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## Microbial Responses to Wheel-Traffic in Conventional and No-Tillage Systems

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### ABSTRACT

Traffic-induced soil compaction and tillage systems can impact the productivity and sustainability of agricultural soils. The objective of this study was to assess the response of soil microbial populations to wheel-traffic in two tillage systems on a Norfolk loamy sand (Typic Kandiodults; FAO classification Luvisc Ferralsols). Experimental variables were with and without traffic under conventional tillage

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(disk harrow twice, chisel plow, field cultivator-planter) vs. no tillage employed in a split-plot design with four replications; main plots were traffic and subplots were tillage. Soil samples were collected from 0–2 and 2–4-cm depths, sieved (2 mm), and used to assess soil-water content, microbial biomass nitrogen (N), dehydrogenase, and microbial characterization using phospholipid ester-linked fatty acid (PLFA) analysis. Traffic increased soil-water content, had little effect on microbial biomass N, and increased microbial activity (no-till plots only) likely due to increased amounts of residue. Soil-water content, microbial biomass N, PLFA estimates of microbial biomass, and microbial activity were all consistently higher in no-till compared to conventional tillage plots. Data from this study suggest that conventional tillage results in a lower, more static, possibly more mature community of microbes while the microbial community under no-till appears to be a younger, more viable growing population. Finally, these data suggest that overall soil quality, at least in the surface soil layer, is improved in agricultural systems employing no-till operations.

*Key Words:* Dehydrogenase; Microbial biomass; Phospholipid fatty acid; Residue management; Soil compaction.

## INTRODUCTION

Traffic-induced soil compaction can negatively impact crop productivity due to restrictions in root growth. It has also been suggested that compaction may affect soil microbial populations, impacting the decomposition of plant materials and subsequent cycling of nutrients required for plant growth.<sup>[1]</sup> Lee et al.<sup>[2]</sup> reported higher levels of microbial biomass carbon (C) associated with trafficked compared with nontrafficked areas.

Reduced soil productivity and increased erosion, associated with intensive tillage operations, have prompted interest in reduced-tillage and no-tillage farming practices. In no-till systems, plant residues remain on the soil surface (as opposed to being incorporated during tillage operations) thereby slowing decomposition, which results in higher levels of soil C and N.<sup>[3,4]</sup> Generally, tillage events result in a large (albeit temporary) increase in microbial biomass and/or activity due to the physical incorporation of organic substrates into the soil.<sup>[2,5]</sup> However, following tillage, measures of microbial communities tend to be higher under no-till conditions due to the generally more favorable

soil conditions.<sup>[2]</sup> Adoption of no-tillage farming systems may enhance soil quality, in part through their impacts on soil microbes.

Soil microbial populations may act as early indicators of changes in soil quality as they can respond much more rapidly to perturbations than other indicators such as soil C or N.<sup>[6]</sup> The size and activity of the soil microbial population is critical to overall soil use and sustainability. Soil organisms contribute to the maintenance of soil quality through their control of many key processes, such as decomposition, nutrient cycling and availability, and soil aggregation, which affects erodibility, water infiltration and storage, and carbon sequestration.<sup>[6]</sup> Understanding the interactive effects of compaction from wheel-traffic and tillage systems, and their impacts on microbial responses, is crucial for proper management and improvement of highly degraded soils in the southeastern U.S. The objective of this study was to assess the response of microbial populations to wheel-traffic in two tillage systems on a coarse-textured soil.

## MATERIALS AND METHODS

### Study Site and Design

This research was conducted as part of a continuing, long-term, traffic/tillage study (which has been previously described<sup>[7,8]</sup>) on a Norfolk loamy sand at the E.V. Smith Research Center of the Alabama Agriculture Experiment Station in east-central Alabama, USA (32°25.461' N, 85°53.403' N). The soil is highly compactible and has a well-developed hardpan at the 18–30 cm depth. Soil bulk density in the hardpan ranges from 1.51 to 1.76 Mg m<sup>-3</sup> with a predominance of sand in the profile. Other soil and residue properties for this study site have been previously described.<sup>[9]</sup>

Crop rotation consisted of corn, followed by a winter cover crop of crimson clover, then soybean, followed again by a winter cover crop of crimson clover. Aboveground soybean nongrain biomass averaged 3400 kg ha<sup>-1</sup> the previous fall and was not readily apparent at the start of this study due to overwinter decomposition. Cover crop was terminated with a burn-down herbicide (glufosinate-ammonium). Fertilizer and lime recommendations were based on standard soil-testing recommendations.

