OCCASIONAL TILLAGE OF NO-TILL SYSTEMS TO IMPROVE CARBON SEQUESTRATION, AND SOIL PHYSICAL AND MICROBIAL PROPERTIES

by

J. Andrés Quincke

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Juan Andrés Quincke, Ph.D.

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Stratification of soil chemical, physical and microbial properties results from continuous, long-term no-till (NT) management. The top 5 cm of soil is typically improved for plant growth, but unimproved at deeper soil depths. This stratification may slow or limit long-term soil organic carbon (SOC) accumulation, increase runoff phosphorus (P) concentrations and losses, and may eventually constrain crop performance. Occasional tillage of NT systems refers to a one-time tillage operation, conducted once in 12 or more years to redistribute SOC and nutrients, and improve overall soil physical properties. It was hypothesized that occasional tillage can further improve agronomic and environmental performance of NT systems. Two experiments in long-term NT fields were conducted under rainfed corn or sorghum rotated with soybean in eastern Nebraska. Tillage treatments included: continuous no-till, tandem disk, 10-cm wide twisted shank-chisel, moldboard plow and mini-moldboard plow. The four studies presented in this dissertation addressed soil physical, chemical and micro-biological properties to (1) identify which tillage implements most effectively redistribute the soil properties, and (2) evaluate if occasional tillage causes short-term detrimental effects to the soil. Moldboard plowing was most effective in redistribution of soil properties. Labile organic matter pools and total SOC were lower in the 0- to 5-cm layer, and an enrichment in soil organic matter was achieved in the 5- to 20-cm layer with moldboard plow tillage. Total SOC and nitrogen in the profile were not reduced by tillage. In a simulated rainfall study, dissolved phosphorus concentration in runoff was reduced with moldboard plow tillage. The soil microbial community was affected by tillage, and its return towards the NT baseline in the years following tillage was quicker in the 0-5 cm depth than in deeper soil. Moldboard plowing caused a loss in mycorrhizal fungi, which did not recover after 2 years post-tillage.

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Lay abstract: No-till is practiced on 25% of Nebraska's cropland, and nearly 1 million hectares may have 15 years or more of continuous no-till by 2010. Soil organic matter and nutrients tend to accumulate in the top 5 cm of soil, often with depletion at deeper soil depths. Additionally, excessive phosphorus at the soil surface may increase potential for surface water pollution due to off-farm phosphorus losses, especially where high rates of manure are broadcast applied on no-till fields. Redistribution of the stratified soil layers may be a means to further improve no-till. *Occasional tillage of no-till systems*, conducted once in 12 or more years to invert the high soil organic carbon surface layer with less improved deeper soil, is proposed as a means to improve no-till systems. Two experiments in long-term no-till fields were installed under rainfed corn or sorghum rotated with soybeans in Eastern Nebraska. Tillage treatments included tillage with continuous no-till, tandem disk, 10-cm wide twisted shank-chisel, moldboard plow and mini-moldboard plow. The studies presented in this dissertation measured soil physical, chemical and micro-biological properties to (1) evaluate if occasional tillage causes short-term soil degradation; and to (2) identify which tillage implement(s) are most effective for improving long-term no-

till systems. A measurable enrichment in soil organic matter in deeper layers was achieved with the moldboard plow that lasted throughout the study. New accumulation of soil organic matter, however, could not be verified, but this may materialize only after several years. Moldboard plowing reduced the concentration of dissolved phosphorus in runoff. The soil microbial community was affected by tillage, and its return towards the NT baseline in the years following tillage was quicker in the 0-5 cm depth than in deeper soil. Moldboard plowing caused a loss in mycorrhizal fungi, which did not recover after 2 years post-tillage. However, there was no evidence that this effect was detrimental to soil fertility.

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GENERAL INTRODUCTION

> "Then I discovered, through certain tests, that the trouble lay in the operation which preceded all of the tests, namely plowing. (…) The moldboard plow has shown to be the villain of the world's agricultural drama." Edward H. Faulkner, 1943.

No-tillage agriculture: past and present

The above quotation from the book "Plowman's folly" is relevant for the history of no-till because it triggered a fundamental change in the way of thinking *a propos* agriculture. The stage was set for pioneering farmers and scientists to start experimenting with reducing tillage and striving to completely eliminate tillage in crop production.

The conversion to no tillage (NT) systems has resulted in benefits to the farmer including increased yields, improved soil quality, reduced production costs, and more time for fewer field operations (Behn, 1977; Phillips and Phillips, 1984). Moreover, the use of NT has shown off-farm benefits, such as less fuel consumption, reduced sediments and nutrients entering surface water bodies, and increased C sequestration during the initial period after conversion from conventionally tilled systems (Six et al., 2000).

No-till is practiced on 25% of the cropland, or on about 1.6 million hectares, in Nebraska as of 2004 (CTIC, 2004). From the NT-area in 1995 and assuming a 90% rate of adherence to continuous NT, we estimate that by 2010, nearly 1 million hectares will already have 15 years or more under continuous NT. Considering the Midwest region, the equivalent figure corresponds to 17.8 million hectares under NT in 2004 (24.8%), and by 2010 an estimated 11.2 million hectares will have 15 years or more of continuous NT (CTIC, 2004).

Inherent ecological limitations of long-term no-tillage

Long-term studies regarding ecological aspects have revealed that NT fields still remain far from ideal. Runoff concentrations of certain phosphorus (P) fractions, such as dissolved reactive P, can be significantly higher in NT than tilled soils (Sharpley and Smith, 1994; Daverede et al., 2003; Gaynor and Findlay, 1995). No-till tends to accumulate P and reduce P sorption capacity at the surface compared with deeper soil layers (Sims et al., 1998). Crop residues also contribute significant quantities of P to agricultural runoff (Schreiber and McDowell, 1985). Moreover, tillage studies are showing that the ecologically relevant process of C sequestration in NT soils may not continue in the long-term (Paustian et al., 1997). In 25-year-old tillage experiments, the most rapid changes in C levels under NT occurred during the first 10 years (Dick et al., 1991). Typically, after this early rapid gain in C following conversion to NT, the rate of C increase is much slower or even stabilizes (Paustian et al., 1997). Due to the fact that in NT above-ground C inputs are left on the soil surface, the differences in C content between NT and conventional till are greatest near the surface (Angers et al., 1997; Paustian et al., 1997). Six et al. (2002) suggested that increases in soil organic matter with increased residue inputs are limited by the capacity of the soil to store carbon in protective pools. Verma et al. (2005) measured CO_2 exchange and soil C stocks during 3 years in production-scale fields and concluded that NT agriculture did not result in soil C sequestration or possibly even causing losses of SOC.

The concept of "occasional tillage" of long term no-till systems

We use the term 'occasional tillage' to refer to the practice of conducting a single, one-time tillage operation in a system that is otherwise maintained without tillage. Occasional tillage in long-term no-till systems has not been explored as a way to further improve overall productivity and soil quality of agroecosystems.

There is limited information on the effects of a single tillage operation on soil that has been under continuous no-till. Pierce et al. (1994) proposed that plowing periodically should "break up" the typical nutrient and C stratification of NT, and found in the following year of a one-time moldboard plowing that soil organic carbon in the 0- to 5-cm layer decreased, while it increased in the 5- to 15-cm depth. However, these increases observed in lower depths had disappeared by year 5. In a study in the semiarid climate of western Nebraska, Kettler et al. (2000) assessed the influence of a one-time plowing (plus secondary tillage operations), for the purpose of weed control, on soil quality attributes of a silt loam soil that had been cropped in a sub-till or no-till system for more than 20 yr. Five years after tillage, soil organic carbon declined 20% in the 0- to 7.5-cm layer compared with undisturbed NT, but increased 15% in the 7.5- to 15-cm depth. Stockfisch et al. (1999) applied a single moldboard tillage event to a silt loam soil after 20 yr under minimum tillage in Germany, and observed after 5 months that SOC significantly declined by 5.3 Mg C ha^{-1} (11% reduction from the original C level) at the 0 – 30 cm depth. These authors concluded that organic matter gained as a result of long-term minimum tillage was completely lost by a single application of inversion tillage. VandenBygaart and Kay (2004), however, did not find a change in SOC, except for one sandy loam plot with low SOC, at 18 months after a one-time plowing of a field with 22 yrs of continuous NT in southern Ontario, Canada.

In general, after a field is converted to NT, the farmer expects to avoid tillage from then on, because the gains in soil quality are viewed as a trade-off with potential yield losses until the soil “reaches a new equilibrium” (Staley et al. 1988). *“While this new stage usually takes years to materialize, gained soil organic matter and the ‘new equilibrium’ are lost rapidly if tillage is resumed.”* This statement constitutes a common wisdom among NT-advocates, including consultants and scientists. However, it is all too vague in terms of the time frame in question, and nothing is said about how long the discontinuation of no-tillage has to be in order for the detrimental effects to take place. Interestingly, Faulkner’s success in recovering and managing soil was not based on avoiding tillage, but on adding large amounts of organic matter that was periodically incorporated with tillage.

The purpose of our research was to further enhance NT systems agronomically and environmentally, by managing the NT-inherent stratification of soil properties through a one-time tillage operation conducted infrequently.

Research approach

Tillage implements differ greatly in type and depth of soil disturbance. While there may be benefits to a deep, high mixing tillage operation, it may cause excessive soil disturbance. It is relevant to compare several tillage options. Two experimental sites were chosen for this study on fields under dryland long-term NT systems in eastern Nebraska. A randomized complete block design with split plots and four replications was used. The main factor was tillage treatment, with NT being the control, and the split-plot factor was broadcasted feedlot manure application before tillage.

The comprehensive hypothesis is that occasional tillage in NT can be conducted with little degradation of soil quality and mineralization of soil organic matter and that it will eventually result in net gains in soil quality and C sequestration.

Four areas of research were identified to test the above hypothesis: 1) soil organic C-pools and CO_2 emissions; 2) soil organic carbon sequestration; 3) soil hydraulic properties and potential losses of P to runoff; and 4) shifts in the composition of soil microbial communities. Specific hypotheses on these areas constitute the basis of the present research, and are addressed as chapters in this dissertation. Briefly, the topics and their respective significance are:

Chapter 1: *Occasional tillage of no-till systems: short-term losses of CO_2 and changes in labile carbon pools in the surface soil.* Soil disturbance caused by tillage is expected to bring about a short term increase in CO_2 emission and a change in SOC fractions. These are related to increased aeration of the soil and accelerated microbial decomposition of labile organic matter pools. However, can tillage be managed with negligible losses of organic carbon?

Chapter 2: *Potential gains in total soil organic carbon (SOC) with occasional tillage of long-term no-till systems*. Soil organic matter was successfully reallocated from the soil surface to deeper layers, leaving the surface soil less C-saturated. Therefore, the hypothesis is that C storage can be increased after the 2nd or 3rd crops following occasional tillage.

Chapter 3: *Soil hydraulic properties and phosphorus run-off as affected by one-time tillage of no-till.* Reducing P concentration in the soil surface layer can reduce potential losses of P caused by runoff, but benefits also depend on physical properties that govern infiltration. Tillage disrupts macropores through the action of disturbing and mixing soil, and may leave a less-aggregated soil on the surface. Therefore, the hypothesis is that tillage may have a counter effect on the potential losses of P in runoff. To test this hypothesis, soil infiltration rate and soil sorptivity were measured *in situ* and runoff samples were collected under simulated rainfall conditions.

Chapter 4: *Soil microbial community change and recovery dynamics after one-time tillage of a long-term no-till system*. A one-time tillage event is known to modify the composition of microbial biomass (Drijber, 2002). The hypothesis is that the largest impact of tillage will be noticed within the surface layer in the first year post-tillage, and that resuming no-till cropping will allow the soil microbial community to recover toward the continuous no-till condition.

Finally, in the synthesis of this dissertation, the results and observations are put into perspective. After these first few years of exploring the feasibility of further enhancing long-term NT systems by means of occasional tillage, can we still agree with Edward Faulkner’s statement that the moldboard plow is “the villain of the world’s agricultural drama”?

References

Angers, D.A., M.A. Bolinder, M.R. Carter, E.G. Gregorich, C.F. Drury, B.C. Liang, R.P. Voroney, R.R. Simard, R.G. Donald, R.P. Beyaert, and J. Martel. 1997. Impact of tillage practices on organic carbon and nitrogen storage in cool, humid soils of eastern Canada. Soil Tillage Res. 41:191-201.

Behn, E.E. 1977. More profit with less tillage. Wallace-Homestead Book Co. Des Moines, Iowa, U.S.A.

CTIC, 2005. National Crop Residue Management Survey Conservation Tillage Data. Conservation tillage information center, W Lafayette, IN, USA. http://www.ctic.purdue.edu/CTIC/CRM.html.

Daverede, I.C., A.N. Kravchenko, R.G. Hoeft, E.D. Nafziger, D.G. Bullock, J.J. Warren, and L.C. Gonzini. 2003. Phosphorus runoff: effect of tillage and soil phosphorus levels. J. Environ. Qual. 32:1436-1444.

Denef, K., J. Six, R. Merckx, and K. Paustian. 2004. Carbon sequestration in microaggregates of no-tillage soils with different clay mineralogy. Soil Sci. Soc. Am. J. 68:1935-1944.

Dick, W.A., E.L. McCoy, W.M. Edwards, and R. Lal. 1991. Continuous application of no-tillage to Ohio soils. Agron. J. 83:65-73.

Drijber, R.A. 2002. Microbial Signatures for Crop Production Systems. Proceedings of the OECD Workshop on Innovative Soil-Plant Systems for Sustainable Agricultural Practices, Izmir, June 3-7, 2002. J.M. Lynch, J.S. Schepers and I. Ünver (Eds). Tübitak, Turkey. pp. 132-146.

Faulkner, E.H. 1943. Plowman’s folly. University of Oklahoma Press. Norman, Oklahoma, U.S.A.

Gaynor, J.D., and W.I. Findlay. 1995. Soil and phosphorus loss from conservation and conventional tillage in corn production. J. Environ. Qual. 24:734-741.

Kettler, T.A., D.J. Lyon, J.W. Doran, W.L. Powers, and W.W. Stroup. 2000. Soil quality assessment after weed-control tillage in a no-till wheat-fallow cropping system. Soil Sci. Soc. Am. J. 64:339-346.

Paustian, K., H.P. Collins, and E.A. Paul. 1997. Management controls in soil carbon. p. 15-49. *In* E.A. Paul et al. (ed.) Soil organic matter in temperate ecosystems: Long term experiments in North America. CRC Press, Boca Raton, FL.

Pierce, F.J., M.-C. Fortin, and M.J. Staton. 1994. Periodic plowing effects on soil properties in a no-till farming system. Soil Sci. Soc. Am. J. 58:1782-1787.

Phillips, R.E., and S.H. Phillips. 1984. No-tillage agriculture, principles and practices. Van Nostrand Reinhold Company Inc. New York, USA.

Schreiber, J.D., and L.L. McDowell. 1985. Leaching of nitrogen, phosphorus and organic carbon from wheat straw residues: I. Rainfall intensity. J. Environ. Qual. 14:251-256.

Sharpley, A.N., and S.J. Smith. 1994. Wheat tillage and water quality in the Southern Plains. Soil Tillage Res. 30:33-48.

Sims, J.T., R.R. Simard, and B.C. Joern. 1998. Phosphorus loss in agricultural drainage: Historical perspective and current research. J. Environ. Qual. 27:277-293.

Six, J., E.T. Elliot, and K. Paustian, 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biology & Biochemistry 32:2099-2103.

Staley, T.E., W.M. Edwards, C.L. Scott, and L.B. Owens. 1988. Soil microbial biomass and organic component alterations in a no-tillage chronosequence. Soil Sci. Soc. Am J. 52:998-1005.

Stockfisch, N., T. Forstreuter, and W. Ehlers. 1999. Ploughing effects on soil organic matter after twenty years of conservation tillage in Lower Saxony, Germany. Soil Till. Res. 52:91-101.

VandenBygaart, A.J., and B.D. Kay. 2004. Persistence of soil organic carbon after plowing a long-term no-till field in southern Ontario, Canada. Soil Sci. Soc. Am. J. 68:1394-4102.

Verma, S.B., A. Dobermann, K.G. Cassman, D.T. Walters, J.M. Knops, T.J. Arkebauer, A.E. Suyker, G.G. Burba, B. Amos, H. Yang, D. Ginting, K.G. Hubbard, A.A. Gitelson, E.A. Walter-Shea. 2005. Annual carbon dioxide exchange in irrigated and rainfed maize-based agroecosystems. Agric. For. Meteorol. 131:77-96.

Chapter 1

Occasional tillage of no-till systems: short-term losses of CO_2 and changes in labile carbon pools in the surface soil.

Abstract: Established no-till systems present a challenging limitation because soil organic carbon (SOC) accumulates mostly in the top 5 cm and most of the soil quality improvement occurs in this surface soil layer. Occasional tillage of no-till systems is proposed as a single, one-time tillage, conducted once in 12 or more years, to mix the high SOC surface layer with less improved deeper soil. This also presents an opportunity to apply and incorporate lime or manure for further improvement of the soil. We hypothesized that occasional tillage will result in increased SOC in the long term, even though significant short-term C losses may occur following tillage. Two experiments in long-term NT fields were installed under rainfed corn or sorghum rotated with soybeans in Eastern Nebraska. Tillage treatments were done in spring or fall and included: continuous no-till, tandem disk, 10-cm wide twisted shank-chisel, moldboard plow and mini-moldboard plow. A portable infrared gas analyzer (Li-Cor 6200) with a 980-cm^3

chamber was used to monitor carbon dioxide (CO_2) efflux before and immediately after tillage and continuing up until planting. Profile distribution of SOC, particulate organic C (POC) and permanganate-oxidizable C were measured on samples taken at planting time at the 0-2.5, 2.5-5.0, 5.0-10, 10-20, and 20-30 cm depths. Some tillage operations effectively redistributed total and labile organic C with negligible CO_2 losses when compared to the undisturbed continuous no-till. Total and labile organic matter pools were reduced by a range of 24 to 88% in the 0-2.5 cm depth and increased by a range of 13 to 381% for the 5-10 cm depth for the various tillage operations, with moldboard plowing having the greatest effect. On an equivalent soil mass basis, tillage did not cause significant losses of organic matter or labile pools between tillage and planting of the next crop. It was concluded that the pronounced stratification of soil organic matter in long-term NT soil could be reduced most effectively by means of occasional moldboard plow tillage, without causing increased losses of labile organic matter pools.

