macroaggregates.

Tillage treatment	Depth	Aggregate-size fraction		
		>250 μm	53–250 μm	<53 μm
	cm	% of macroaggregate fraction		
CT	0–5	51.41 a†	40.43 b*	8.16 c
	5–20	46.30 b	45.25 a	8.45 c
NT	0–5	43.35 a	49.75 a*	6.90 b
	5–20	44.09 a	47.90 a	8.11 b

† Values followed by a different letter indicate a significant difference ($P < 0.05$) across aggregate fractions within a given tillage treatment and depth increment. An * a significant difference between tillage treatments within the same depth increment and aggregate-size class.

Table 3. Amino sugar C concentration in the coarse particulate organic matter (POM) (>250 μm), microaggregate (53–250 μm), and silt + clay (<53 μm) fractions isolated from small macroaggregates.†

Tillage treatment	Fraction size	Glc	Gal	MurA	Total
	μm	mg kg^{-1} macroaggregate fraction			
		0- to 5-cm depth			
CT	>250	39 a‡	11 a	1.5 a	51 a
	53–250	47 a*	29 a*	5.0 a*	80 a*
	<53	35 a*	21 a	3.0 a	59 a
NT	>250	63 b	21 b	3.0 b	86 b
	53–250	293 a*	145 a*	9.0 a*	446 a*
	<53	74 b*	26 b	3.0 b	102 b
		5- to 20-cm depth			
CT	>250	9 b	4 c	0.5 c	14 c
	53–250	58 a	35 a	5.0 a	97 a
	<53	36 a	20 b	3.0 b	59 b
NT	>250	7 c	2 c	0.3 c	9 c
	53–250	85 a	43 a	4.0 a	132 a
	<53	30 b	15 b	3.0 b	48 b

† Glc, Glucosamine; Gal, Galactosamine; MurA, Muric acid.
‡ Values followed by a different letter indicate a significant difference ($P < 0.05$) across aggregate fractions within a given tillage treatment and depth increment. An * indicates a significant difference between tillage treatments within the same depth increment and aggregate size class.

Due to the greater proportion of macroaggregate-derived microaggregates (Table 2) and the greater concentration of Glc and Gal they contained (Table 3), this fraction contributed more amino sugar-C on a whole soil basis than the other fractions in NT surface soil (Fig. 4). The coarse POM (>250 μm) and silt + clay (<53 μm) fractions contained similar and significantly lower amounts of Glc and Gal relative to the microaggregate fraction in NT. The coarse POM fraction was associated with sand and had a low concentration of amino sugars, supporting earlier observations that amino sugars are clay, and not sand associated (Ladd et al., 1996; Zhang et al., 1998). Amino sugars did not differ significantly by tillage when compared on a POM-C basis. At the 0- to 5-cm depth, we observed enrichments of 22.6 and 25.1 mg amino sugar C g^{-1} POM-C for CT and NT, respectively, and 19.9 and 14.3 mg amino sugar C g^{-1} POM-C at the 5- to 20-cm depth.

We hypothesized that the amino sugar fraction within macroaggregates would be dominated by FAS-C since fungi play a key role in the binding of microaggregates into stable macroaggregates (Tisdall and Oades, 1982;

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Table 4. Percentage of the fraction of total organic C (TOC) as fungal-derived (FAS) versus bacterial-derived (BAS) C in conventional (CT) and no-tillage (NT) soil at the Horseshoe Bend site.†

		Aggregate-size class				Macroaggregate-derived fraction		
Amino sugar C	Tillage	>2000 μm	250–2000 μm	53–250 μm	<53 μm	>250 μm	53–250 μm	<53 μm
				0- to 5-cm depth				
%TOC as FAS-C	CT	1.36	1.69	1.43	2.21	1.66	1.08	1.69
	NT	1.69	2.24*	1.96	3.17*	1.75	2.42*	2.67
%TOC as BAS-C	CT	0.79	0.99	0.76	1.62	0.60	0.85	1.42
	NT	1.09	1.30	1.24	1.89*	0.77	1.36	1.49
				5- to 20-cm depth				
%TOC as FAS-C	CT	2.21	2.20	1.91	2.16	1.30	1.66	2.00
	NT	2.03	1.71	1.82	2.05	1.09	2.08	1.59
%TOC as BAS-C	CT	1.24	1.45	1.19	1.66	0.69*	1.27	1.50
	NT	1.34	1.17	1.24	1.77	0.35	1.34	1.22