The experimental layout and design were previously described in detail by Reeves et al.<sup>[7]</sup> Experimental variables were with vs. without traffic and conventional tillage (disk harrow twice, chisel plow, field

cultivator) vs. no tillage. Thus, there were four combinations of traffic and tillage arranged in a split-plot design with four replicates; main plots were traffic and subplots were tillage.

Conventional spring tillage included disking twice to 10–12 cm, chisel plowing to 15–18 cm, and field cultivation to 10 cm. All plots received 25 mm irrigation water on 4 April 1995 (Day of Year [DOY] 94) between the disking and chisel plow operations.<sup>[9]</sup> The no-tillage treatment required no surface tillage. In both conventional and no-till plots, an eight-row (76-cm row width) no-till planter was used immediately behind the field cultivator to simulate the planting operation (planters were not loaded with seed). The planter was equipped with interlocking steel-fingered row cleaners set to float just above the soil surface to skim excessive residues from a 10-cm bandwidth over the planting row.

All tillage and planting operations for the without-traffic plots were done with an experimental wide-frame tractive vehicle (6.1 m wide) described by Monroe and Burt.<sup>[10]</sup> In the trafficked plots, a 4.6 Mg tractor with tires (470 mm × 970 mm) inflated to an average pressure of 125 k Pa immediately followed the wide-frame tractive vehicle to simulate tractor traffic in a field operation.

### Soil Sampling and Microbial Analysis

Soil samples from 0–2- and 2–4-cm depths were collected using a 17-mm diameter soil probe prior to spring tillage operations (DOY 90) and following disking (DOY 93), chisel plowing (DOY 94), and cultivator/planting operations (DOY 95) for a total of four sampling periods. Approximately 500 g of soil was collected by systematic sampling in an M-pattern across each plot at each sampling period. Soils were sealed in plastic bags and stored on ice until transported to the laboratory for analysis.

Soils were sieved (2 mm) and divided into four aliquots: one for determination of soil water content; one for determination of microbial biomass N; one for determination of dehydrogenase activity; and one for microbial characterization, including phospholipid ester-linked fatty acid (PLFA) analysis.<sup>[11]</sup>

Soil-water content was determined by placing approximately 1 g fresh-soil weight into an aluminum weighing pan, oven-drying at 105°C for 3 days, and recording the oven-dry weight. Percent of soil-water content was calculated as:  $(\text{fresh weight} - \text{oven-dry weight}) / \text{oven-dry weight} \times 100$ . Three replicate soil samples were used for each plot.

Microbial biomass N was determined using chloroform fumigation/extraction techniques as described by Horwath and Paul.<sup>[12]</sup> Fifty gram fresh soil was placed into 125 mL flasks, flasks were placed into vacuum desiccators with 50 mL chloroform, a vacuum was placed on the desiccator until the chloroform boiled ( $\approx 22$  mm Hg), and the desiccator was then sealed and incubated (25°C) for 24 h. Following removal of the chloroform, desiccators were flushed with clean air for a minimum of six times. Soil samples were removed, 50 mL of 0.5 M  $K_2SO_4$  were added to each flask, and flasks were placed on a rotary shaker at 200 rpm for 30 min. The resulting soil suspensions were then filtered through Whatman No. 42 filter paper in plastic funnels with the solution captured in 50 mL plastic vials. Vials were capped and frozen until N was determined using standard Kjeldahl procedures. Nitrogen was also determined on a replicate set of nonchloroform incubated soil samples following  $K_2SO_4$  extraction. Microbial biomass N was calculated as incubated N minus nonincubated N and expressed as  $\mu\text{g N}$  per gram soil dry weight. Three replicate soil samples were used for each plot at each sampling date.