Abbreviations: ARDC, Agricultural Research & Development Center; Ch20, 10-cm wide twisted shanks at 20 cm depth; Ch30, 10-cm wide twisted shanks at 30 cm depth; cPOM, coarse particulate organic matter (2000-250 μm); Disk, tandem disk; fPOM, fine particulate organic matter (250-53 μm); MiniMP, mini-moldboard plow; MP, moldboard plow; NT, continuous no-till; OxidC, permanganate-oxidizable carbon (0.02 *M* $KMnO_4$ solution); P, phosphorus; POM, particulate organic matter; RMF, Rogers Memorial Farm; SOC, soil organic carbon; SOM, soil organic matter; totPOM, total particulate organic matter (2000-53 μm).

1.1. INTRODUCTION

It is widely accepted that the conversion of conventional tillage systems to no tillage (NT) has resulted in benefits to the farmer including increased yields, improved soil quality, and reduced costs and time requirement due to fewer field operations (Behn, 1977; Phillips and Phillips, 1984). Off-farm benefits have also been acknowledged, such as less fuel consumption and increased C sequestration relative to tilled systems during the initial period after conversion from conventionally tilled systems (Six et al., 2000).

Ecological problems as well as benefits may result from no-till systems. Runoff concentrations of certain P fractions, such as dissolved reactive P, can be significantly higher in NT than tilled soils (Sharpley and Smith, 1994; Gaynor and Findlay, 1995; Daverede et al., 2003). No-till tends to accumulate P and reduce P sorption at the surface compared with deeper layers in the soil (Sims et al., 1998). Crop residues also contribute significant quantities of P to agricultural runoff (Schreiber and McDowell, 1985). Moreover, some tillage studies show that long-term C sequestration in NT soils does not occur (Paustian et al., 1997). In

25-year-old tillage experiments, the most rapid changes in C levels under NT, relative to conventional tillage, occurred during the first 10 years (Dick et al., 1991). Typically, after this early rapid gain in C following conversion to NT, the rate of C increase is much slower or stabilizes, and it is unlikely that using NT practices alone can achieve sustained C sequestration (Paustian et al., 1997). Because of the fact that in NT above-ground organic inputs are left on the soil surface, the differences in soil organic carbon (SOC) content between NT and conventional till are greatest near the surface (Doran, 1987; West and Post, 2002). Verma et al. (2005) measured CO_2 exchange and soil C stocks during 3 years in production-scale fields and concluded that NT agriculture did not result in soil C sequestration and possibly caused losses of SOC. However, this does not contradict findings that C balance is more positive, or less negative, with NT than with conventionally tilled systems.

We use the term ‘occasional tillage’ to refer to the practice of a single, one-time tillage operation, conducted once in 10-15 years, in a system that is otherwise maintained without tillage. There is limited information on the effects of a single tillage operation on soils that had not been tilled for 10 or more years. Pierce et al. (1994) proposed that plowing periodically should “break up” the typical stratification of soil properties that develops with NT. They measured soil properties at different depths after a one-time moldboard plowing of a loamy soil of Michigan that had been under NT for 7 years. One year after, SOC was 10% less in the plowed than the NT treatment for the 0- to 5-cm depth, and remained so in year 5 post-tillage. In lower depths (5-10 cm and 10-15 cm), SOC was significantly higher than NT one year after plowing, and more than 60% of this difference persisted by year 5. In a study in the semiarid climate of western Nebraska, Kettler et al. (2000) assessed the influence of plowing and secondary tillage operations, for the purpose of weed control, on soil quality attributes of a silt loam soil that had been cropped in sub-till or no-till systems for more than 20 years. Five years after tillage, SOC declined 20% in the 0- to 7.5-cm layer compared with undisturbed NT, but increased 15% in the 7.5- to 15-cm depth. Stockfisch et al. (1999) observed in Germany a significant decline in concentration of SOC after subjecting a silt loam soil that was 20 yr under minimum tillage to a one-time moldboard plowing. This decline amounted to about 50% within the 0-5 cm stratum, and less than 30% in the 10-20 cm layer. These authors concluded that organic matter gained as a result of long-term minimum tillage was completely lost by a single application of inversion tillage. On the other hand, VandenBygaart and Kay (2004) did not find a change in SOC, except for one sandy loam with low SOC, at 18 months after a one-time plowing of a field with 22 yrs of continuous NT in southern Ontario, Canada. The sandy loam reportedly had most of the gain in SOC under NT in the occluded particulate fraction (Yang and Kay, 2001). Therefore, SOC in this soil likely was more susceptible to tillage-induced mineralization, in comparison to fine-texture soils, which had most of SOC gains under NT in the humified fraction.

CO_2 flux measurements

The *closed chamber method* is a simple technique for the quantification of gas flux from soil (Parkin et al., 1996). Evolved CO_2 can be trapped in an alkali solution, and the amount of CO_2 determined by titration or by weighing soda lime traps. One advantage of the closed chamber method with alkali absorption is that it allows an integrated estimate of respiration during 8- to 12-h exposure periods (Franzluebbers et al., 2002). However, several sources of measurement bias exist with this technique (Parkin et al., 1996). Carbon dioxide accumulation in closed chambers is not linear, but rather the rate decreases with time due to reduced CO_2 gradient, because of CO_2 accumulation within the chamber headspace. Some methodological comparison studies found that the static chamber method with alkali absorption gave lower values compared with dynamic chamber methods under conditions of high soil respiration (Nay et al., 1994). In the early 1990s, the latter systems gained rapid acceptance when accurate and portable CO_2 analyzers became widely available (Hutchinson and Rochette, 2003).

A practical and cost-effective technique is the *Li-Cor 6200* infrared gas analyzer (LiCor Corp., Lincoln NE). This portable instrument takes real-time determinations of CO_2 flux by circulating the chamber headspace gas through the analyzer, requiring about 2 minutes per reading. As readings are taken *in situ*, no samples are taken, which implies the exemption from further lab work and from the risk of mishandling samples. Some disadvantages are known to the portable gas analyzer technique. For a quantitative estimation of the total CO_2-

loss over the course of the sampling period, several CO_2 determinations have to be taken. Calculations are based on linear interpolation and integration of the CO_2 flux over time. However, when changes in actual CO_2 flux occur faster than the time interval between readings, linear interpolations between readings will incorrectly resemble the true CO_2–release curve. As illustrated in Fig. 1.1, not measuring the flux at inflection points of the curve may result in both under- and overestimations.

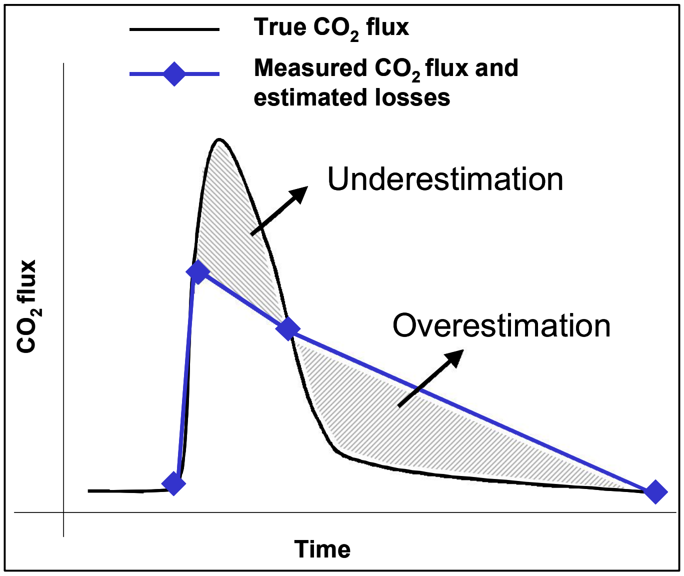

Figure 1.1. Under- and overestimations of tillage-induced CO_2 losses that can take place when using a dynamic chamber technique.

Moreover, a chamber of 10-cm diameter is small, considering the scale of ridges and soil clods that result from the use of different tillage implements (Reicosky and Lindstrom, 1993; Reicosky et al., 1997). A means to increase the sampling area and cope with higher variability is to take measurements at more points in the plot.

Soil organic matter fractions

Labile fractions of soil organic matter are more easily decomposed by soil microbes and lost due to tillage when compared to humified fractions (Woomer et al. 1994). Two labile organic C pools are commonly defined, with different decomposition rates and turnover times (Parton et al., 1987). The active C pool is composed mainly of microbial biomass, soluble carbohydrates, and exocellular enzymes. The second, slower functional pool is particulate organic matter (POM) and is defined as between 0.053 and 2.0 mm in size. Active organic C may be most affected by tillage in the short term (Weil et al., 2003), while microbial decomposition of POM may not become evident immediately after tillage. However, POM is much more sensitive to change than total soil organic matter, responding relatively rapidly to changes in land use and soil management (Gajda et al., 2001).

This study is one of a series to determine effects of occasional tillage on long-term no-till systems. The hypothesis is that with proper tillage operation(s) to reduce stratification of surface soil properties, net gains in soil quality, C sequestration, and crop yields can be achieved. This requires that the tillage operation needs to be relatively deep and inverting, but with minimal short-term losses of soil organic matter. Therefore, the specific objectives of this study were to determine tillage-induced CO_2 losses and to evaluate how labile carbon pools are affected by different tillage operations.

1.2. MATERIALS AND METHODS

1.2.1. Site descriptions and management

Field research was conducted at two sites in eastern Nebraska with long-term dryland NT-systems. Both soils are deep, well or moderately well drained, formed in loess on uplands, with moderately slow permeability. For both sites, mean annual precipitation is 737 mm (29 in) and mean annual temperature is 11 ºC. The sites differ in soil type and crop management history.

The first site was located at the Rogers Memorial Farm (RMF) of the University of Nebraska–Lincoln (UN–L) and approximately 16 km east of Lincoln, NE (40º50’44” N lat, 96º28’18” W long, 380 m altitude). The soil was a Sharpsburg silty clay loam (fine, smectitic, mesic Typic Argiudolls). The site occupied the area between two parallel steep-back sloped terraces that had been established in the mid-1960s. Conversion to NT occurred in 1991 with a soybean crop. The NT rotation was predominantly corn [*Zea mays* (L.)] and sorghum [*Sorghum bicolor* (L.) Moench.] rotated with soybeans [*Glycine max* (L.) Merr.]. The last crop before the establishment of the occasional tillage experiment was soybean. Controlled traffic has been practiced in order to minimize soil compaction. Lime has been surface broadcast applied without incorporation in the fall of 1997, 1999 and 2001 at a rate of 4.5 Mg ha^{-1} to correct pH problems. At this site, the research was under a grain sorghum – soybean rotation.

The second site was located at UN–L's Agricultural Research and Development Center (ARDC) near Mead, NE, and about 48 km north of Lincoln (41º10'48" N lat, 96º28'40" W long, 358 m altitude). The soil was a Yutan silty clay loam (fine-silty, mixed, superactive, mesic Mollic Hapludalfs). This soil series was originally classified as fine, montmorillonitic, mesic Typic Argiudolls but reclassified due to loss of the thick dark mollic surface horizon due to severe erosion. The site occupied a nonirrigated corner of a center pivot-irrigated field under a corn – soybean rotation, which was completely converted to NT in 1996. Since 1988 however, most but not all crops had also been under NT. No manure had been applied, but cattle have grazed on corn stalks. The only fertilizer applied in the system was anhydrous ammonia knifed in between rows, at an average rate of 146 kg N ha^{-1}. The last crop before the establishment of the occasional tillage experiment was corn.

1.2.2. Experimental design

The experimental design at both sites was a randomized complete block design with split plots and four replicates. Tillage treatment was the main plot factor at both sites, and time of tillage was another main plot factor at RMF. Before tillage, 87.4 kg P ha^{-1} was applied to sub-plots as composted feedlot manure to determine the interaction of a heavy P application with tillage.

1.2.3. Tillage treatments

The tillage treatments at the RMF site were moldboard plowing (MP); chisel plowing with 10-cm wide twisted shanks at either 20 or 30-cm depth (Ch20 or Ch30); tandem disking (Disk); and continuous no-till (NT) as the control. The second main plot factor at RMF was spring vs fall tillage. The no-till treatment had a manure-P treatment for the spring, but not for the fall. Main plots were 24 m long and 4.6 m wide.

At the ARDC site, tillage treatments were: moldboard plowing (MP); a chisel plowing with 10-cm wide twisted shanks at 30-cm depth (Ch30); tandem disking (Disk); mini moldboard plowing (miniMP); and continuous no-till (NT). Tillage was done only in the fall. Main plots are 24 m long and 6.1 m wide. A description of tillage treatments is given in Table 1.1 and pictures for the twisted shank chisel and the mini-moldboard plow are given in Figures 1.2 and 1.3.

Tillage operations were completed on 26 March 2003 (DOY 85) for the spring tillage (RMF-site only) and a one-pass tandem disk was done on the MP-plots on 23 April to reduce surface roughness. Fall-tillage treatments were performed on 24 Oct. 2003 (DOY 297) and on 26 Nov. 2003 (DOY 330) at the RMF- and ARDC-sites, respectively.

Table 1.1. Summary of tillage treatments and implements used at Roger Memorial Farm (RMF) and the Agricultural and Research Development Center (ARDC).

Tillage implement	Abbre-viation	Description	Depth (cm)	Speed of travel (km/h)	Site / season where implements were used †		
					RMF Spring	RMF Fall	ARDC Fall
Disk	Disk	Pull-type, tandem disk.	7 – 10	5.8 – 6.4	√	√	√
Twisted-shank chisel plow	Ch20	Mounted, with seven 10-cm wide twisted shanks on 36-cm centers, staggered on three bars.	20	6.3 – 7.2	√	√	
Twisted-shank chisel plow	Ch30	Mounted, with seven 10-cm wide twisted shanks on 36-cm centers, staggered on three bars.	30	1.5	√	√	√
Mini-moldboard plow	miniMP	Semi-mounted moldboard plow, with eight 41-cm wide bottoms. Moldboards are of reduced size and cause only partial inversion of soil.	13 – 20	4.2 – 4.4			√
Moldboard plow	MP	Mounted moldboard plow, with four 41-cm-wide bottoms.	20	4.6 – 4.8	√‡	√	√
Continuous no-till	NT	Control. Soil and residue as left by harvest equipment from preceding crop.	-	-	√	√	√

† Dates of tillage were respectively Mar. 26 and Oct. 24 2003 for spring and fall tillage at Rogers Memorial Farm (RMF/Spring and RMF/Fall), and Nov. 26 2003 at UN–L’s Agricultural Research & Development Center (ARDC/Fall).

‡ In the case of RMF/Spring, a tandem disk harrow was needed after MP to smooth the soil surface prior to planting. No secondary tillage was needed after fall moldboard plowing.

Figure 1.2. The chisel plow to 20-cm and 30-cm (Ch20 and Ch30) used in this study was equipped with 10-cm-wide twisted shanks that bring some deep soil to the surface.

Figure 1.3. The mini-moldboard plow (miniMP) in operation adjacent to a plot tilled with a regular moldboard plow (MP). Note the difference in residue cover and soil roughness with the two implements.

1.2.4. Field and laboratory procedures

Tillage-induced CO_2-efflux: A LI-6200 Portable Photosynthesis System was connected to a 995-cm^3 chamber with a 78.5-cm^2 circular cross section (Norman et al., 1992). This portable gas exchange system measures the CO_2 concentration of air passing through an analyzer by comparing the amount of infrared radiation absorbed to that absorbed by a reference cell of known CO_2

concentration. The beveled bottom rim of the chamber was pressed 0.5 cm directly into the soil during measurements. After placing the chamber on the soil, the CO_2 concentration within the system was drawn below ambient by passing the air coming from the chamber through a soda lime scrub. Soil surface CO_2 flux was determined by calculating the slope of the CO_2 concentration versus time curve over three 15-second intervals when the CO_2 concentration of the system was near ambient. Within each plot, the first set of flux measurements was completed on three measurement points within the first 5 min of tillage operation. These were marked with flags so that subsequent measurements could be repeated at the same points within each plot. In general, 3 to 5 cycles of measurements were made on the day of tillage, after which one cycle was done every 2 – 7 days until planting.

Soil sampling and analyses: Soil samples were taken from all treatments prior to or shortly after planting at depths of 0-2.5, 2.5-5, 5-10, 10-20, and 20-30 cm. Twelve cores were taken per plot with a 1.8-cm diameter probe.

Particulate organic matter (POM): Determination of coarse (2000-250 μm) and fine (250-53 μm) particulate organic matter (cPOM and fPOM, respectively) was assessed by weight-loss on ignition (450 ºC) according to Cambardella et al. (2001) for each. Briefly, 30-g air-dried soil was dispersed with 90 ml of 5-g L^{-1} sodium hexametaphosphate in 140-ml containers, and shaken on a reciprocal shaker overnight at about 140 rpm. The dispersed soil was passed through a nest of the 2000, 250, and 53 μm sieves by rinsing with tap water sequentially from the larger to smaller mesh sieves until rinsate was clear. Plant fragments (>2 mm), as well as material that passed through the 53-μm sieve, were discarded since they were not defined as POM. The material retained in the 250- and 53-μm sieves was back-washed into pre-weighed small aluminum pans and dried at 55 ºC for at least 24 h and resulting fractions were cPOM and fPOM, respectively. The exact weight of each dried sample was recorded to 10^{-4} g, and samples were then placed in a muffle furnace and heated for 4 h after the oven temperature reached 450 ºC. After cooling in a desiccator containing dry silica gel, the weight of the ignited sample was recorded to 10^{-4} g. The loss of mass after ignition (i.e. the subtraction of the two recorded weights) represents the mass of POM obtained in the corresponding fraction from the soil sample mass initially dispersed. POM content was calculated as the ratio between POM and soil sample masses, and expressed as mg POM g^{-1} soil. The summation of fPOM and cPOM contents gave the total POM content (totPOM) for the soil sample.