* Indicates a significant difference ($P < 0.05$) between tillage treatments within the same depth increment and aggregate size class.
† FAS-C = Fungal-derived glucosamine-C; BAS-C = Bacterial-derived glucosamine-C + galalactosamine-C + muramic acid-C.

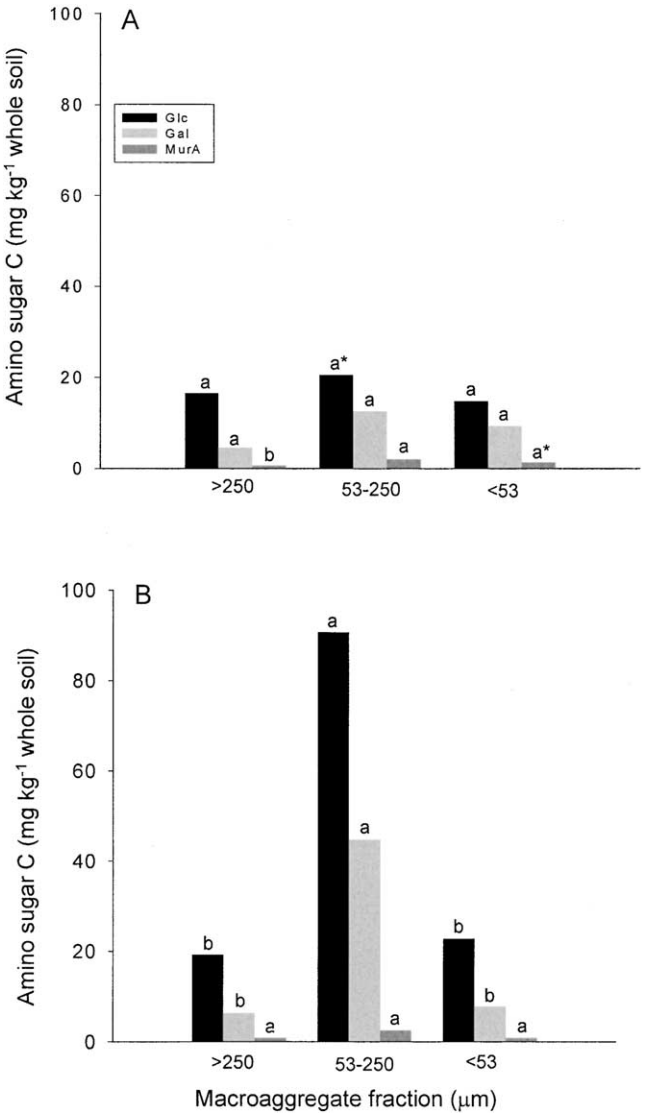

Fig. 4. Amino sugar C in macroaggregate-derived size classes on a whole soil basis for (A) conventional tillage (CT) and (B) no-tillage (NT) soils at Horseshoe Bend, GA (0–5 cm depth). Bars with different letters are significantly different ($P < 0.05$) across aggregate size classes for a given amino sugar. An * indicates a significant difference between CT and NT within an aggregate size class and amino sugar.

Beare et al., 1994; Bossuyt et al., 2001). The percentage of total soil C comprised of amino sugars ranged from 1.0 to 5.7% and differed little across aggregate size classes, indicating that there was little difference in SOM quality with respect to amino sugars among aggregate sizes. Nevertheless, the few significant differences observed in FAS-C and BAS-C enrichments between tillage systems support our hypothesis (Table 4). The FAS-C, as a proportion of total C, was only significantly different between tillage systems for the small macroaggregates in surface soil and the accumulation of FAS-C in NT was mostly pronounced in the microaggregates occluded in these small macroaggregates. These results support the mechanism of microbial-derived C accumulation in NT soils due to the increased fungal binding of microaggregates to form stable macroaggregates.