Dehydrogenase activity, a measure of microbial respiration and a reliable index of microbial activity in soil,<sup>[13]</sup> was determined from modified procedures described by Tabatabai.<sup>[14]</sup> Sieved soil ( $\approx 1$  g) was placed in test tubes (15 mm × 100 mm), covered with 1 mL of 3% aqueous (w/v) 2,3,5-triphenyltetrazolium chloride and stirred with a glass rod. After a 96-h incubation (27°C), 10 mL of methanol was added to each test tube and the suspension was vortexed for 30 s. Tubes were then incubated for 1 h to allow suspended soil to settle. The resulting supernatant ( $\approx 5$  mL) was carefully transferred to clean test tubes using Pasteur pipets. Absorbance was read spectrophotometrically at 485 nm and formazan concentration was calculated using a standard curve produced from known concentrations of triphenyl formazan. Dehydrogenase activity was expressed as  $\mu\text{g}$  formazan per gram soil dry weight. Three replicate soil samples were used for each plot at each sampling date.

Phospholipid ester-linked fatty acid analysis was performed using the methods described by White and Ringelberg.<sup>[15]</sup> Briefly, soils were extracted with the single-phase chloroform-methanol-buffer system. The total lipid extract was fractionated into neutral lipids, glycolipids, and polar lipids by silicic acid column chromatography. Polar lipids were transesterified to the fatty acid methyl esters and analyzed by gas chromatography/mass spectroscopy using an Agilent 6890 series gas chromatograph interfaced to an Agilent 5973 mass selective detector with a 50-m nonpolar column (0.2 mm I.D., 0.11  $\mu\text{m}$  film thickness). Analysis was conducted using a temperature program of 100°C initial temperature, increased  $10^\circ \text{min}^{-1}$  to 150°C for 1 min, then by  $3^\circ \text{min}^{-1}$

to 282°C for 5 min with injector temperature at 270°C and detector temperature at 290°C. Total analysis time was 55 min.

### Data Analysis

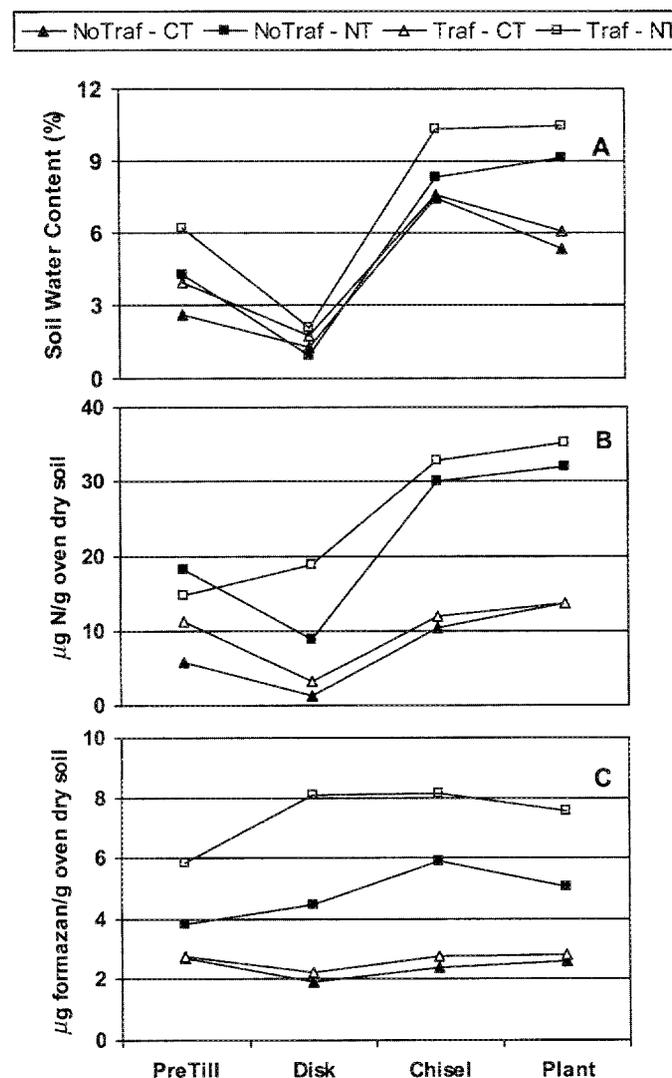
Data from the three replicate samples were averaged prior to analysis. All analyses were performed using the mixed procedure of the Statistical Analysis System.<sup>[16]</sup> Error terms appropriate to the split-plot design were used to test the significance of main-effects variables and their interactions. In all cases, differences were considered significant at the  $P < 0.05$  level; values that differed at the  $0.05 < P < 0.15$  level were considered trends.