Permanganate-oxidizable carbon (OxidC): The procedure developed by Weil et al. (2003), modified from Blair et al. (1995) was used to measure permanganate-oxidizable carbon. Briefly, 5 g of air-dried soil were shaken vigorously (about 100 strokes min^{-1}) for 2 min in 20 mL of a solution containing 0.02 M $KMnO_4$ and 0.1 M $CaCl_2$. The $CaCl_2$ in the solution causes the soil to flocculate, leaving a clear supernatant solution after 5–10 min of settling. Absorbance at 550 nm was recorded from this solution after diluting it 100 times. The bleaching of the purple

$KMnO_4$ color (reduction in absorbance) is proportional to the amount of oxidizable C in soil so that the lower the absorbance reading, the greater the amount of oxidizable C in the soil. To estimate the amount of C oxidized, it was assumed that 1 mol MnO_4 was consumed (reduced from Mn^{7+} to Mn^{2+}) in the oxidation of 0.75 mol (9000 mg) of C (Blair et al., 1995).

Soil bulk density: Preliminary observations on our experimental sites indicated the unsuitability of the soil core method for surface layers in plots that had been tilled. Considerable compression occurred on the surface soil when the probe was pushed into the soil. Therefore, the following sand-method was used for the strata 0- to 2.5-cm and 2.5- to 5-cm. If necessary, the site's soil surface was gently leveled with the edge of a wooden board before a metal cylinder (15 cm inner diameter and 2.5 cm height) was pressed into the soil. The soil was then carved out with a hand shovel and a spatula and placed separately in labeled paper bags. While the metal cylinder prevented adjacent soil from falling into the formed cavity, the latter was then lined with a fine plastic sheet and filled with sand. The volume of the removed soil was estimated from the volume of the sand used, which was measured by pouring it from the plastic sheet into a graduated cylinder. This procedure was repeated for the 2.5- to 5-cm increment. A push probe with an inserted plastic tube liner was used for the next increments, 5- to 10-cm, 10- to 20-cm, and 20- to 30-cm. After taking the core, the plastic tube was removed with the soil cylinder inside. Empty space in the tubes was filled with cotton balls to prevent the core from falling apart during handling, and then the tubes were capped and labeled. In the laboratory, soil cores were slid out of the tube and cut into segments according to the corresponding increments. Since the last increment was frequently less than 10 cm, its length was measured with a ruler to calculate more precisely its volume. The mass of the soil from all five depths was obtained after oven-drying at 105 ºC for 48 h. Measurements and samples were taken at three locations per plot and the arithmetic mean was computed for each plot.

Table 1.2. Bulk density for fall tillage treatments for each sampling stratum at Rogers Memorial Farm (RMF) and the Agricultural and Research Development Center (ARDC)†.

Depth	Soil bulk density					
	No-till	Disk	Chisel 20‡	Chisel 30	mini-Moldboard‡	Moldboard
cm	---------- g cm^{-3} ----------					
	RMF/Fall§					
0 – 2.5	0.87	0.80	0.82	0.84	-	0.89
2.5– 5	1.19	0.93	0.88	0.92	-	0.86
5 – 10	1.44	1.30	1.28	1.18	-	1.15
10 – 20	1.32	1.30	1.31	1.23	-	1.19
20 – 30	1.33	1.33	1.29	1.28	-	1.30
	ARDC/Fall					
0 – 2.5	0.84	0.78	-	0.80	0.82	0.80
2.5– 5	1.09	1.15	-	0.95	0.95	1.02
5 – 10	1.33	1.33	-	1.19	1.21	1.18
10 – 20	1.30	1.28	-	1.28	1.22	1.16
20 – 30	1.31	1.31	-	1.31	1.30	1.36

† Dates of tillage were respectively Mar. 26 and Oct. 24 2003 for spring and fall tillage at Rogers Memorial Farm (RMF/Spring and RMF/Fall), and Nov. 26 2003 at UN–L's Agricultural Research & Development Center (ARDC/Fall).

‡ Chisel plow at 20 cm was not used at ARDC, while mini-moldboard plow was not used at RMF.

§ Bulk density values for RMF/Spring were assumed as equal to RMF/Fall.

Soil bulk density was assessed at the same increments as for total and labile organic matter pools for depths of 0-2.5, 2.5-5, 5-10, 10-20, 20-30 cm. Two blocks in each site were sampled in summer 2004 for the non-manured sub-plots of fall-tillage treatments. The bulk density was averaged across blocks for each depth, tillage treatment and site (Table 1.2). For spring-tillage treatments at RMF in 2003, we used bulk density data from the respective operations in the fall.

1.2.5. Calculations and Statistical Analyses

The CO_2 fluxes were calculated on a unit horizontal land area assuming that soil roughness within the chamber's measurement area (78.5 cm^2) is representative of the entire tilled area. The cumulative amount of CO_2 evolved after tillage was calculated for 5 min, 4 h, 6 d and for 30 d following tillage using numerical integration (trapezoid rule). This method assumes linear interpolation between the measured fluxes over the time interval (Reicosky, 1997).

Soil organic matter, totPOM, fPOM, cPOM and OxidC data were used in combination with bulk density data (obtained for same strata) to estimate the mass that is contained in the topsoil per unit area. Two distinct methods were used. The common approach is the fixed-depth method and simply uses the concentration of the element or pool of interest, bulk density and the thickness of each sampling stratum:

$$Stock_D = \sum (\text{conc}_i * \rho_i * t_i) \qquad \text{[Eq. (1)]}$$

where Stock_D is the mass of SOM, fPOM, cPOM or OxidC contained per unit area to a soil depth D; each stratum i has a specific concentration (conc_i), bulk density (ρ_i) and thickness (t_i). Soil depth D was established at 20 cm and required using 4 of the 5 sampled strata (recall that the 5th stratum was 20- to 30-cm). Values for t_i were 2.5, 2.5, 5 and 10 cm for the 1st to the 4th strata (for a total depth of 20 cm). Note that the product ($\rho * t_i$) represents the mass of soil per unit area [(Mg m^{-3})* m * (10^3 kg Mg^{-1})= kg soil m^{-2}] in each sampling stratum, and is multiplied by conc_i (g SOM kg^{-1} soil) to give g m^{-2}.

Treatments with higher bulk density contain a higher soil mass per unit area within a given sampling depth, as is explicit in Eq. (1). As a result, stock estimations on a fixed-depth basis are biased in favor of those conditions that increase soil bulk density (Ellert and Bettany, 1995; VandenBygaart and Kay, 2004). The equivalent soil-mass method has been developed to correct for this limitation by inter- or extrapolating a calculated stock to an established dry soil mass per unit area (kg dry soil m^{-2}). Calculations for this method were following Gifford and Roderick (2003). To maintain consistency of abbreviations in the present paper, their equation is rewritten as follows:

$$Stock_{250kg} = Stock_{10cm} + \frac{Stock_{20cm} - Stock_{10cm}}{Ms_{20cm} - Ms_{10cm}}(250 - Ms_{10cm}) \qquad \text{[Eq. (2)]}$$

where $Stock_{250kg}$ is the mass of SOM, fPOM, cPOM or OxidC contained within a cumulative soil mass of 250 kg m^{-2}; $Stock_{10cm}$ and $Stock_{20cm}$ are the cumulative masses of these pools to a fixed depth of 10 and 20 cm, respectively [calculated according to Eq. (1)]; Ms_{20cm} and Ms_{10cm} are cumulative dry soil masses to a fixed depth of 10 and 20 cm (in units of kg m^{-2}); and 250 kg cumulative dry soil mass per m^2 is the reference soil mass chosen for this study and approximately corresponds to 20 cm depth.

Data for these variables, and for soil C variables, were analyzed using analysis of variance (ANOVA) and mixed model procedures in SAS (SAS Inst., 1989) appropriate for a randomized complete block split plot design with four replications. Separate ANOVAs were run for each sampling depth and site, treating replications as random effects, while tillage and manure treatments were fixed effects. Whenever a significant ANOVA occurred, means were separated using the LSD option (α=0.05).

1.3. RESULTS

1.3.1. Cumulative CO_2 losses

Cumulative CO_2 losses were calculated for 5 minutes, 4 hours, 6 days and 30 days after tillage, and are presented in Table 1.3. **Five-minute losses** (i.e. immediately after tillage) were greater with all tillage operations than with no-till in the fall at RMF. The results were similar at the ARDC with higher CO_2 losses with moldboard (MP) and mini-moldboard (miniMP) tillage, but not for spring tillage at RMF. **Four-hour losses** followed this trend, but losses with MP tillage were not significantly higher than with NT. The miniMP had the highest 4-h losses and was statistically different than NT. Chisel plowing at 20 cm depth (Ch20) at RMF in the spring and chisel plowing at 30 cm depth (Ch30) at the ARDC site had higher 4-h losses than NT. Cumulative losses during **six days** were not affected by tillage treatment except for increased loss with spring Ch20 at RMF. Ch20 had the highest **thirty-day losses**, calculated for spring and fall at RMF. Disk and Ch30 in the fall were also higher than NT. Both in the spring and fall at RMF, MP tended to have lower 30-d losses than NT, although this effect was not statistically significant.

Table 1.3. Cumulative CO_2 losses after a one-time tillage event at three site/seasons of this study†.

			Integration time since tillage operation							
Site / tillage time	Tillage implement		5 min		4 h		6 d		30 d	
			----------------------		g CO_2 m^{-2}		----------------------			
RMF / Spring 2003	No-till		0.022		1.29	b‡	30	b	115	bc
	Disk		0.030		1.50	b	34	b	131	b
	Chisel 20 cm		0.038		2.10	a	41	a	181	a
	Chisel 30 cm		0.032		1.50	b	34	b	115	bc
	Moldboard plow		0.029		1.58	b	35	ab	93	c
RMF / Fall 2003	No-till		0.021	b	0.82		23		82	b
	Disk		0.048	a	1.66		32		120	a
	Chisel 20 cm		0.054	a	1.80		38		121	a
	Chisel 30 cm		0.060	a	1.60		31		120	a
	Moldboard plow		0.057	a	1.54		23		65	b
ARDC / Fall 2003	No-till		0.006	c	0.34	c	8		-	
	Disk		0.015	bc	0.41	bc	8		-	
	Chisel 30 cm		0.027	abc	0.88	ab	10		-	
	Mini-moldboard plow		0.036	ab	1.04	a	11		-	
	Moldboard plow		0.051	a	0.86	abc	14		-	

† Dates of tillage were respectively Mar. 26 and Oct. 24 2003 for spring and fall tillage at Rogers Memorial Farm (RMF/Spring and RMF/Fall), and Nov. 26 2003 at UN–L's Agricultural Research & Development Center (ARDC/Fall).

‡ Letters denote significant differences between tillage treatments within a site/season (α=0.05), and no letters were used where the effect of tillage was not significant.

Table 1.4. Summary of the analysis of variance for the effects of tillage implement, manure application and their interaction at three site/seasons †.

Sampling depth	RMF / Spring†			RMF / Fall			ARDC / Fall		
cm	Tillage	Manure	Interaction	Tillage	Manure	Interaction	Tillage	Manure	Interaction
	Soil Organic Matter								
0 – 2.5	>0.001	0.320	0.564	<0.001	0.572	0.463	<0.001	0.337	0.382
2.5 – 5	>0.001	0.054	0.478	0.005	0.448	0.769	0.042	0.229	0.388
5 – 10	0.004	0.185	0.817	0.082	0.952	0.674	0.143	0.218	0.241
10 – 20	0.005	0.492	0.834	0.965	0.617	0.055	0.084	0.221	0.010
20 – 30	0.783	0.916	0.015	0.721	0.890	0.173	0.441	0.415	0.135
	Total Particulate Organic Matter								
0 – 2.5	0.003	0.316	0.153	>0.001	0.888	0.896	<0.001	0.009	0.713
2.5 – 5	0.001	0.469	0.644	0.033	0.633	0.575	0.018	0.381	0.940
5 – 10	0.128	0.648	0.150	0.005	0.969	0.825	0.073	0.475	0.214
10 – 20	0.002	0.236	0.154	0.651	0.385	0.386	0.539	0.051	0.033
20 – 30	0.667	0.676	0.085	0.625	0.595	0.277	0.534	0.485	0.713
	Fine Particulate Organic Matter								
0 – 2.5	0.004	0.957	0.130	0.002	0.995	0.843	0.034	0.059	0.731
2.5 – 5	0.001	0.682	0.650	0.124	0.769	0.552	0.129	0.610	0.725
5 – 10	0.141	0.231	0.099	0.005	0.712	0.446	0.513	0.452	0.133
10 – 20	0.018	0.055	0.093	0.689	0.385	0.382	0.624	0.113	0.119
20 – 30	0.804	0.664	0.013	0.668	0.688	0.396	0.306	0.668	0.3491
	Coarse Particulate Organic Matter								
0 – 2.5	0.018	0.025	0.717	<0.001	0.461	0.938	<0.001	0.048	0.024
2.5 – 5	0.011	0.256	0.589	0.059	0.491	0.579	0.027	0.299	0.280
5 – 10	0.177	0.267	0.799	0.036	0.426	0.910	0.001	0.831	0.946
10 – 20	0.019	0.232	0.287	0.106	0.671	0.861	0.006	0.100	0.016
20 – 30	0.565	0.307	0.504	0.972	0.291	0.392	0.513	0.577	0.779
	Oxidizable Carbon								
0 – 2.5	<0.001	0.139	0.387	<0.001	0.596	0.766	0.017	0.182	0.982
2.5 – 5	<0.001	0.504	0.665	0.009	0.630	0.725	0.044	0.855	0.896
5 – 10	0.003	0.827	0.479	<0.001	0.617	0.601	0.268	0.434	0.298
10 – 20	0.411	0.443	0.385	0.083	0.298	0.275	0.042	0.305	0.305
20 – 30	0.159	0.861	0.084	0.811	0.584	0.018	0.251	0.678	0.235

† Dates of tillage were respectively Mar. 26 and Oct. 24 2003 for spring and fall tillage at Rogers Memorial Farm (RMF/Spring and RMF/Fall), and Nov. 26 2003 at UN–L's Agricultural Research & Development Center (ARDC/Fall).

A separate ANOVA was done for each depth and site/season.

Table 1.5. Concentration of soil organic matter, total POM, fine POM, coarse POM and oxidizable C within each sampling depth as affected by tillage implement at the three sites/season of this study†.

Sampling depth	RMF / Spring					RMF / Fall					ARDC / Fall				
cm	NT	Disk	Ch20	Ch30	MP	NT	Disk	Ch20	Ch30	MP	NT	Disk	Chisel	MiniMP	MP
	Soil Organic Matter (SOM), mg SOM g^{-1} soil														
0 – 2.5	43 a‡	44 a	39 b	38 b	33 c	37 ab	39 a	35 bc	33 c	28 d	34 a	35 a	33 a	31 b	28 b
2.5 – 5	35 b	40 a	39 a	40 a	33 c	32 bc	34 ab	35 a	36 a	30 c	28 b	30 ab	32 a	31 a	29 b
5 – 10	32 b	34 a	35 a	35 a	35 a	28	29	29	31	31	27	26	29	28	29
10 – 20	31 bc	33 a	30 c	31 ab	32 ab	28	28	28	29	28	25	25	26	25	27
20 – 30	29	30	29	28	28	26	25	26	25	24	22	21	21	21	20
	Total Particulate Organic Matter (totPOM), mg POM g^{-1} soil														
0 – 2.5	15.0 ab	17.9 a	9.9 bc	10.9 b	4.7 c	13.7 ab	15.5 a	9.6 b	10.7 b	2.9 c	8.2 a	8.2 a	8.4 a	5.7 b	2.7 c
2.5 – 5	7.0 bc	10.5 a	9.9 ab	9.7 ab	4.1 c	5.7 b	7.5 ab	9.6 a	10.1 a	4.2 b	2.9 bc	3.6 abc	4.8 a	4.4 ab	2.3 c
5 – 10	3.6	4.3	6.1	6.1	4.7	2.5 b	2.6 b	2.4 b	5.3 a	5.9 a	1.5	1.1	2.2	2.5	2.7
10 – 20	2.2 b	2.1 b	1.9 b	2.4 b	3.9 a	3.9	2.1	1.6	2.7	3.0	1.1	1.0	1.0	1.0	1.7
20 – 30	2.2	2.8	4.5	2.7	2.4	1.7	1.8	1.8	2.2	2.5	1.1	0.8	0.7	0.7	1.1
	Fine Particulate Organic Matter (fPOM), mg POM g^{-1} soil														
0 – 2.5	11.4 ab	13.5 a	7.1 c	7.7 bc	4.0 c	10.5 a	10.8 a	7.0 a	8.0 a	2.5 b	5.8 a	5.3 a	5.6 a	4.0 ab	2.1 b
2.5 – 5	5.9 a	8.3 a	7.6 a	6.9 a	2.8 b	4.9	5.7	7.4	7.8	3.4	2.2	2.5	3.3	2.9	1.6
5 – 10	2.7	3.6	4.7	4.6	3.7	2.2 b	2.2 b	1.8 b	4.1 a	4.2 a	1.3	0.9	1.6	1.7	1.8
10 – 20	1.9 b	1.9 b	1.6 b	1.9 b	3.0 a	3.5	1.9	1.3	2.2	2.3	1.0	0.8	0.8	0.8	1.4
20 – 30	1.6	2.6	2.4	2.2	2.0	1.5	1.6	1.5	1.9	2.3	2.0	0.8	0.6	0.6	0.8
	Coarse Particulate Organic Matter (cPOM), mg POM g^{-1} soil														
0 – 2.5	3.5 a	4.5 a	2.8 a	3.2 a	0.6 b	3.2 b	4.7 a	2.6 b	2.5 b	0.4 c	2.4 ab	2.9 a	2.8 a	1.6 bc	0.6 c
2.5 – 5	1.1 c	2.2 ab	2.3 a	2.8 a	1.2 bc	0.8 b	1.8 ab	2.2 a	2.3 a	0.8 b	0.6 b	1.1 ab	1.5 a	1.5 a	0.7 b
5 – 10	0.9	0.7	1.5	1.5	1.0	0.3 b	0.4 b	0.6 b	1.2 ab	1.7 a	0.2 b	0.2 b	0.6 ab	0.9 a	0.9 a
10 – 20	0.3 b	0.2 b	0.3 b	0.5 b	0.9 a	0.3	0.2	0.3	0.4	0.7	0.1 b	0.2 b	0.2 b	0.2 b	0.4 a
20 – 30	0.6	0.2	2.0	0.3	0.4	0.2	0.2	0.3	0.3	0.3	0.2	0.2	0.1	0.1	0.2
	Oxidizable Carbon (OxidC), mg C g^{-1} soil														

0 – 2.5		0.51	a	0.50	a	0.43	b	0.41	b	0.28	c		0.58	ab	0.59	a	0.53	bc	0.50	c	0.32	d		0.45	a	---		---		0.36	b	0.27	b
2.5 – 5		0.38	a	0.43	a	0.41	a	0.43	a	0.27	b		0.45	bc	0.45	bc	0.55	a	0.54	ab	0.37	c		0.34	b	---		---		0.43	a	0.31	b
5 – 10		0.29	b	0.28	b	0.36	a	0.36	a	0.34	a		0.35	b	0.35	b	0.41	a	0.42	a	0.43	a		0.27		---		---		0.30		0.33	
10 – 20		0.25		0.24		0.23		0.21		0.24			0.29		0.31		0.33		0.35		0.34			0.21	ab	---		---		0.15	b	0.26	a
20 – 30		0.16		0.19		0.19		0.14		0.13			0.24		0.22		0.24		0.24		0.23			0.12		---		---		0.08		0.13	

† Dates of tillage were respectively Mar. 26 and Oct. 24 2003 for spring and fall tillage at Rogers Memorial Farm (RMF/Spring and RMF/Fall), and Nov. 26 2003 at UN–L's Agricultural Research & Development Center (ARDC/Fall).