In addition to the accumulation of FAS-C in microaggregates within macroaggregates, we observed an accumulation of both FAS-C and BAS-C in the silt + clay fractions of NT surface soil and an accumulation of BAS-C in the subsurface layer of CT. The latter result is probably related to fresh residue with readily available C being plowed into the subsurface layer of the CT soil, which is in agreement with Bossuyt et al. (2002) who observed that more young C is accumulated in the subsurface soil of CT compared with NT. The accumulation of FAS-C and BAS-C in the silt + clay fraction is most probably a result of the organic matter stabilizing effect by the reactive surfaces of silt and especially clay particles.

In conclusion, total amino sugar C in the plow layer was significantly higher in NT than in CT soils, due primarily to a greater accumulation of amino sugars stabilized in microaggregates contained within macroaggregates in NT surface soil (0–5 cm). The significant increase in FAS-C within the microaggregates occluded in small macroaggregates of NT surface soil corroborates our hypothesis of a preferential fungal-derived C accumulation in NT soils due to the important role fungi play in the binding of microaggregates into macroaggregates.

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REFERENCES

Amelung, W., A. Miltner, X. Zhang, and W. Zech. 2001. Fate of microbial residues during litter decomposition as affected by minerals. Soil Sci. 166:598–606.

Amelung, W., X. Zhang, K.W. Flach, and W. Zech. 1999. Amino sugars in native grassland soils along a climosequence in North America. Soil Sci. Soc. Am. J. 63:86–92.

Arshad, M.A., M. Schnitzer, D.A. Angers, and J.A. Ripmeester. 1990. Effects of till versus no till on the quality of soil organic matter. Soil Biol. Biochem. 22:595–599.

Ball, B.C., M.V. Cheshire, E.A.G. Robertson, and E.A. Hunter. 1996. Carbohydrate composition in relation to structural stability, compactiblility and plasticity of two soils in a long-term experiment. Soil Tillage Res. 39:143–160.

Beare, M.H. 1997. Fungal and bacterial pathways of organic matter decomposition and nitrogen mineralization in arable soil. p. 37–70. *In* L. Brussaard and R. Ferrera-Cerrato (ed.) Soil ecology in sustainable agricultural systems. Lewis Publ., CRC Press, Boca Raton, FL.

Beare, M.H., P.F. Hendrix, and D.C. Coleman. 1994. Water-stable aggregates and organic matter fractions in conventional and no-tillage soils. Soil Sci. Soc. Am. J. 58:777–786.

Beare, M.H., S. Hu, D.C. Coleman, and P.F. Hendrix. 1997. Influences of mycelial fungi on soil aggregation and organic matter storage in conventional and no-tillage soils. Appl. Soil Ecol. 5:211–219.

Beare, M.H., R.W. Parmelee, P.F. Hendrix, W. Cheng, D.C. Coleman, and D.A. Crossley, Jr. 1992. Microbial and faunal interactions and effects on litter nitrogen and decomposition in agroecosystems. Ecol. Monogr. 62:569–591.

Bossuyt, H., K. Denef, J. Six, S.D. Frey, R. Merckx, and K. Paustian. 2001. Influence of microbial populations and residue quality on aggregate stability. Appl. Soil Ecol. 16:195–208.

Bossuyt, H., J. Six, and P.F. Hendrix. 2002. Aggregate-protected carbon in no-tillage and conventional tillage agroecosystems using carbon-14 labeled plant residue. Soil Sci. Soc. Am. J. 66:1965–1973.

Brock, T.D., and M.T. Madigan. 1988. Biology of microorganisms. 5th ed. Prentice Hall, Englewood Cliffs, NJ.

Cambardella, C.A., and E.T. Elliott. 1994. Carbon and nitrogen dynamics of soil organic matter fractions from cultivated grassland soils. Soil Sci. Soc. Am. J. 58:123–130.

Chantigny, M.H., D.A. Angers, D. Prevost, L. Vezina, and F. Chalifour. 1997. Soil aggregation and fungal and bacterial biomass under annual and perennial cropping systems. Soil Sci. Soc. Am. J. 61: 262–267.