## RESULTS AND DISCUSSION

Soil microbial measurements were consistently higher in the 0–2-cm compared to the 2–4-cm depth. Further, as no affect of treatment variables and no interactions were observed on any of the soil microbial assays or on soil-water content at the 2–4-cm depth, all data presented herein deal exclusively with the 0–2-cm soil depth.

Soil-water content was significantly higher in trafficked areas than in nontrafficked areas prior to spring tillage ( $P=0.03$ ) and following disking ( $P=0.01$ ). Traffic had no affect on soil-water content at the final two sampling periods, which occurred following irrigation (Fig. 1A). Compaction, due to wheel-traffic, can reduce soil porosity<sup>[17]</sup> and may have decreased water movement through the soil profile. No-till plots had higher soil-water content than conventional plots prior to tillage ( $P<0.01$ ), following chisel plowing ( $P=0.06$ ), and following the cultivator/planting operation ( $P<0.01$ ). Soil-water content was not different following disking due to the fact that the soil was extremely dry at this time ( $\approx 1.5\%$ ). Higher soil-water content in no-till plots is likely a result of extra residue from no-till operations, which can reduce evaporative soil-water loss.<sup>[18]</sup> Interactions between traffic and tillage on soil-water content were not significant at any sampling period. Soil-water content decreased up to the irrigation event, increased following irrigation, then began to decrease in conventional tillage plots but remained high in no-till plots (Fig. 1A). Again, this is most likely due to lowered water loss resulting from increased residue in no-till plots.

Traffic had little affect on microbial biomass N at any sampling period; however, there was a trend ( $P=0.08$ ) for trafficked areas to have



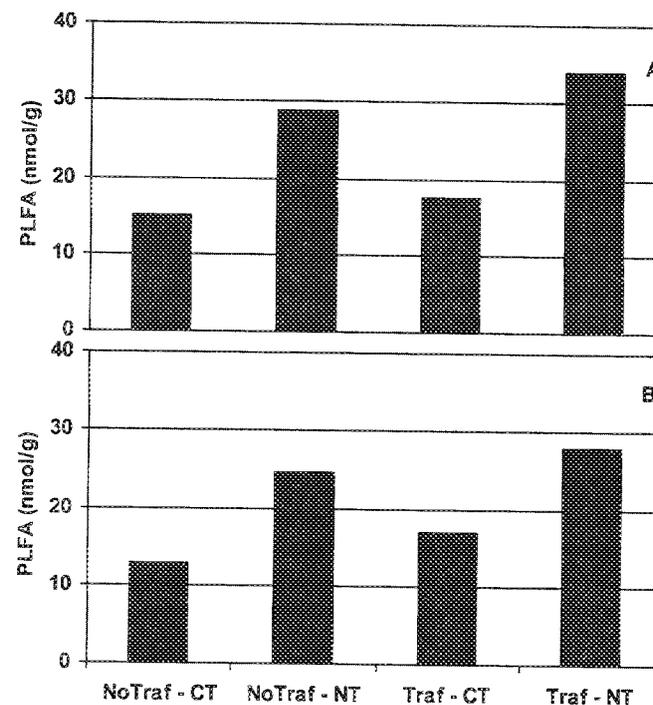
**Figure 1.** Interactive effects of traffic (NoTraf = no traffic; Traf = traffic) and tillage system (CT = conventional tillage; NT = no tillage) on soil water content (A), soil microbial biomass nitrogen (B), and dehydrogenase (C). Sampling periods on the X-axis are prior to spring tillage (PreTill), following disking (Disk), following chisel plowing (Chisel), and following cultivator/planter operation (Plant).