‡ Letters denote significant differences between tillage treatments within a site/season and within a sampling stratum (α=0.05).

1.3.2. Redistribution of soil organic matter within the profile

Tillage implements and manure application were compared by depth increments for concentration of: total soil organic matter (SOM); total, fine and coarse particulate organic matter (totPOM, fPOM and cPOM, respectively); and oxidizable C (OxidC) (Table 1.4). Because the interaction of tillage with manure application was not often significant for all soil organic matter fractions, differences between tillage treatments can be visualized by exploring simple effects (Table 1.5.).

Total soil organic matter concentration in the 0- to 2.5-cm stratum was lower for MP than NT in all three sites/tillage times and in the 2.5- to 5-cm stratum with spring MP tillage at RMF (Table 1.5). At the 5- and 20-cm depths, however, MP tended to increase SOM. Tillage with miniMP reduced SOM in the 0- to 2.5-cm stratum when compared to NT but increased SOM in the 2.5- to 5-cm increment. Chisel plowing also reduced SOM in the 0- to 2.5-cm stratum in both spring and fall, but not at ARDC. In the 2.5- to 5-cm stratum, Ch20 and Ch30 consistently had higher SOM than NT and MP. Disking did not reduce SOM in the 0- to 2.5-cm layer and tended to increase it in the 2.5- to 5-cm stratum. In soil strata between 5 and 30 cm, SOM for Disk, Ch20, Ch30 and miniMP tended to have SOM intermediate between NT and MP but without significant differences.

Total POM was lower for MP than NT in the surface stratum in all three sites/tillage times. In the next stratum (2.5- to 5-cm), MP tended to reduce totPOM relative to NT but the difference was not significant. Between 5- and 20-cm, totPOM was occasionally higher with MP than NT. With miniMP, totPOM was less than with NT but more than MP. In the 2.5- to 5-cm stratum, miniMP had higher totPOM than MP. Chisel plowing showed higher totPOM than MP and miniMP, although it tended to be lower than NT. In the 2.5- to 5-cm layer, Ch20 and Ch30 had higher totPOM than NT and MP. In strata from 5 to 30 cm, totPOM for Ch20, Ch30 and miniMP did not differ from NT. Likewise, disking did not cause totPOM to change compared to NT at any depth.

Fine POM was lower for MP than NT in the surface stratum in all three sites/tillage times. This reduction in the next stratum (2.5- to 5-cm) was not significant except for RMF site with spring tillage. Between 5- and 20-cm, MP tended to increase fPOM relative to NT. Mini-moldboard, Ch20, Ch30 and Disk did not reduce fPOM when compared to NT in the surface layer. An increase in fPOM in the 2.5- to 5-cm layer was noticeable for these treatments with respect to NT, but not statistically significant. Likewise, in strata from 5 to 30 cm, fPOM for Disk, Ch20, Ch30 and miniMP did not differ from NT.

Coarse POM was lower for MP than NT in the surface stratum, but not in the 2.5- to 5-cm stratum. Between 5- and 20-cm, MP increased cPOM relative to NT, but had no effect in the 20- to 30-cm stratum. Mini-moldboard was not different from NT and MP in the surface layer. Likewise, Ch20 and Ch30 did not reduce cPOM in the surface layer with respect to NT, but increased it in the 2.5- to 5-cm layer.

No differences were detected between NT and Ch20 or Ch30 below 5 cm depth. Disking did not cause differences compared to NT in any stratum, although cPOM tended to be higher than NT in the layers between 0 and 5 cm.

Oxidizable C was lower for MP than NT in the surface stratum, and for one site it was also lower in the 2.5- to 5-cm stratum. Between 5- and 10-cm, MP increased OxidC relative to NT, but not at the 10- to 30-cm depths. Mini-moldboard reduced OxidC with respect to NT in the surface layer, and increased it compared to NT and MP in the 2.5- to 5-cm layer. In the 10- to 20-cm stratum, miniMP had lower OxidC than MP. Chisel plowing (Ch20 and Ch30) reduced OxidC in the surface layer relative to NT. Conversely, Ch20 and Ch30 increased OxidC at the 2.5- to 5-cm and 5- to 10-cm layers, although significant only in the latter. Disking did not cause differences compared to NT in any stratum.

1.3.3. Total SOM and labile pools on a mass-per-area basis

On a **fixed-depth basis** (i.e. not equivalent-mass-basis), moldboard and mini-moldboard plowing reduced soil organic matter stocks when compared with NT (Table 1.6). Chisel plowing at 30 cm depth did not reduce SOM at the ARDC, but did so for both tillage events at the RMF site. Disking did not reduce SOM stocks in any tillage event. Tillage did not affect total, fine or coarse POM stocks at any of the three tillage events. Stocks of OxidC were lower after MP when compared to NT at both tillage events at RMF site. Disk, Ch20 and Ch30 also had lower OxidC than NT with the spring tillage at RMF.

Table 1.6. Soil organic matter, total POM, fine POM, coarse POM and Oxidizable C on a mass-per-area basis as affected by tillage implement at the three sites/season of this study†. Calculations were using two contrasting approaches: fixed depth (20 cm) or equivalent soil mass (250 kg m^{-2}).

		Fixed depth (20 cm)								Equivalent soil mass (250 kg m^{-2})						
Site / tillage time †	Tillage implement	Soil mass ‡	SOM		totPOM	fPOM	cPOM	OxidC		SOM		totPOM	fPOM	cPOM	OxidC	
		kg m^{-2}	--							g m^{-2} --						
RMF / Spring	No-till	256	8356	a§	1087	874	213	76	a	8171	b	1074	862	211	75	
	Disk	238	8255	a	1213	1001	212	69	bc	8662	a	1245	1030	214	72	
	Chisel 20 cm	238	7795	b	1066	822	244	71	b	8160	b	1089	841	248	74	
	Chisel 30 cm	226	7645	b	1107	825	282	65	c	8389	ab	1163	870	293	70	
	Moldboard plow	220	7280	c	906	723	203	60	c	8236	b	1019	813	228	67	
RMF / Fall	No-till	256	7473	a	881	718	162	90	ab	7304		870	710	160	88	c
	Disk	238	7045	ab	922	732	191	84	bc	7392		949	755	194	88	c
	Chisel 20 cm	238	7047	ab	777	596	181	92	a	7394		797	612	185	96	ab
	Chisel 30 cm	226	6896	b	1093	896	191	91	a	7575		1158	953	201	99	a
	Moldboard plow	220	6406	c	852	646	206	80	c	7237		941	714	227	91	bc
ARDC / Fall	No-till	245	6547	a	500	401	102	65		6674		506	407	103	66	
	Disk	243	6485	a	464	341	126	--		6663		472	348	127	--	
	Chisel 30 cm	231	6551	a	534	389	145	--		7046		552	404	148	--	
	Mini-moldboard plow	227	6071	b	496	349	147	54		6638		519	368	151	58	
	Moldboard plow	220	6047	b	604	463	148	62		6835		674	517	163	70	

† Dates of tillage were respectively Mar. 26 and Oct. 24 2003 for spring and fall tillage at Rogers Memorial Farm (RMF/Spring and RMF/Fall), and Nov. 26 2003 at UN–L's Agricultural Research & Development Center (ARDC/Fall).

‡ Soil mass was calculated to a depth of 20cm from bulk density and help to understand the discrepancy found between fixed depth basis and equivalent soil mass methods.

§ Letters denote significant differences between tillage treatments within a site/season stratum (α=0.05).

On an **equivalent soil mass basis**, SOM stocks were not reduced by tillage relative to NT in any of the three tillage events (Table 1.6.). Disking increased SOM stock with respect to NT only with spring tillage at RMF. Likewise, total, fine or coarse POM stocks, as well as OxidC stocks were not lower than NT at any of the three tillage events. Interestingly, Ch20 and Ch30 increased stock of OxidC at RMF site when chisel plowing was done in the fall.

1.4. DISCUSSION

1.4.1 Accuracy of CO_2 flux measures

Upon placing the chamber on the soil surface, the CO_2 concentration can build up in the chamber headspace and reduce the concentration gradient between soil air and the atmosphere. The LI-6200 allows for scrubbing part of the CO_2 within the system so that the operator can monitor the CO_2 concentration and initiate flux measures when it is about to equal ambient CO_2 concentration. Because this is more difficult to achieve when soil has just been disturbed (Rochette et al., 1997), measurements were frequently made at higher CO_2 concentrations. Therefore, flux measures on tilled soils may have been slightly underestimated as a result of negative feedback from higher-than-ambient CO_2 concentrations in the chamber.

The chamber was placed directly on the soil surface and lack of good seal may have resulted in leaks from the chamber space to the ambient air (Rochette et al., 1997). Leakage may be greater on windy days on bare soils that have pores and cracks exposed to the surface (Matthias et al., 1980). Although tilled surfaces may have been subject to underestimations as a result of higher leaks, measurements were not taken under especially windy conditions.

The small size of CO_2 chamber may have resulted in under-representing the roughness of the soil surface and causing a bias towards lower CO_2 flux estimations on the rougher tilled soils (Fig. 1.4). In order to theoretically quantify this bias, let the following be soil surface roughness (Reicosky and Lindstrom, 1993), A_s :

$$A_s = \frac{A_{\exp}}{A_{horiz}}$$

where A_{exp} (m^2) is the exposed soil surface area and A_{horiz} (m^2) is the horizontal area. Because soil roughness features may be of a larger size than the area of the sample, A_s needs to be defined for the scale of the sampling area ($A_{s\ sample}$):

$$A_{s.sample} = \frac{A_{\exp.sample}}{A_{horiz}}$$

The true CO_2 flux can now be calculated from the sampled CO_2 flux as follows and allows accounting for soil roughness that cannot be captured by the sampling area due to reasons of scale:

$$Flux = Flux_{sample} \frac{\frac{A_{\exp}}{A_{horiz}}}{\frac{A_{\exp.sample}}{A_{horiz}}} = Flux_{sample} \frac{A_s}{A_{s.sample}}$$

No quantitative measure of soil roughness was made in this study, but Reicosky and Lindstrom (1993) attempted an approximation to A_s using elevation data on a two-dimensional grid (1 by 1 m; grid spacing 2.5 by 5 cm). They calculated the length of the hypotenuse from individual points along a row of 40 pins and averaged for 20 rows. This is an extremely conservative estimation because (1) it only considers roughness along *one* dimension instead of two (i.e. along rows in one direction of the grid), and (2) the hypotenuse is the shortest distance between two points. In order to use their data and considering the grid spacing of this procedure, we could assume the soil surface roughness within the area of the chamber ($A_{s\text{-sample}}$) to be equal to 1. According to the A_s values reported by Reicosky and Lindstrom (1993) the correction factor F would be 1.6 and 1.5 for the moldboard and the chisel plow, respectively.

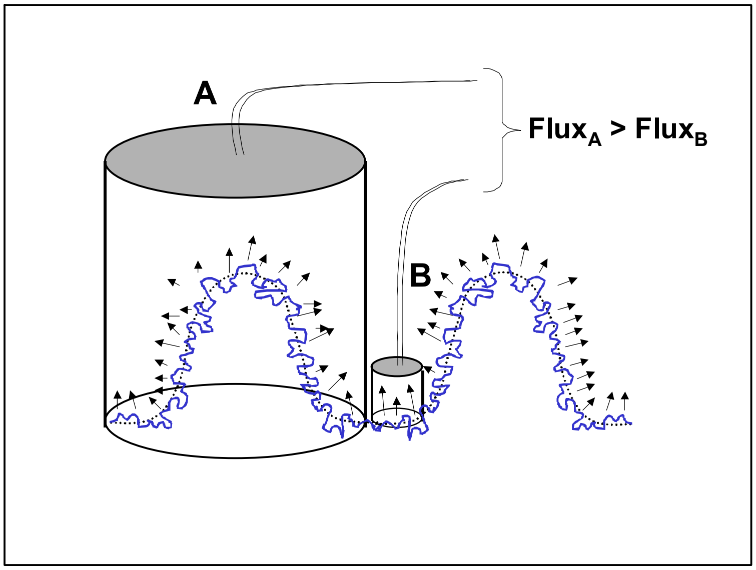

Figure 1.4. Roughness related bias of CO_2 flux measurements. [Note: Chamber **A** represents a sufficient size for this soil surface, while **B** represents a chamber that is smaller than certain features of the soil surface (i.e. ridges or big clods). If CO_2 fluxes were expressed per unit area of *exposed*, rather than *horizontal* area, measurements with chambers **A** or **B** should be the same. The ratio between exposed and horizontal areas (the soil surface roughness index A_s used in the text) is smaller for chamber **B**, because ridges are of bigger scale than the sampling area. While **A** adequately estimates the CO_2 flux (per unit horizontal area), **B** underestimates it].

These possible sources of error may have contributed to the relatively high standard error in the measurement of CO_2-flux and the detection of few statistically significant tillage effects.

1.4.2. Immediate CO_2 flush

A sharp but short increase in CO_2 flux from the soil immediately after soil disturbance has been commonly observed, when a soil chamber of similar type and size as in this study (area = 7.85 $x10^{-3}$ m^2) was used (Prior et al., 1997 and 2004; Rochette and Angers, 1999).

A CO_2 spike was also consistently measured immediately upon tillage when a "canopy chamber" of 2.67 m^2 equipped with a pair of fans to ensure adequate mixing within the chamber head space was used (Reicosky and Lindstrom, 1993; Reicosky et al., 2005). As discussed above, one reason for higher flux measures with the "canopy chamber" is that soil surface area after tillage was considerably greater under the canopy chamber (Reicosky et al., 1997). These authors also recognized that increased turbulence and dynamic pressure differences inside the canopy chamber produced by the mixing fans may cause gaseous mass flow and bias their flux measurements (Hanson et al., 1993; Dugas et al., 1997). Kimball and Lemon (1971) found that air turbulence effects on soil gas exchange were larger with large pore sizes. This explains why soil and canopy chambers yield comparable results when flux data were acquired on undisturbed soil surfaces, but when measuring recently plowed soil, CO_2 flux measured with the canopy chamber increased 10 times with respect to the soil chamber (Reicosky et al., 1997).

1.4.3. The nature of the immediate CO_2 spike

Earlier studies ascribed the CO_2 losses measured immediately after tillage to increased microbial activity (i.e. Reicosky and Lindstrom, 1993), adducing that tillage makes “fresh” and occluded organic matter available to soil microbes. This hypothesis is supported for instance by earlier work of Rovira and Greacen (1957) on the effect of aggregate disruption on soil microbial activity, finding that 21 kg C ha^{-1} could be released due to increased decomposition of soil organic matter.

However, an increasing number of studies support the hypothesis that the immediate release of CO_2 upon tillage is mostly of physical, rather than biological nature. The concentration of CO_2 in soil air usually reaches its maxima of 5-12 $x10^4$ $\mu L\ L^{-1}$ during the summer, while in the fall or in the spring, measurements rarely are above 2 $x10^4$ $\mu L\ L^{-1}$. The loss of CO_2 upon tillage is proportional with depth and soil disturbance (Reicosky and Lindstrom, 1993), but passing the equipment several times does not substantially increase CO_2 losses (Ellert and Janzen, 1999). These findings support the hypothesis of passive degassing of CO_2 from soil pores and solution.

Reicosky et al. (1997) indirectly demonstrated that biological activity is not involved in CO_2 losses immediately after tillage when they measured increased short-term losses of CO_2 after plowing or chiseling, but failed in detecting concurrent changes in inorganic N content. Also, Wuest et al. (2003) disturbed soil that had been previously sterilized and found that the resulting CO_2 spike was comparable to the spikes observed from fresh soil.