Elliott, E.T. 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Sci. Soc. Am. J. 50:627–633.

Frey, S.D., E.T. Elliott, and K. Paustian. 1999. Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. Soil Biol. Biochem. 31:573–585.

Guggenberger, G., S.D. Frey, J. Six, K. Paustian, and E.T. Elliott. 1999. Bacterial and fungal cell-wall residues in conventional and no-tillage agroecosystems. Soil Sci. Soc. Am. J. 63:1188–1198.

Gupta, V.V.S.R., and J.J. Germida. 1988. Distribution of microbial biomass and its activity in different soil aggregate size classes as affected by cultivation. Soil Biol. Biochem. 20:777–786.

Hendrix, P.F. 1997. Long-term patterns of plant production and soil carbon dynamics in a Georgia Piedmont agroecosystem. p. 235–245. *In* E.A. Paul et al. (ed.) Soil organic matter in temperate agroecosystems: Long-term experiments in North America. Lewis Publ., CRC Press, Boca Raton, FL.

Iqbal, Z., J.M. Midgley, and D.G. Watson. 1996. Synthesis of 3,5-di(trifluoromethyl)benzyl hydroxylamine hydrochloride (DTFMBO), a new derivatizing agent for gas-chromatography-mass spectrometry of ketosteroids. Iran. J. Chem.Chem. Eng. 15:108–113.

Ladd, J.N., R.C. Foster, P. Nannipieri, and J.M. Oades. 1996. Soil structure and biological activity. p. 23–78. *In* G. Stotzky, and J.M. Bollag (ed.) Soil biochemistry. 9th ed. Marcel Dekker, New York.

Metherell, A.K. 1992. Simulation of soil organic matter dynamics and nutrient cycling in agroecosystems. Ph.D. Diss. Colorado State University, Ft. Collins.

Parsons, J.W. 1981. Chemistry and distribution of amino sugars in soils and soil organisms. p. 197–227. *In* E.A. Paul, and J.N. Ladd (ed.) Soil biochemistry. Marcel Dekker, New York.

SAS Institute. 1999. SAS procedures guide. Vol. 2. Version 8 ed. SAS Inst., Cary, NC.

Six, J., E.T. Elliott, and K. Paustian. 1999. Aggregate and soil organic matter dynamics under conventional and no-tillage systems. Soil Sci. Soc. Am. J. 63:1350–1358.

Six, J., E.T. Elliott, and K. Paustian. 2000a. Soil macroaggregate turnover and microaggregate formation: A mechanism for C sequestration under no-tillage agriculture. Soil Biol. Biochem. 32:2099–2103.

Six, J., G. Guggenberger, K. Paustian, L. Haumaier, E.T. Elliott, and W. Zech. 2001. Sources and composition of soil organic matter fractions between and within soil aggregates. Eur. J. Soil Sci. 52:607–618.

Six, J., K. Paustian, E.T. Elliott, and C. Combrink. 2000b. Soil structure and organic matter: I. Distribution of aggregate-size classes and aggregate-associated carbon. Soil Sci. Soc. Am. J. 64:681–689.

Tisdall, J.M., and J.M. Oades. 1982. Organic matter and water-stable aggregates in soils. J. Soil Sci. 3:141–161.

Turrion, M.-B., B. Glaser, and W. Zech. 2002. Effects of deforestation on contents and distribution of amino sugars within particle-size fractions of mountain soils. Biol. Fertil. Soils 35:49–53.

Wander, M.M., and G.M. Bidart. 2000. Tillage practice influences on the physical protection, bioavailability and composition of particulate organic matter. Biol. Fertil. Soils 32:360–367.

Zhang, X., and W. Amelung. 1996. Gas chromatographic determination of muramic acid, glucosamine, mannosamine, and galactosamine in soils. Soil Biol. Biochem. 28:1201–1206.

Zhang, X., W. Amelung, Y. Yuan, and W. Zech. 1998. Amino sugar signature of particle-size fractions in soils of the native prairie as affected by climate. J. Soil Sci. 163:220–229.

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