higher microbial biomass N following the disking treatment (Fig. 1B). Similarly, Lee et al.<sup>[2]</sup> observed higher microbial biomass carbon in trafficked compared with nontrafficked areas following tillage operations. Soil compaction can decrease available pore space, which slows the rate at which organic substrates are incorporated into and released from microbial biomass.<sup>[19]</sup> Microbial biomass N tended to be higher ( $P=0.12$ ) in no-till plots prior to spring tillage. Higher microbial biomass under no-till treatment has been previously reported<sup>[5]</sup> and is likely due to increased amounts of surface residue and its impacts on soil-moisture retention. Generally, microbial biomass increases following tillage events.<sup>[2,5]</sup> However, in the present study, concurrent measurements of microbial biomass N were consistently higher ( $P < 0.01$  in all cases) in no-till compared with conventional tillage plots (Fig. 1B). Extremely low soil-water content following disking likely restricted microbial response to this tillage operation (Fig. 1A). A similar explanation for a lack of response in soil CO<sub>2</sub> efflux following disking was reported by Reicosky et al.<sup>[9]</sup> Microbial biomass N increased in all plots following irrigation and subsequent tillage operations; however, the increase was much greater in no-till compared to conventional tillage plots. Again, the effects of no-till on soil-water content and surface residues is most likely responsible for this increase in microbial biomass N. No significant traffic by tillage interactions were observed for microbial biomass N at any sampling period.

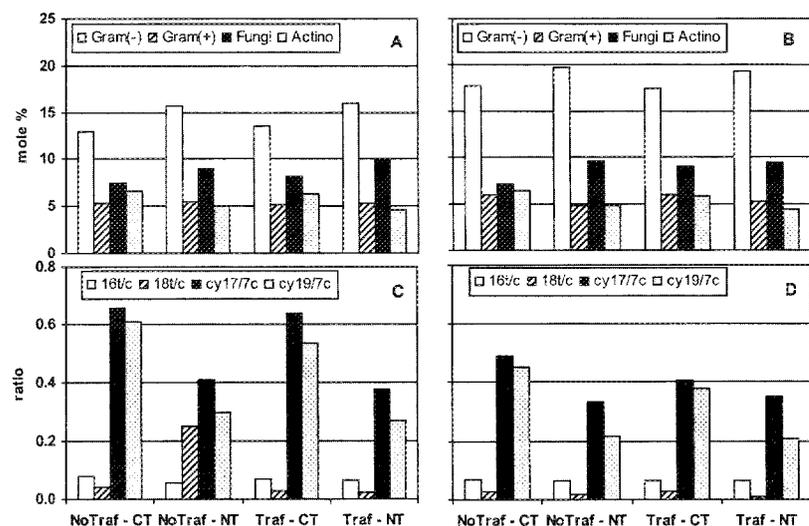
Microbial respiration, as determined by the dehydrogenase assay, can reflect changes in the size of the microbial population and/or can reflect changes in the respiratory activity of a given population size in response to changes in the soil environment. Microbial activity tended to remain relatively stable over time in the conventional tillage plots, indicating little impact of tillage events on either population size or respiratory activity (Fig. 1C). Significant traffic by tillage interactions for microbial activity were observed at all sampling periods except following chisel plowing; traffic had no effect in the conventional tillage plots, but this measure was significantly higher in trafficked areas compared with nontrafficked areas in the no-till plots ( $P \leq 0.01$  prior to tillage and following disking and cultivation/planting;  $P=0.07$  following chisel plowing). The increase in microbial respiration following the final two tillage events reflected the increase in microbial biomass, which occurred following irrigation. No-till plots generally exhibited significantly higher microbial activity than conventional tillage plots in both trafficked ( $P < 0.01$  in all cases) and nontrafficked areas ( $P=0.01$  to  $0.09$ ); however, the difference due to the tillage system tended to be greater in the trafficked areas. The higher soil-water content and greater amounts

of residue in no-till plots are most likely the reasons for higher microbial activity in these plots.