1.4.4. Cumulative CO_2 losses

Following the immediate CO_2 release, Wuest et al. (2003) observed a gradual increase in CO_2 flux. In contrast, the flux rate from sterilized soil dropped to the predisturbance level and stayed there for the duration of the experiment. This has been attributed to enhanced microbial respiration due to increased availability of organic matter (Buyanovski et al., 1986). A similar CO_2 response of biotic nature was also measured by Kessavalou et al. (1998) at 24 h following soil wetting.

Therefore, we expected to measure higher cumulative CO_2 emissions after tillage. Four hours after tillage, however, cumulative CO_2 emissions showed no difference between disking or moldboard plowing and no-till, presumably because the emission of inorganic CO_2 due to tillage was largely completed. The lack of difference in cumulative CO_2 emissions over time between tillage and NT was probably due to low soil temperatures that constrained microbial activity and respiration.

It is interesting to note that cumulative emissions at 6 and 30 d after tillage at RMF were higher for chisel and disk, but not for moldboard plow. If, despite of the low soil temperatures, these emissions were indeed of biological nature, it could be hypothesized that the increased soil microbial decomposition is located mostly in the surface layer, where soil temperature would be least limiting for microbial activity. Our finding that soil organic matter, particulate organic matter, and oxidizable C in the surface layer were lowest after moldboard plowing supports this hypothesis. It is also possible that these tillage operations did more to aerate the surface soil than plowing.

1.4.5. Redistribution of soil organic matter within the profile

Observations from comparing NT versus tilled soil within the same sampling stratum have to be examined with caution because soil bulk density is a distorting factor that may lead to misinterpretations. This may be of particular relevance in long-term NT soils that have a relatively dense soil and stratification of organic matter (especially POM). Let’s consider a situation in which a soil is sampled by fixed depth increments, showing a high stratification for a certain property tested (Figure 1.5.). The soil is subjected to a theoretical tillage with the only effect of reducing the soil bulk density by 25%, while neither the soil profile is mixed, nor the tested property per se undergoes changes. Each original sampling stratum has become thicker and a part of it is now sampled in the next stratum. Consequently, soil testing of subsurface sampling strata can show an *increase* with respect to the undisturbed soil sample. The implication of this is that certain tillage implements may be regarded as homogenizing the soil profile: the effect is mistakenly ascribed to the implement’s mixing action, while in fact an enrichment in deeper layers is simply observed as per this effect illustrated in Figure 1.5.

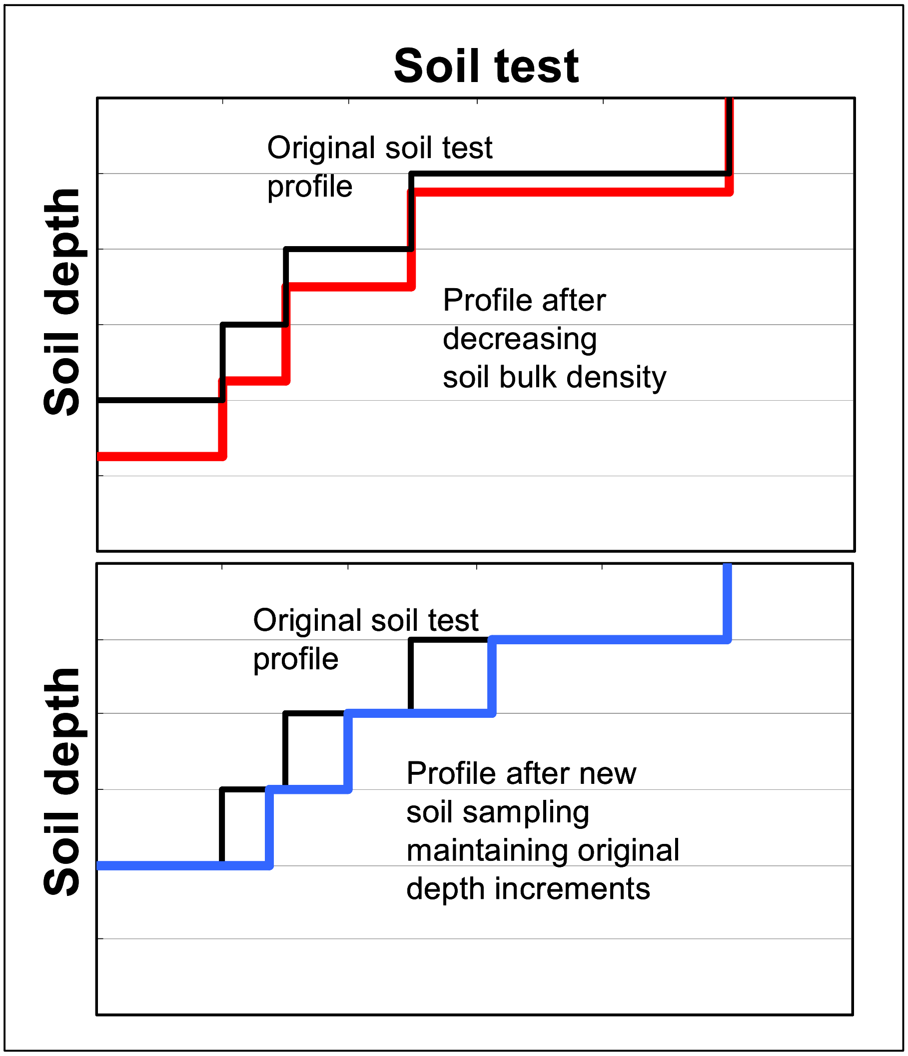

Figure 1.5. Theoretical demonstration that soil test values can *increase* in subsurface strata after tillage disturbance solely because of reduced soil bulk density. [**Explanation**: Soil sampling is done in equal depth increments (i.e.: 0-5, 5-10, 10-15, and 15-20 cm) and the undisturbed profile shows a continuous decline with depth that may be typical for long-tem no-till (in black). An idealized disturbance reaches exactly to the third layer (i.e. 15 cm) and decreases soil bulk density by 25%. It is assumed that neither the soil profile is mixed, nor the tested property per se undergoes changes (i.e. no accelerated decomposition of soil organic matter). Despite of that, a second soil sampling after disturbance (maintaining the same sampling increments) shows an *increase* with respect to the undisturbed soil samples (in blue). This is because each original sampling stratum has become thicker and a part of it is now sampled in the next lower stratum. Note that the fourth layer also shows a higher soil test, even though it was not disturbed in this example].

Results in this study likely were subject to this source of error. For instance, SOM values after disking in the spring are *higher* than NT in the 10- to 20 cm depth. It is virtually impossible that the disk mixed this stratum with surface soil because it is a shallow tillage implement.

Changes in the vertical distribution of soil organic matter and other pools in response to different tillage operations have been reported in previous studies (Angers et al., 1997; Lorenz and Lal, 2005). Most significant effects were observed in the layers between 0 and 5 cm. Values of SOM, fPOM, cPOM and OxidC in the 0- to 2.5-cm stratum were lowest for MP, and was consistently different from NT. Moldboard plow was the only implement that resulted in equal or lower test values than NT in the stratum 2.5- to 5-cm. Because of the soil inversion caused by MP, the highest test values for these organic matter fractions were in the 5- to 10-cm and 10- to 20-cm depths. The miniMP caused the next highest reduction of SOM and POM in the 0- to 2.5-cm layer, but increased SOM and POM in the 2.5- to 5-cm layer. Additionally, miniMP was not as effective as MP with regards to the incorporation of manure. The vertical distribution of coarse POM (Fig. 1.6.) indicated that MP incorporation of manure increased cPOM in the 10- to 20-cm depth while miniMP incorporation of manure increased cPOM between 0 and 5 cm depth but not in deeper strata. Despite soil disturbance to a considerable depth, chisel plows are basically a vertical tillage tool that leave a high amount of residues on the surface. Although Ch20 or Ch30 rarely reduced organic matter fractions in the 0- to 2.5-cm stratum, an increase in the 2.5- to 5-cm stratum was often observed. The shallow disk tillage did not reduce the stratification of SOM and labile pools, while POM fractions were increased in the 2.5- to 5-cm, but not in deeper layers.

Collectively, the data suggest the following ranking of tillage implements for their effectiveness for reducing the stratification of chemical properties that is typical of long term NT systems:

MP > miniMP > Ch30 = Ch20 > Disk = NT

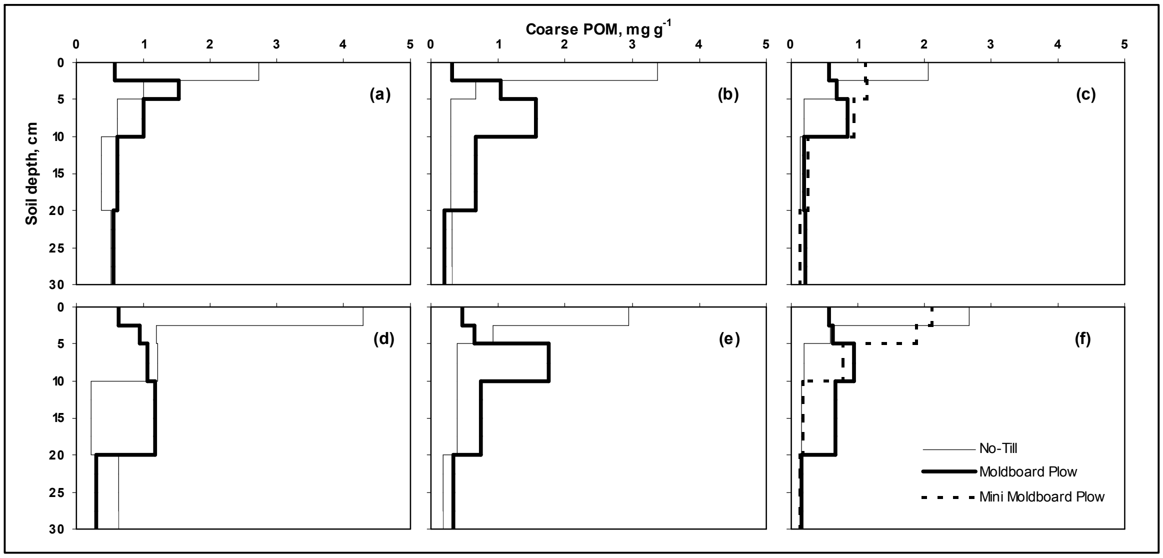

Figure 1.6. Soil coarse particulate organic matter profile at the 3 sites for no-till (fine line), moldboard plow (bold line) and mini-moldboard plow (dashed line). Figures a, b, c are non-manured, and d, e, f received broadcast manure before tillage. Spring-tillage at Rogers Memorial Farm (a and d), fall-tillage at RMF (b and e), and ARDC (c and f).

1.4.6. Total SOM and labile fractions on a mass-per-area basis

The mass of total and fractions of soil organic matter was determined per unit area using the more traditional ‘fixed-depth’ and the ‘equivalent-soil mass’ methods. These two methods gave contrasting results as expected. Given the effect of tillage on soil bulk density, the soil mass per unit area to a fixed depth of 20 cm was consistently lower for tilled than for NT soils (Table 1.6.). As a result, stock estimations for SOM (to a depth of 20 cm) were biased against plowing with chisel, mini-moldboard and moldboard. After adjusting the sampling depth to attain a soil mass of 250 kg soil m^{-2} in each tillage treatment, the differences in SOM stock between NT and tilled soils were reduced. This is graphically illustrated for NT versus MP in Fig. 1.7. Similar findings were reported by Ellert and Bettany (1995) after reassessing previously published data on C storage for no-till versus plowed soils. Differences in SOM caused by tillage were reduced when comparing treatments on an equivalent soil mass basis.

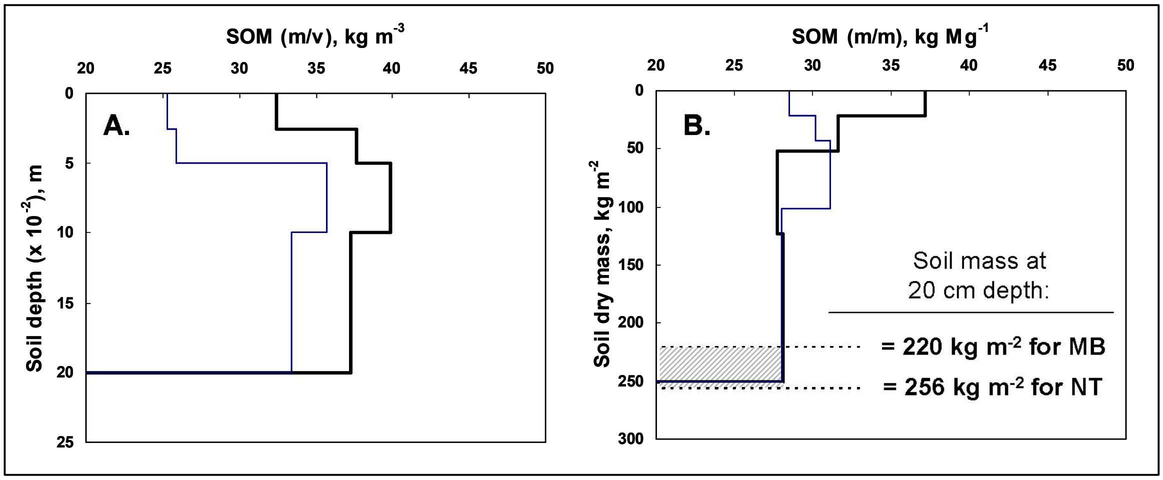

Figure 1.7. Graphical illustration of the two methods used for calculating soil organic matter stocks. The comparison is for no-till (bold line) and fall moldboard plow (fine line) at Rogers Memorial Farm. **Note**: The area under each curve represents the SOM stock for each case (kg SOM m^{-2}). Fixed depth data (plot A) indicated a statistically significant loss of SOM due to moldboard plowing. After adjusting data to an equivalent soil mass of 250 kg m^{-2} (plot B), SOM stocks were not different (see also Table 1.6.). The slashed area represents the difference in SOM stock in the case that no adjustment for soil mass was made. Bulk density (ρ) is implicit in both methods in different ways: for the fixed depth method, ρ is used to calculate SOM on a mass-per-soil volume basis [SOM (m/v) = SOM (m/m) x ρ], while for the equivalent soil mass method, ρ is used to convert depth increments to soil mass [soil dry mass = increment x ρ].

There was a minor discrepancy between organic matter stocks and measured CO_2 losses. We expected that higher CO_2 losses would correspond with lower stocks of certain labile organic matter pools. For instance, if Ch20 had *higher* cumulative losses of CO_2 after 30 d at RMF than with NT (Table 1.3.), stock estimations for SOM or labile pools should have been *lower* than NT. One possible explanation as to why these discrepancies occurred is that these determinations covered a time period of different length. While CO_2 flux measures were done to cover a maximum of 30 d after tillage, soil sampling to determine reductions in stocks of organic matter pools allowed up to six months for effects to take place. A second explanation is that measured labile pools are not the only possible substrates that were subject to microbial decomposition and release of CO_2. Cellulose and cellular contents of plant and animal residues have a turnover time of 0.1-0.5 yr (Woomer et al., 1994) and are therefore more labile than POM fractions. Some tillage operations may have caused more decomposition of this more labile pool, which was not measured in this study.

1.5. CONCLUSIONS

The stratification inherent to long-term no-till systems can be reduced by means of a one-time tillage in the late fall or early spring. Moldboard plow and mini-moldboard plow were the most effective in reducing the high organic matter content of the surface strata, while chisel plow and disk caused essentially no redistribution within the soil profile.

Measures of CO_2 fluxes confirmed that a CO_2 flush immediately follows tillage as a result of increased diffusion of CO_2 from soil air. However, cumulative losses during up to one month after tillage were similar for moldboard plow and no-till.

On an equivalent soil mass basis, tillage did not cause significant losses of organic matter or labile pools between tillage and planting of the next crop. Therefore, it was concluded that the pronounced stratification of soil organic matter in long-term NT soil could be reduced most effectively by means of occasional moldboard plow tillage, without causing increased losses of labile organic matter pools.

1.6. REFERENCES

Angers, D.A., M.A. Bolinder, M.R. Carter, E.G. Gregorich, C.F. Drury, B.C. Liang, R.P. Voroney, R.R. Simard, R.G. Donald, R.P. Beyaert, and J. Martel. Impact of tillage practices on organic carbon and nitrogen storage in cool, humid soils of eastern Canada. Soil Tillage Research 41:191-201.

Behn, E.E. 1977. More profit with less tillage. Wallace-Homestead Book Co. Des Moines, Iowa, U.S.A.

Blair, G.J., R.D.B. Lefroy, and L. Lise. 1995. Soil carbon fractions based on their degree of oxidation, and the development of a carbon management index for agricultural systems. Australian J. Agric. Res. 46:1459–1466.

Buyanowski, G.A., G.H. Wagner, and C.J. Gantzer. 1986. Soil respiration in a winter wheat ecosystem. Soil Sci. Soc. Am. J. 50:338-344.

Cambardella, C.A., A.M. Gajda, J.W. Doran, B.J. Wienhold, and T.A. Kettler. 2001. Estimation of particulate and total organic matter by weight loss-on-ignition. p. 349-359. In: R. Lal, J.M. Kimble, R.F. Follet and B.A. Stewart (eds.), Assessment Methods for Soil Carbon. Lewis Publishers, Boca Raton, FL.

Daverede, I.C., A.N. Kravchenko, R.G. Hoeft, E.D. Nafziger, D.G. Bullock, J.J. Warren, and L.C. Gonzini. 2003. Phosphorus runoff: effect of tillage and soil phosphorus levels. J. Environ. Qual. 32:1436-1444.

Dick, W.A., E.L. McCoy, W.M. Edwards, and R. Lal. 1991. Continuous application of no-tillage to Ohio soils. Agron. J. 83:65-73.

Doran, J.W. 1987. Microbial biomass and mineralizable nitrogen distributions in no-tillage and plowed soils. Biol. Fertil. Soils 5:68-75.

Dugas, W.A., D.C. Reicosky, and J. 1997. Chamber and micrometeorological measurements of CO_2 and H_20 fluxes for three C4 grasslands. Agric. For. Meteorol. 83:113.133.

Ellert, B.H., and J.R. Bettany. 1995. Calculation of organic matter and nutrients stored in soils under contrasting management regimes. Can. J. Soil Sci. 75:529-538.

Ellert, B.H., and H.H. Janzen. 1999. Short-term influence of tillage on CO_2 fluxes from a semi-arid soil on the Canadian prairies. Soil Tillage Research 50:21-32.

Franzluebbers, K., A.J. Franzluebbers, and M.D. Jawson. 2002. Environmental controls on soil and whole-ecosystem respiration from a tallgrass prairie. Soil Sci. Soc. Am. J. 66:254–262.

Gajda, A.M., J.W. Doran, T.A. Kettler, B.J. Wienhold, J.L. Pikul, Jr., and C.A. Cambardella. 2001. Soil quality evaluations of alternative and conventional management systems in the Great Plains. P. 381-400. In: R. Lal, J.M. Kimble, R.F. Follet and B.A. Stewart (eds.), Assessment Methods for Soil Carbon. Lewis Publishers, Boca Raton, FL.

Gaynor, J.D., and W.I. Findlay. 1995. Soil and phosphorus loss from conservation and conventional tillage in corn production. J. Environ. Qual. 24:734-741.

Gifford, R.M., and M.L. Roderick. 2003. Soil carbon stocks and bulk density: spatial or cumulative mass coordinates as a basis of expression? Global Change Biology 9:1507-1514.

Hanson, P.J., S.D. Wullschleger, S.A. Bohlman, and D.E. Todd. 1993. Seasonal and topographic patterns of forest floor CO2 efflux from an upland oak forest. Tree Physiology 13: 1-15.

Hutchinson, G.L., and P. Rochette. 2003. Non-flow-through steady-state chambers for measuring soil respiration: numerical evaluation of their performance. Soil Sci. Soc. Am. J. 67:166–180.

Kanamasu, E.T., W.L. Power, and J.W. Sij. 1974. Field chamber measurement of CO2 flux from soil surface. Soil Sci. 118:223-237;

Kettler, T.A., D.J. Lyon, J.W. Doran, W.L. Powers, and W.W. Stroup. 2000. Soil quality assessment after weed-control tillage in a no-till wheat-fallow cropping system. Soil Sci. Soc. Am. J. 64:339-346.

Kessavalou, A., J.W. Doran, A.R. Mosier, and R.A. Drijber. 1998. Greenhouse gas fluxes following tillage and wetting in a wheat-fallow cropping system. J. Environ. Qual. 27:1105-1116.

Kimbal, B.A., and E.R. Lemon. 1971. Air turbulence effects upon soil gas exchange. Soil Sci. Am. Proc. 35:16-21.

Lorenz, K., and R. Lal. 2005. The depth distribution of soil organic carbon in relation to land use and management and the potential of carbon sequestration in subsoil horizons. Adv. Agronomy 88:35-66.

Matthias, A.D., A.M. Blackmer, and J.M. Bremner. 1980. A simple chamber technique for field measurement of emissions of nitrous oxide from soils. J. Environ. Qual. 9:251-256.

Nakayama, F.S., and B.A. Kimble. 1988. Soil carbon dioxide distribution and flux within the open-top chamber. Agron. J. 80:394-398;

Nay, S.M., K.G. Mattson, and B.T. Bormann. 1994. Biases of chamber methods for measuring soil CO2 efflux demonstrated with a laboratory apparatus. Ecology 75:2460–2463.

Norman, J.M., R. Garcia, and S.B. Verma. 1992. Soil surface CO_2 fluxes and the carbon budget of a grassland. Journal of Geophysical Research 97:18845-18853.

Omonode, R.A., A. Gal, D.E. Stott, T.S. Abney, and T.J. Vyn. 2006. Short-term versus continuous chisel and no-till effects on soil carbon and nitrogen. Soil Sci. Soc. Am J. 70:419-425.

Parkin, T.B., J.W. Doran, and E. Franco-Vizcaino. 1996. Field and laboratory tests of soil respiration. pp. 231-245. *In* J.W. Doran and A.J. Jones (ed.) Methods for Assessing Soil Quality. SSSA Special Publ. 49. SSSA, Madison, WI.

Parton, W.J., D.S. Schimel, C.V. Cole, and D.S. Ojima. 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. Soil Sci. Soc. Am. J. 51:1173-1179.

Paustian, K., H.P. Collins, and E.A. Paul. 1997. Management controls in soil carbon. p. 15-49. *In* E.A. Paul et al. (ed.) Soil organic matter in temperate agroecosystems: Long term experiments in North America. CRC Press, Boca Raton, FL.

Phillips, R.E., and S.H. Phillips. 1984. No-tillage agriculture, principles and practices. Van Nostrand Reinhold Company Inc. New York, USA.

Pierce, F.J., M.C. Fortin, and M.J. Staton. 1994. Periodic plowing effects on soil properties in a no-till farming system. Soil Sci. Soc. Am. J. 58:1782-1787.

Prior, S.A., R.L. Raper, and G.B. Runion. 2004. Effect of implement on soil CO_2 efflux: fall vs. spring tillage. Transactions of the ASAE 47:367-373.

Prior, S.A., H.H. Rogers, G.B. Runion, H.A. Torbert, and D.C. Reicosky. 1997. Carbon dioxide enriched agro-ecosystems: Influence of tillage-induced short-term soil carbon dioxide efflux. J. Environ. Quality 26:244-252.

Reikosky, D.C. 1997. Tillage-induced CO_2 emmision from soil. Nutr. Cycling Agroecosys. 49:273-285.

Reicosky, D.C, W.A. Dugas, and H.A. Torbert. 1997. Tillage-induced soil carbon dioxide loss from different cropping systems. Soil Tillage Res. 41:105-118.

Reicosky, D.C. 2001. Effects of conservation tillage on soil organic carbon dynamics: fields experiments in the U.S. corn belt. P 481-485. *In* D.E. Stott, R.H. Mohtar and G.C. Steinhardt (eds.) Sustaining the Global farm. Selected papers from the 10th International Soil Conservation Organization Meeting held in May 24-29, 1999 at Purdue University and the USDA-ARS National Soil Erosion Research Laboratory.

Reicosky, D.C. and M.J. Lindstrom. 1993. Fall tillage method: effect on short-term carbon dioxide flux from soil. Agron. J. 85:1237-1243.

Reicosky, D.C., M.J. Lindstrom, T.E. Schumacher, D.E. Lobb, and D.D. Malo. 2005. Tillage induced CO_2 loss across an eroded landscape. Soil Tillage Res. 81:183-194.

Roberts, W.P., and K.Y. Chan. 1990. Tillage-induced increases in carbon dioxide loss from soil. Soil Tillage Res. 17:143-151.

Rochette, P., and D.A. Angers. 1999. Soil surface carbon dioxide fluxes induced by spring, summer, and fall moldboard plowing in a sandy loam. Soil Sci. Soc. Am. J. 63:621-628.

Rochette, P., B. Ellert, E.G. Gregorich, R.L. Desjardins, E. Pattey, R. Lessard, and B.G. Johnson. 1997. Description of a dynamic closed chamber for measuring soil respiration and its comparison with other techniques. Ca, J. Soil Sci. 77:195-203.

Rovira, A.d. and E.L. Greacen. 1957. The effect of aggregate disruption on the activity of microorganisms in the soil. Aust. J. Agric. Res. 8:659-673.

SAS Institute Inc., 1989. SAS/STAT® User's Guide, Version 6, 4th ed., vol. 1 & 2. SAS Institute Inc. Cary, NC.

Schreiber, J.D., and L.L. McDowell. 1985. Leaching of nitrogen, phosphorus and organic carbon from wheat straw residues: I. Rainfall intensity. J. Environ. Qual. 14:251-256.

Sharpley, A.N., and S.J. Smith. 1994. Wheat tillage and water quality in the Southern Plains. Soil Tillage Res. 30:33-48.

Sims, J.T., R.R. Simard, and B.C. Joern. 1998. Phosphorus loss in agricultural drainage: Historical perspective and current research. J. Environ. Qual. 27:277-293.

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

Six, J., R.T. Conant, E.A. Paul, and K. Paustian. 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant Soil 241:155-176.

Stockfisch, N., T. Forstreuter, and W. Ehlers. 1999. Ploughing effects on soil organic matter after twenty years of conservation tillage in Lower Saxony, Germany. Soil Till. Res. 52:91-101.

VandenBygaart, A.J., and B.D. Kay. 2004. Persistence of soil organic carbon after plowing a long-term no-till field in southern Ontario, Canada. Soil Sci. Soc. Am. J. 68:1394-4102.

Verma, S.B., A. Dobermann, K.G. Cassman, D.T. Walters, J.M. Knops, T.J. Arkebauer, A.E. Suyker, G.G. Burba, B. Amos, H. Yang, D. Ginting, K.G. Hubbard, A.A. Gitelson, E.A. Walter-Shea. 2005. Annual carbon dioxide exchange in irrigated and rainfed maize-based agroecosystems. Agric. For. Meteorol. 131:77-96.

Weil, R.R., K.R. Islam, M.A. Stine, J.B. Gruver, and S.E. Samson-Liebig. 2003 Estimating active carbon for soil quality assessment: a simplified method for laboratory and field use. Am. J. Alt. Agric. 18:3-17.

West, T.O., and W.M. Post. 2002. Soil organic carbon sequestration rates by tillage and crop rotation: A global data analysis. Soil Sci. Soc. Am. J. 66:1930-1946.

Woomer, P.L., A. Martin, A. Albrecht, D.V.S. Resck, and H.W. Scharpenseel. 1994. The importance and management of soil organic matter in the tropics. p. 47-80. *In P.L. Woomer and M.J. Swift* (eds.) The biological management of tropical soil fertility. John Wiley and Sons, Chichester, UK.

Wuest, S.B., D. Durr, and S.L. Albrecht. 2003. Carbon dioxide flux measurement during simulated tillage. Agron. J. 95:715-718.

Chapter 2

Potential gains in total soil organic carbon (SOC) with occasional tillage of long-term no-till systems

Abstract: Established no-till systems present a challenging limitation because soil organic carbon (SOC) accumulates mostly in the top 5 cm and most of the soil quality improvement occurs in this surface soil layer. Occasional tillage for no-till systems is proposed as a one-time tillage, conducted once in 12 or more years, to invert the high SOC surface layer with less improved deeper soil. We hypothesized that occasional tillage will result in increased SOC in the long term. Two experiments in long-term no-till fields were conducted under rainfed corn or grain sorghum rotated with soybeans in Eastern Nebraska. Tillage treatments were done in spring or fall and included: continuous no-till, tandem disk, 10-cm wide twisted shank-chisel, moldboard plow and mini-moldboard plow. After harvest of the second or third crop post-tillage, concentration of SOC was assessed for three sampling depths: 0-5, 5-20, and 20-30 cm. Total stock of SOC were calculated on an equivalent soil mass basis for 60, 250, and 400 kg soil m^{-2} in approximate correspondence to the sampling depths in the NT soil. Moldboard plowing reduced the concentration of SOC compared to no-till in the 0-5 cm soil

depth, but SOC was similar for the other tillage options. Therefore, stocks for the surface 60 kg soil m^{-2} for moldboard plow were lower than no-till by 9 to 21%. However, at a soil depth of 5-20 cm, moldboard plowing increased SOC concentration relative to no-till, and SOC stocks for 250 kg soil m^{-2} and 400 kg soil m^{-2} (approximately 0-20 cm and 0-30 cm, respectively) were not different from no-till. A one-time moldboard plow tillage effectively redistributed SOC through the soil profile by incorporating surface SOC to lower depths without causing losses of SOC due to increased decomposition. Plowing greatly reduced SOC in the surface 5 cm, without causing SOC loss, Although no increase in C stock was measured in soil that received occasional tillage, it is concluded that occasional tillage increased the potential for higher C-sequestration rate under continuous no-till.

Abbreviations: ARDC, Agricultural Research & Development Center; Ch20, 10-cm wide twisted shank chisel at 20 cm depth; Ch30, 10-cm wide twisted shank chisel at 30 cm depth; Disk, tandem disk; MiniMP, mini-moldboard plow; MP, moldboard plow; NT, continuous no-till; RMF, Rogers Memorial Farm; SOC, soil organic carbon; SOM, soil organic matter.

2.1. INTRODUCTION

Adoption of no tillage (NT) systems has resulted in benefits to the farmer including increased yields, enhanced efficiency of fertilizers, improved soil quality, and reduced costs and time requirement due to fewer field operations (Behn, 1977; Phillips and Phillips, 1984). No-till increases soil organic carbon (SOC) when agricultural fields are converted from conventional tillage (Six et al., 2000). Because C sequestration by agricultural soil is viewed as an effective strategy for the mitigation of greenhouse gases, trading of C credits has recently become an added profit opportunity for NT farmers.

Three distinct mechanisms are generally recognized for the stabilization of organic matter in the soil, and are commonly defined based on their chemical, physical, or biochemical nature (Jastrow and Miller, 1998). Briefly, *chemical stabilization* refers to the binding between SOM and clay and silt particles; *physical protection* is exerted by micro- and macroaggregates, which restricts access of microbes to substrate due to pore size exclusion; and *biochemical recalcitrance* is due to the resistant and/or recalcitrant chemical nature of the substrate, such as lignin and polyphenols.

Gains in SOC due to long term NT has been ascribed to a higher accumulation of particulate organic matter (POM), particularly POM occluded in microaggregates (Denef et al., 2004; Six et al, 1999). This implies that physical protection is an important mechanism for SOM stabilization under NT conditions. However, chemical analyses have also indicated that POM, while occluded within aggregates, undergoes a process of selective decomposition of easily decomposable carbohydrates (i.e. O-alkyl C) and preservation of recalcitrant long-chained C (i.e. alkyl C) (Golchin et al., 1998). When comparisons between NT and tilled soils included the humified fraction, this pool accounted for an important portion of the difference in SOC between NT and tilled soils (Yang and Kay, 2001).

However, tillage studies showed that C sequestration in NT soils may not continue in the long-term (Paustian et al., 1997). Although many long term experiments show a proportional relationship between C input rates and SOC, in situations where SOC approaches high levels, two to three fold increases in C inputs may not result in additional SOC accumulation (Campbell et al., 1991). Paustian et al. (1997) postulated that “there is a maximum amount of C which can be stabilized in organo-mineral complexes that are resistant to decomposition and that, once this capacity is saturated, additional residues remain accessible to rapid microbial decomposition and add little to the total soil C storage”. Six et al. (2002) reviewed and summarized a number of studies on SOM dynamics and stabilization in order to examine evidence in favor of the C-saturation hypothesis. With regards to chemical stabilization, a direct relationship exists between SOC and silt plus clay content, suggesting that the size of the chemically protected SOC pool depends on the silt and clay proportion in soil.

The physically protected pool depends mostly on the degree of microaggregation, which is affected by clay content and clay type. The biochemically stabilized pool turns over very slowly because its complex chemical composition inhibits decomposition; Six et al. (2002) found no indication that this pool could accumulate indefinitely, but has a limited size.

Carbon accumulation in NT systems becomes much slower after an early rapid gain in C that typically lasts about 10 years following conversion from conventional tillage (Dick et al., 1991; Omonode et al., 2006). This can be explained by means of the C saturation postulation as follows: crop-derived C inputs are deposited within the soil surface layer (i.e. 0-5 cm) and causes SOC to increase until this shallow soil zone reaches C saturation (Paustian et al., 1997; Doran, 1987). Deeper soil usually does not gain SOC with no-till management, because this soil layer does not receive higher C inputs. In consequence, C sequestration of the entire soil eventually ceases, even though soil at the 5-30 cm remains below the C saturation limit. Therefore, sustained C sequestration is unlikely with continuous no-till (Paustian et al., 1997).

A hypothesis can be formulated, therefore, that more SOC could be stored in the profile if the vertical SOC distribution can be manipulated to reallocate SOC from surface to deeper soil layers. In doing so, the soil surface would become C-unsaturated and conditions would be given for resuming C sequestration under no-till management. One proposed method to achieve or develop a less C-saturated surface soil is occasional tillage of no-till.

Occasional tillage refers to the practice of a one-time tillage operation, conducted once in maybe 10-15 years, in an otherwise continuous no-till system. We hypothesized that a one-time tillage can effectively reallocate SOC from the surface into deeper soil layers and subsequently increase the C storage capacity of the surface soil. In Chapter 1 of this dissertation it was shown that soil organic matter in the surface 2.5 cm of soil was significantly lower after moldboard plowing when compared to continuous no-till. In the short-term after tillage, it was concluded that occasional tillage in NT resulted in negligible losses of soil organic matter. If the enrichment of deeper soil layers can be maintained despite of soil disturbance, the C-sequestration capacity of these soils can be increased.

There is limited information on the effects of a single tillage operation on soils that have been under continuous no-till. One year after one-time tillage with a moldboard plow, Pierce et al. (1994) found that SOC in the 0- to 5-cm layer decreased, while it increased in the 5- to 15-cm depth. However, these increases observed in lower depths had disappeared by year 5. In the semiarid climate of western Nebraska, Kettler et al. (2000) assessed the influence of plowing and secondary tillage operations, for the purpose of weed control, on properties of a silt loam soil that had been cropped in a sub-till or no-till system for more than 20 yr. Five years after tillage, SOC had declined by 20% in the 0- to 7.5-cm layer compared with undisturbed NT, but increased by 15% in the 7.5- to 15-cm depth. Stockfisch et al. (1999) observed in Germany a significant decline in concentration of SOC after moldboard plowing once a silt loam soil that was under minimum tillage for 20 years. This decline amounted to about 50% within 0-5cm stratum, and less than 30% in the 10-20cm layer. These authors concluded that organic matter gained as a result of long-term minimum tillage was completely lost by a single application of inversion tillage. On the other hand, VandenBygaart and Kay (2004) did not find a change in SOC, except for one sandy loam plot with low SOC, at 18 months after a one-time plowing of a field with 22 yrs of continuous NT in southern Ontario, Canada. The sandy loam reportedly had most of the gain in SOC under NT in the occluded particulate fraction (Yang and Kay, 2001). Therefore, SOC in this soil likely was more susceptible to tillage-induced mineralization, in comparison to fine-texture soils, which had most of SOC gains under NT in humified fraction.