Soil samples were analyzed for PLFA for the sampling periods following disking and chisel plowing only. Conventional tillage reduced PLFA estimates of microbial biomass compared with the no-till treatment; PLFA estimates of microbial biomass were not affected by traffic (Fig. 2). Phospholipid ester-linked fatty acid estimates of microbial biomass were highly correlated with both microbial biomass N and dehydrogenase activity at both sampling periods ( $r^2 \geq 0.95$ ). Phospholipid ester-linked fatty acid analysis also demonstrated subtle shifts in microbial community composition due to differences in tillage systems. No-till plots tended to have higher populations of Gram(-) bacteria but lower populations of actinomycetes; Gram(+) bacteria and



**Figure 2.** Interactive effects of traffic (NoTraf = no traffic; Traf = traffic) and tillage system (CT = conventional tillage; NT = no tillage) on microbial biomass estimates based on phospholipid ester-linked fatty acid (PLFA) analysis following disking (A) and chisel plowing (B).



**Figure 3.** Interactive effects of traffic (NoTraf=no traffic; Traf=traffic) and tillage system (CT=conventional tillage; NT=no tillage) on relative microbial community composition (Gram(-) bacteria, Gram(+) bacteria, fungi, and actinomycetes) following disking (A) and chisel plowing (B), based on phospholipid ester-linked fatty acid (PLFA) analysis and on microbial physiological status following disking (C), and chisel plowing (D) using ratios of specific phospholipid fatty acids.

fungi were not significantly affected by tillage treatments (Fig. 3). Associated with increased biomass and relative percentage of Gram(-) bacteria, ratios of specific PLFAs suggested a decrease in the stress ratios for this functional group. No-till practices produced lower cyclopropyl/monoenoic precursor ratios, which generally correspond to a viable growing population. Conversely, higher ratios (as seen in conventional plots) are typically associated with old or stationary-phase organisms. Further, it has been shown that release of CO<sub>2</sub> per unit microbial biomass is higher for "young" compared with "mature" sites.<sup>[20]</sup> These factors might aid explanation of the dehydrogenase data discussed previously. That is, the low and stable microbial activity under conventional tillage might reflect a mature microbial population in a stationary phase of growth, while the increase under no-till would reflect a younger, more viable growing population. Phospholipid ester-linked fatty acid ratios tended to decrease in conventional plots between the disking and chisel

plowing treatments, possibly suggesting a change in the microbial population toward a more active phase of growth as a result of tillage.

Although *soil quality* is a very broad term relating to the chemical, physical, and biological properties of soil,<sup>[21]</sup> the size and activity of the soil microbial population is critical to overall soil use and sustainability.<sup>[6]</sup> Soil organisms contribute to the maintenance of soil quality through their control of many key processes (e.g., decomposition, nutrient cycling and availability, and soil aggregation) and may act as early indicators of changes in soil quality.<sup>[6]</sup> Microbial data from this study suggest that overall soil quality is improved in agricultural systems employing no-till operations.

## CONCLUSIONS

Traffic increased soil-water content prior to the irrigation event but had little effect on microbial biomass N. Traffic increased microbial activity only in no-till plots, which was likely a result of increased amounts of residue in these plots in conjunction with the more favorable soil-moisture conditions. The largest differences in microbial response observed in this study occurred between the conventional tillage and the no-till systems; soil-water content, microbial biomass N, PLFA estimates of microbial biomass, and microbial activity were all higher in no-till compared to conventional tillage plots. It was expected that tillage operations would increase soil microbe populations and/or activity and, while an increase in microbial biomass N was observed following chisel plowing, the low soil water-content prior to irrigation and during disking likely restricted this response. Data from this study suggest that conventional tillage results in a lower, more static, possibly more mature community of microbes while the microbial community under no-till appears to be a younger, more viable growing population. Finally, it appears that overall soil quality is improved by using no-till farming practices, at least in the upper layer of soil.

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