The general objective of this research was to assess the effect of a one-time tillage operation on SOC concentration after one or two crops had been grown again with no-till (i.e. two or three years after the one time tillage). Specific objectives were (1) to compare C stocks following one-time tillage with continuous no-till soils, and (2) to evaluate changes in the vertical distribution of SOC that may indicate potential increases in C sequestration.

2.2. MATERIALS AND METHODS

2.2.1. Site descriptions and management

Field research was conducted at two sites in eastern Nebraska with long-term, dryland no-till history (Table 2.1). Both soils are deep, well or moderately well drained, formed in loess on uplands, with a moderately slow permeability. For both sites, mean annual precipitation is 737 mm (29 in) and mean annual temperature is 11 ºC. The sites differ for soil type and crop management history.

Table 2.1. General description of experimental sites.

Experimental Site	Continuous No-Till before start of experiment	Date of Occasional Tillage	Crops that followed occasional tillage	Soil Series	Soil Classification
Rogers Memorial Farm	12 years	Spring 2003 (Mar-26)	<u>Sorghum</u>† - Soybeans - Sorghum	Sharpsburg Si-Cl-Loam	Typic Argiudoll
Rogers Memorial Farm	13 years	Fall 2003 (Oct 24)	<u>Soybeans</u> - Sorghum	Sharpsburg Si-Cl-Loam	Typic Argiudoll
Agric. R&D Center	7 years	Fall 2003 (Nov-26)	<u>Soybeans</u> - Maize	Yutan Si-Cl-Loam	Mollic Hapludalf

† Underlined crop is the <u>first crop</u> after occasional tillage.

The site at the Rogers Memorial Farm (RMF) of the University of Nebraska–Lincoln (UN–L) was located approximately 16 km east of Lincoln, NE (40º50’44” N lat, 96º28’18” W long, 380 m altitude). The soil was a Sharpsburg silty clay loam (fine, smectitic, mesic Typic Argiudolls). The site occupied the area between two parallel steep-back sloped terraces that were established in the mid-1960s. Conversion to NT occurred in 1991 with a soybean crop. The NT rotation included small grain cereals and corn rotated with soybeans. Controlled traffic has been practiced in order to minimize soil compaction. Lime was broadcast applied without incorporation in the fall of 1997, 1999 and 2001 at a rate of 4.5 Mg ha^{-1} to correct soil pH problems. At this site, the first crop following the application of occasional tillage was sorghum (2003, for spring-tillage) and soybeans (2004, for fall-tillage).

The site at UN–L's Agricultural Research and Development Center (ARDC) was located near Mead, NE, about 48 km north of Lincoln (41º10'48" N lat, 96º28'40" W long, 358 m altitude). The soil was a Yutan silty clay loam (fine-silty, mixed, superactive, mesic Mollic Hapludalfs). This soil series was originally classified as fine, montmorillonitic, mesic Typic Argiudolls but reclassified due to loss of the thick dark mollic surface horizon due to severe erosion. The site occupied an unirrigated corner of a center pivot-irrigated field under a corn – soybean rotation, which was completely converted to NT in 1996. From 1988 to 1996, most but not all crops were also under NT. No manure had been applied, but cattle grazed corn stalks during the winter. The only fertilizer applied in the system was anhydrous ammonia knifed in between rows during corn years, at an average rate of 146 kg N ha^{-1} yr^{-1}. At this site, the first crop following the application of occasional tillage was soybeans (2004).

2.2.2. Experimental design

The experimental design at both sites was a randomized complete block design with split plots and four replicates. Tillage treatment was a main plot factor at both sites, and time of tillage was another main plot factor at RMF. Split plot treatments were a broadcast application of composted feedlot manure (at 87.4 kg P ha^{-1}) before tillage, and the corresponding control without manure. For this study, only the non-manured split-plots were used, and results were analyzed as a randomized complete block design.

The tillage treatments at the RMF site were moldboard plowing (MP); chisel plowing with 10-cm wide twisted shanks at either 20- or 30-cm depth (Ch20 or Ch30); tandem disking (Disk); and continuous no-till (NT) as the control. The second main plot factor at RMF was spring vs. fall tillage. Main plots were 24 m long and 4.6 m wide.

At the ARDC site, tillage treatments were: moldboard plowing (MP); a chisel plowing with 10-cm wide twisted shanks at 30-cm depth (Ch30); tandem disking (Disk); mini moldboard plowing (miniMP); and continuous no-till (NT). Main plots were 24 m long and 6.1 m wide. A thorough description of tillage treatments is given in Chapter 1 of this dissertation.

Tillage operations were completed on 26 March 2003 (DOY 85) for the spring tillage (RMF-site only) and a one-pass tandem disk was done on the MP-plots on 23 April. Fall-tillage treatments were performed on 24 Oct. 2003 (DOY 297) and on 26 Nov. 2003 (DOY 330) at the RMF- and ARDC-sites, respectively.

2.2.3. Field and laboratory procedures

Total carbon: Soil samples were collected in November 2005 from the following three depth increments: 0 - 5, 5 - 20, and 20 - 30 cm. Eight cores were taken per plot with a 1.8-cm diameter probe, plus four cores with a 3.2 cm diameter probe for the first sampling stratum. After grinding the entire air-dried sample with a soil grinder (>2mm), a sub-sample of 6 - 10g was finely ground in square glass bottles during 6 hours in a roller-mill (Arnold and Schepers, 2004). About 4 g of the finely ground sample was stored in 6-mL screw-capped glass vials. Total carbon was analytically determined with an automatic dry combustion C and N analyzer that was interfaced with a continuous-flow mass spectrometer (Schepers et al., 1989). For this, between 25 and 26 mg of the finely ground sample was weighed (to 10^{-3} mg) and wrapped in tin-capsules. Values for C concentration were reported on a gravimetric basis.

Inorganic carbon: Because of the previous liming history of Rogers Memorial Farm, inorganic C concentration was accounted for in the calculation of SOC. Four surface soil samples (0–5 cm) from no-till plots were analyzed for pH (1:1 soil:water) and inorganic C (reacting the soil with 0.25 *M* HCl and subsequent titration with 0.3 *M* NaOH). The resulting linear relationship between inorganic C and pH was used to estimate inorganic C in the remaining samples that had pH above 7.0 (% inorganic C = 1.4078 * pH – 9.8918, r^2 =0.999). For the site at ARDC, it was assumed that organic C was equal to total C, because available knowledge about the site's history, as well as pH determinations of earlier studies indicated that soil inorganic C was negligible.

Soil bulk density: A 3.2 cm diameter push probe was used to collect soil samples for bulk density from the surface stratum (0 - 5 cm). Special care was given while pushing the probe into the soil in order to avoid compressing the surface soil. A known volume of soil was systematically collected by cutting the cores of exact length, using a spatula and reference marks on the probe. For the next two sampling depths (5 - 20 and 20 - 30 cm), a push probe with an inserted plastic tube liner was used. A small hole was made with a hand shovel to approximately 5 cm depth, and the probe was inserted at the bottom of this hole. After taking the core, the plastic tube was removed with the soil cylinder inside. Empty space in the tubes was filled with cotton balls to prevent the core from falling apart during handling, and then the tubes were capped and labeled. In the laboratory, soil cores were slid out of the tube and cut into segments according to the corresponding increments. The mass of the soil from all three depths was obtained after oven-drying it in the lab at 105ºC for 48 h. Two blocks at each site were sampled and the average was assumed to represent bulk density of each tillage treatment. Samples for bulk density were from the inter-row, systematically sampling both trafficked- and non-trafficked areas.

Table 2.2. Bulk density† estimations for three depth increments in each treatment at both sites.

	Depth increment (cm)		
Tillage, Season	0- 5	5 - 20	20 - 30
	-----------	Mg m^{-3}	-------------
		Rogers Memorial Farm	
No-till	1.17	1.31	1.34
Disk, Spring-tillage	1.23	1.33	1.36
Disk, Fall-tillage	1.21	1.30	1.34
Chisel 20cm, Spring-tillage	1.25	1.29	1.33
Chisel 20cm, Fall-tillage	1.23	1.28	1.31
Chisel 30cm, Spring-tillage	1.23	1.32	1.34
Chisel 30cm, Fall-tillage	1.22	1.32	1.30
Moldboard plow, Spring-tillage	1.23	1.28	1.29
Moldboard plow, Fall-tillage	1.25	1.26	1.31
		Agric. R&D Center	
No-till	1.17	1.38	1.39
Disk	1.16	1.42	1.40
Chisel at 30cm	1.07	1.25	1.44
Mini moldboard plow	1.06	1.29	1.37
Moldboard plow	1.08	1.32	1.37

† Bulk density estimations were averages from 2 blocks for each site.

2.2.4. Calculations and Statistical Analyses

Soil organic carbon data were used in combination with bulk density data to estimate C stocks on an equivalent mass basis. These were calculated for 60 kg soil m^{-2} (~5 cm depth), 250 kg soil m^{-2} (~20 cm), and 400 kg soil m^{-2} (~30 cm), according to equations (1a-c) (after Gifford and Roderick, 2003).

$$Stock_{60kg} = Stock_{5cm} + \frac{Stock_{20cm} - Stock_{5cm}}{Ms_{20cm} - Ms_{5cm}}(60 - Ms_{5cm})$$ [Eq. (1a)]

$$Stock_{250kg} = Stock_{5cm} + \frac{Stock_{20cm} - Stock_{5cm}}{Ms_{20cm} - Ms_{5cm}}(250 - Ms_{5cm}) \qquad \text{[Eq. (1b)]}$$

$$Stock_{400kg} = Stock_{20cm} + \frac{Stock_{30cm} - Stock_{20cm}}{Ms_{30cm} - Ms_{20cm}}(400 - Ms_{20cm}) \qquad \text{[Eq. (1c)]}$$

where $Stock_{60kg}$, $Stock_{250kg}$, and $Stock_{400kg}$ are the masses of SOC contained within a soil mass of 60, 250, and 400 kg m^{-2}; respectively; Ms_{5cm}, Ms_{20cm} and Ms_{30cm} are masses of dry soil to a fixed depth of 5, 20 and 30 cm, respectively (in units of kg m^{-2}); and 60, 250 and 400 kg dry soil per m^2 are the reference soil masses chosen for this study and approximately correspond to 5, 20 and 30 cm depth in NT.

$Stock_{5cm}$, $Stock_{20cm}$ and $Stock_{30cm}$ are total SOC stored in the soil at a fixed depth of 5, 20 and 30 cm, and were calculated according to the following general equation:

$$StockD = \sum (conc_i * \rho_i * t_i) \qquad \text{[Eq. (2)]}$$

where each stratum i had a specific concentration ($conc_i$), bulk density (ρ_i) and thickness (t_i). Soil depth (D) was 5, 20 or 30 cm; and thickness of the layer (t_i) was respectively 5, 15 and 10 cm for the 1st, 2nd and 3rd strata, respectively.

Data for SOC concentrations, as well as stocks of SOC were analyzed using analysis of variance (ANOVA) and mixed model procedures in SAS (SAS

Inst., 1989) appropriate for a randomized complete block design with three[1] replications. To test for the season-by-tillage interaction (for the case of RMF only), a subset of data was used that excluded no-till. Because the treatment design differed slightly between sites, separate ANOVAs were run by site in order to compare all treatments of the study. An ANOVA for tillage and site effects was done combining the data of both sites, but excluding miniMP and Ch20, since these were not used at both sites. Replications were always treated as random effects, while tillage treatments were fixed effects. Whenever a significant ANOVA occurred, means were separated using the LSD 0.05 option.

2.3. RESULTS

The effect of tillage season and the season-by-tillage implement interaction were not significant for SOC at Rogers Memorial Farm (Table 2.3). Therefore, data from fall- and spring tillage at RMF were combined in order to determine tillage effects on SOC.

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[1] Although each experiment had four replications, only three were used for this study.

Table 2.3. Summary of ANOVAs to test for statistical significance of tillage season, site and tillage type on soil organic carbon (SOC) at Rogers Memorial Farm (RMF) and the Agricultural Research and Development Center (ARDC) in Eastern NE.

				SOC concentration			SOC stock		
				Sampling stratum (cm):			Equivalent soil mass (kg m^{-2}):		
Data	N	DF error	Effect	0 - 5	5 - 20	20 - 30	60	250	400
				p-value					
RMF †	24	16	Season	0.84	0.95	0.88	0.90	0.98	0.90
			Tillage (T)	<0.01	0.14	0.92	<0.01	0.39	0.67
			Season x T	0.91	0.86	0.82	0.91	0.93	0.87
RMF (fall & spring combined)	27	22	Tillage	<0.01	0.13	0.95	<0.01	0.41	0.72
ARDC	15	10	Tillage	0.13	0.72	0.94	0.14	0.58	0.68
Combined sites ‡	33	25	Site	0.24	0.19	0.07	0.18	0.11	0.05
			Tillage (T)	<0.01	0.37	0.84	<0.01	0.95	0.95
			Site x T	0.17	0.72	0.97	0.19	0.38	0.77

† No-till (NT) data were excluded from the data set when testing for tillage season and the season-by-tillage interaction. Fall- and spring-tillage were then combined in a dataset that included NT.

‡ The combined sites ANOVA for the effect of tillage did not include chisel 20-cm and mini-moldboard because they were not used at both sites.

The **concentration of SOC** in the surface layer (0-5 cm) at RMF was significantly lower for moldboard plow (MP) than no-till (NT). SOC with tandem disk (Disk), chisel plow at 20 cm depth (Ch20) or at 30 cm depth (Ch30) were not different from NT, but had higher SOC than MP. In the next sampling depth (5-20 cm), MP had the highest SOC concentration, although it was not statistically different from that of NT or the other treatments. In the deepest sampling depth (20-30 cm), soc was not affected by tillage at either site (Tables 2.3 and 2.4).

Table 2.4. Effect of tillage type on soil organic carbon (SOC) concentration in three sampling depths for each study site at Rogers Memorial Farm (RMF) and the Agricultural Research and Development Center (ARDC) in Eastern NE.

	Sampling depth (cm):		
Site / Tillage	0 - 5	5 - 20	20 - 30
	Organic C concentration, mg C g soil^{-1}		
RMF			
No-till	19.8 a	15.3	11.8
Disk	19.3 a	14.4	11.8
Chisel 20cm	21.5 a	15.3	12.6
Chisel 30cm	20.0 a	15.3	12.5
Moldboard plow	15.6 b	16.4	11.9
Standard error	2.13	1.22	2.02
ARDC			
No-till	16.1	14.3	10.9
Disk	21.3	14.7	9.9
Chisel 30cm	18.6	14.4	11.1
Mini-moldboard plow	16.5	13.3	10.2
Moldboard plow	14.6	15.2	10.2
Standard error	2.98	1.64	2.10

The amount of SOC stored in the soil per unit area was calculated on an equivalent soil mass basis (Eq. 1a-c). These stocks were significantly affected by tillage when the reference soil mass was 60 kg soil m^{-2} (Table 2.3), which represents approximately the upper 5 cm of soil. The lowest stock of SOC was consistently for MP (Fig. 2.1). Moldboard plow was lower than NT for carbon at the surface, while the remaining tillage treatments were not different from NT (Fig. 2.1).

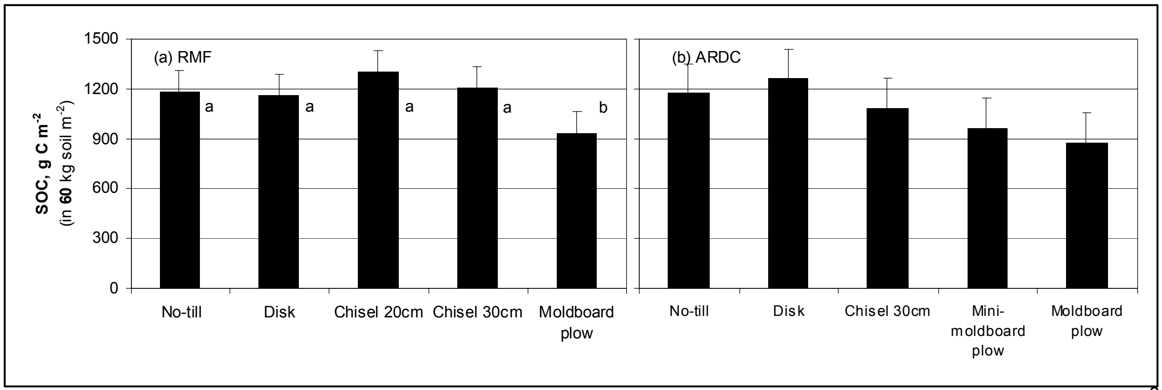

Figure 2.1. Stocks of soil organic carbon (SOC) in an *equivalent soil mass of 60 kg m*$^{-2}$ (~ 0 – 5 cm depth). A one-time tillage operation was done in 2003 and samples were taken in fall 2005. Results from Rogers Memorial Farm (RMF) are in graph (a) and results from Agricultural Research and Development Center (ARDC) are in graph (b). Tillage treatments with different letters were statistically different (α=0.05) and error bars indicate standard errors.

When the reference soil mass was 250 or 400 kg soil m^{-2}, the stocks of SOC were not affected by tillage (Table 2.3; Figure 2.2).

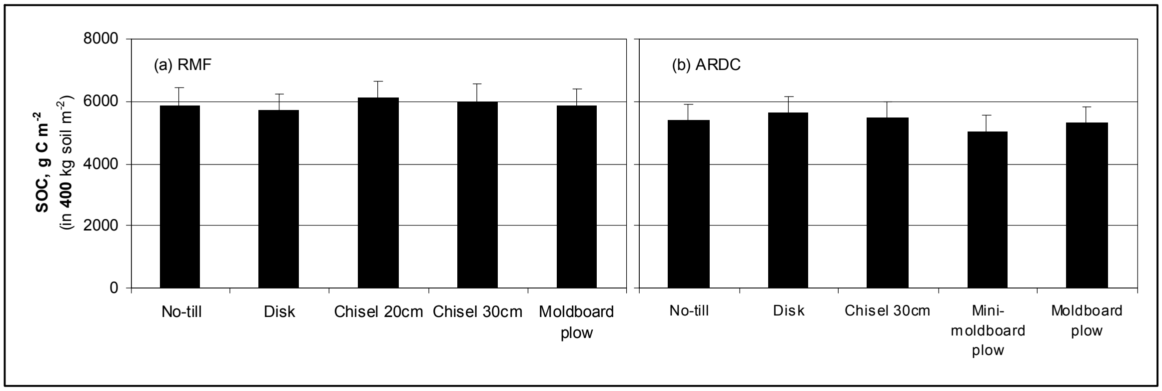

Figure 2.2. Stocks of soil organic carbon (SOC) in an *equivalent soil mass of 400 kg m^{-2}* (~ 0 – 30 cm depth). A one-time tillage operation was done in 2003 and samples were taken in fall 2005. Results from Rogers Memorial Farm (RMF) are in graph (a) and results from Agricultural Research and Development Center (ARDC) are in graph (b). Error bars indicate standard errors of the estimates. No significant differences were found between tillage treatments.

2.4. DISCUSSION

In this study, several tillage implements were tested for their effectiveness in reallocating C from surface to deeper soil layers while causing minimal loss of SOC as this would offset the potential benefits from occasional tillage of long-term no-till.

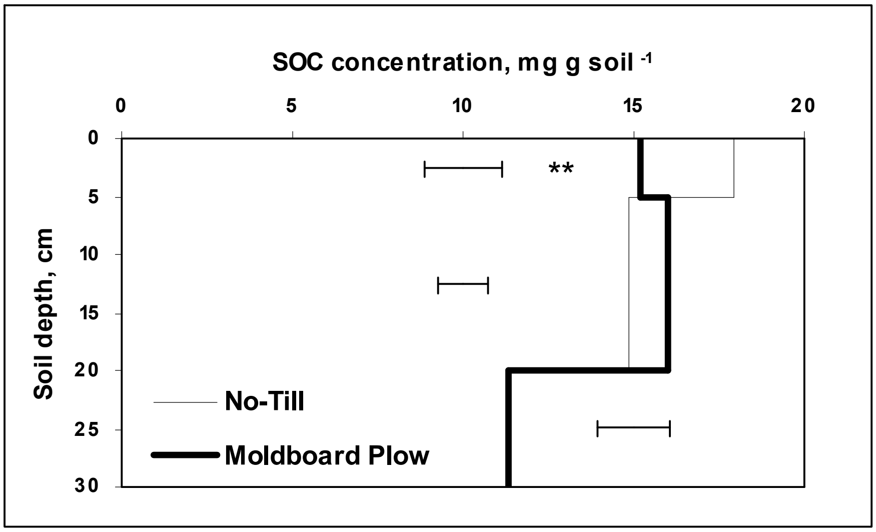

Figure 2.3. Vertical distribution of soil organic carbon (SOC) concentration, comparing a one-time moldboard plow against continuous long-term no-till. Data are the means of three occasional tillage events in 2003 at two sites. Soil samples were taken in fall 2005. Error bars indicate standard errors of the means and significant differences within a depth are denoted with (**).

Occasional tillage with a moldboard plow effectively reallocated SOC from the surface into deeper soil layers (Fig. 2.3). Kettler et al. (2000) and VandenBygaart and Kay (2004) found similar results after using a moldboard plow to subject long-term NT soils to a one-time tillage in Southern Ontario. The findings in the present study indicated also that tandem disk, chisel plow or a mini-moldboard plow tillage did not reduce SOC in the upper soil layer when compared to NT. Disk tillage was ineffective in reallocating C but did not result in significant C loss. On the other hand, the chisel plow and mini-moldboard plow are intentionally designed to cause some soil mixing to 20 cm or deeper while leaving some ground cover by crop residues. For example, Omonode et al. (2006) found that continuous no-till and intermittent chisel plowing (i.e. every second year) resulted in no differences in the profile distribution of SOC to a depth of 30 cm after 7 years.

Stockfisch et al. (1999) reported significant losses of SOC to a depth of 30 cm at 5 months after moldboard plowing a field that had been under long-term minimum tillage. A similar result was reported by Torbert et al. (2004) after subjecting a 40-yr permanent pasture to one or two years of moldboard plow. Both studies, however, calculated the stocks of SOC on a *fixed-depth* basis, which likely lead to an overestimation of stock differences between plowed and minimum or untilled soils (Ellert and Bettany, 1995). In the present study, stock calculations were made on an equivalent soil mass basis in order to account for variations in soil mass in the sampling layer due to tillage effects on soil bulk density. After 2 or 3 years post-tillage, no reduction was found in the amount of SOC that was stored per unit area (in an *equivalent soil mass* of 400 kg soil m^{-2}) in the soil profile (Fig. 2.4). VandenBygaart and Kay (2004) used the *equivalent soil mass* method to calculate and compare SOC stocks, and observed no change in SOC stocks 18 mo after moldboard plowing a 22-yr old NT soil.

Omonode et al. (2006) found that a 24-yr old NT, a 6-yr old NT, and 6-yr old chisel plow system were not statistically different in C stocks to a depth of approximately 30 cm. Finally, Pierce et al. (1994) and Kettler et al. (2000) reported that a one-time plowing did not result in a significant change in SOC storage 4 to 5 yrs after plowing.

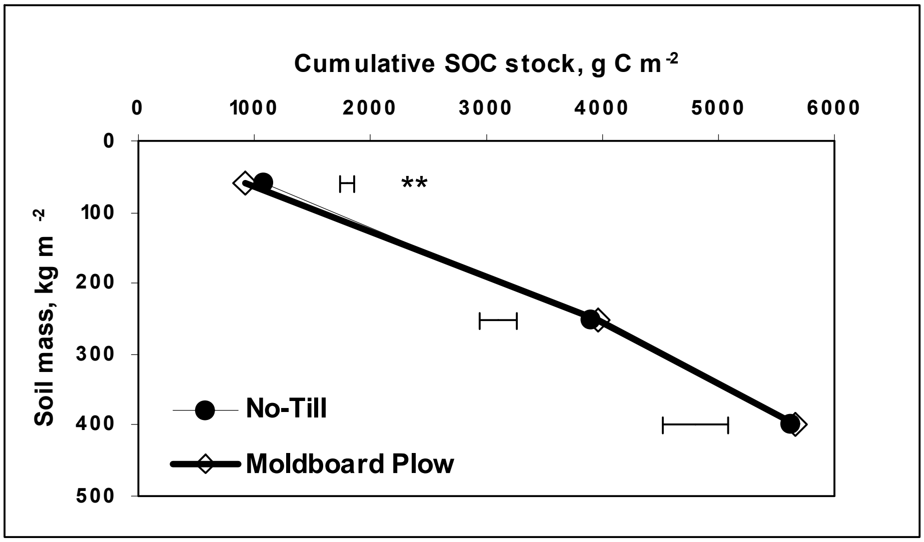

Figure 2.4. Stocks of soil organic carbon (SOC) on an equivalent soil mass basis. A one-time moldboard plow is compared against continuous long-term no-till. SOC stocks were calculated for 60 kg soil m^{-2} (~5cm depth), 250 kg soil m^{-2} (~20 cm), and 400 kg soil m^{-2} (~30 cm). The values presented are the means across three occasional tillage events in 2003 at two sites. Soil samples were taken in fall 2005. Error bars indicate standard errors and significant differences are denoted with (**).

2.5. CONCLUSIONS

The results reported in this study support the hypothesis that a one-time tillage can effectively reallocate SOC from the surface into deeper soil layers without SOC loss. Moldboard plowing redistributed SOC without causing C losses, so that deeper layers in the profile became richer in soil organic carbon.

Plowing greatly reduced SOC in the 0-5 cm layer, leaving a less C-saturated surface soil. Although no increase in C stock was measured in soil that received occasional tillage, it is concluded that occasional tillage increased the potential for higher C-sequestration rate under continuous no-till.

2.6. REFERENCES

Arnold, S.L., and J.S Schepers. 2004. A simple roller-mill grinding procedure for plant and soil samples. Commun. Soil Sci. Plant Anal. 35:537-545.

Behn, E.E. 1977. More profit with less tillage. Wallace-Homestead Book Co. Des Moines, Iowa, U.S.A.

Campbell, C.A., K.E. Bowren, M. Schnitzer, R.P. Zentner, and L. Townley-Smith. 1991. Effect of crop rotations and fertilization on soil biochemical properties in a thick Black Chernozem. Can. J. Soil Sci. 71:377-387.

Denef, K., J. Six, R. Merckx, and K. Paustian. 2004. Carbon sequestration in microaggregates of no-tillage soils with different clay mineralogy. Soil Sci. Soc. Am. J. 68:1935-1944.

Dick, W.A., E.L. McCoy, W.M. Edwards, and R. Lal. 1991. Continuous application of no-tillage to Ohio soils. Agron. J. 83:65-73.

Doran, J.W. 1987. Microbial biomass and mineralizable nitrogen distributions in no-tillage and plowed soils. Biol. Fertil. Soils 5:68-75.

Ellert, B.H., and J.R. Bettany. 1995. Calculation of organic matter and nutrients stored in soils under contrasting management regimes. Can. J. Soil Sci. 75:529-538.

Gifford, R.M., and M.L. Roderick. 2003. Soil carbon stocks and bulk density: spatial or cumulative mass coordinates as a basis of expression? Global Change Biology 9:1507-1514.

Golchin, A., J.A. Baldock, and J.M. Oades. 1998. A model linking organic matter decomposition, chemistry, and aggregate dynamics. p. 245-266. In R. Lal et al. (ed.) Soil processes and the carbon cycle. CRC Press, Boca Raton, FL, U.S.A.

Jastrow, J.D., and R.M. Miller. 1998. Soil aggregate stabilization and carbon sequestration: Feedbacks through organomineral associations. p. 207-223. *In* R. Lal et al. (ed.) Soil processes and the carbon cycle. CRC Press, Boca Raton, FL, U.S.A.

Kettler, T.A., D.J. Lyon, J.W. Doran, W.L. Powers, and W.W. Stroup. 2000. Soil quality assessment after weed-control tillage in a no-till wheat-fallow cropping system. Soil Sci. Soc. Am. J. 64:339-346.

Omonode, R.A., A. Gal, D.E. Stott, T.S. Abney, and T.J. Vyn. 2006. Short-term versus continuous chisel and no-till effects on soil carbon and nitrogen. Soil Sci. Soc. Am J. 70:419-425.

Paustian, K., H.P. Collins, and E.A. Paul. 1997. Management controls in soil carbon. p. 15-49. *In* E.A. Paul et al. (ed.) Soil organic matter in temperate agroecosystems: Long term experiments in North America. CRC Press, Boca Raton, FL.

Phillips, R.E., and S.H. Phillips. 1984. No-tillage agriculture, principles and practices. Van Nostrand Reinhold Company Inc. New York, USA.

Pierce, F.J., M.C. Fortin, and M.J. Staton. 1994. Periodic plowing effects on soil properties in a no-till farming system. Soil Sci. Soc. Am. J. 58:1782-1787.

SAS Institute Inc., 1989. SAS/STAT® User's Guide, Version 6, 4th ed., vol. 1 & 2. SAS Institute Inc. Cary, NC.

Schepers, J.S., D.D. Francis, and M.T. Thompson. 1989. Simultaneous determination of total C, total N, and ^{15}N on soil and plant material. Commun. Soil Sci. Plant Anal. 20:949-959.

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. Elliot, and K. Paustian, 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biology & Biochemistry 32:2099-2103.

Six, J., R.T. Conant, E.A. Paul, and K. Paustian. 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant Soil 241:155-176.

Stockfisch, N., T. Forstreuter, and W. Ehlers. 1999. Ploughing effects on soil organic matter after twenty years of conservation tillage in Lower Saxony, Germany. Soil Till. Res. 52:91-101.

Torbert, H.A., S.A. Prior, and G.B. Runion. 2004 Impact of the return to cultivation on carbon (C) sequestration. Journal of Soil and Water Conservation 59:1-8.

VandenBygaart, A.J., and B.D. Kay. 2004. Persistence of soil organic carbon after plowing a long-term no-till field in southern Ontario, Canada. Soil Sci. Soc. Am. J. 68:1394-4102.

Yang, X.M., and B.D. Kay. 2001. Impacts of tillage practices on total, loose- and occluded-particulate, and humified organic carbon fractions in soils within a field in southern Ontario. Can. J. Soil Sci. 81:149-156.

Chapter 3

Soil hydraulic properties and phosphorus run-off as affected by one-time tillage of no-till

Abstract: Runoff from agricultural land results in reduced availability of water for crop growth and delivery of nutrients to surface waters. In the case of long term no-till management, available soil phosphorus (P) can reach high levels in the surface layer and can subsequently enrich runoff P concentration. This is further aggravated with surface application of manure, and adds to the potential of P delivery to surface waters in runoff. Occasional tillage for improvement of no-till systems is proposed as a single, one-time tillage conducted once in 12 or more years intended to mix the surface layer with less improved deeper soil. We hypothesized that occasional tillage results in decreased P loads in runoff water, even though tillage may disrupt macro-pores and reduce the rate of water infiltration. The objective of this research was to measure runoff and P loads under simulated rainfall from contrasting tillage treatments. Two experiments in long-term no-till fields were installed under rainfed corn or sorghum rotated with soybeans in eastern Nebraska. In the second or third growing seasons post-tillage, a Cornell Sprinkle Infiltrometer was used to simulate a 12-min rainfall event with an intensity of 30 cm h^{-1} (~about twice the intensity for a rainfall that has a recurrence period of 25 years). This rainfall intensity was continued until runoff rate became steady. Sorptivity and field-saturated infiltration rate were determined. Collected runoff was analyzed for total P and dissolved P. Soil aggregation of the surface layer was measured by means of sequentially wet-sieving air–dry soil (0.250- and 0.053-mm openings) and weighing the dry soil mass in each size class of wet-stable aggregates. Soil aggregation was not affected by tillage treatments. Tillage affected sorptivity and infiltration differently at the two locations with more and less runoff with tillage at RMF and ARDC, respectively. Moldboard plowing resulted in less dissolved P loss at both sites and less total P loss at ARDC, although the reductions were small relative to absolute P loss. The benefit of one-time moldboard plow tillage in terms of reduced P loss in runoff was generally positive and presumably would be greater if surface soil P levels were extremely high.

Abbreviations: ARDC, Agricultural Research & Development Center; Disk, tandem disk; DP, dissolved phosphorus; *i*, Field-saturated infiltration rate; MP, moldboard plow; NT, continuous no-till; PP, particulate phosphorus; *r*, measured rainfall rate; RMF, Rogers Memorial Farm; RO, runoff rate; *S*, sorptivity; TP, total phosphorus; t_{RO}, time to runoff.

3.1. INTRODUCTION

It is widely accepted that the conversion to no tillage (NT) systems has resulted in benefits to the farmer including increased yields, improved soil quality, and reduced costs and time requirement because of fewer field operations (Behn, 1977; Doran et al., 1987).

An environmental concern is that runoff water from NT soils can have significantly higher concentrations of dissolved reactive phosphorus than tilled soils (Sharpley and Smith, 1994; Gaynor and Findlay, 1995; Daverede et al., 2003). With NT, P accumulates, and P sorption is reduced, at the surface compared with deeper soil layers (Sims et al., 1998) leading to greater amounts of available P on the soil surface. Also, crop residues contribute significant quantities of P to agricultural runoff (Schreiber and McDowell, 1985). Furthermore, the problem of excessive P concentration in the soil surface is increased in no-till systems when manures are broadcast-applied without incorporation (Griffin et al., 2003; Laboski and Lamb, 2003).

The high P concentration in the shallow soil surface layer can be reduced through the soil mixing action of tillage (Sharpley, 2003). We use the term ‘occasional tillage’ to refer to the practice of a single, one-time tillage operation, conducted once in 10-15 years, in a system that is otherwise maintained without tillage. García (2005) showed that moldboard plowing, but not disking, effectively reduced the concentration of available phosphorus in the surface 2.5 cm of soil, when compared to long-term no-till soil. Furthermore, a broadcast application of 44 kg P ha^{-1} increased the Bray-P1 test in the surface soil by about 40 mg P kg^{-1} soil, unless it was adequately incorporated by means of tillage.

Moldboard plowing could be a means to reduce P concentration in runoff and consequently reduce total off-farm losses of P to runoff. However, tillage alters the physical integrity of the soil, which can result in additional factors and mechanisms controlling off-farm P losses. The diagram in Figure 3.1 summarizes a conceptual framework of effects that can be elicited by a one-time tillage operation.

Water infiltration rate determines the capacity of the soil to absorb water and directly relates to the amount of water that becomes ponded. A high infiltration rate is desirable to reduce the amount of runoff, and is viewed as a relevant soil quality attribute. During the initial phase of a rainfall event, water infiltration is a non-steady process in which water gradually fills void soil pores as the wetting front advances downwards. With continued rainfall, the soil matrix is gradually brought to field-saturation, and the flow of water develops into a steady-state process with a lower, but constant infiltration rate. Both, the early infiltration and the field-saturated infiltration rates are important to runoff management.

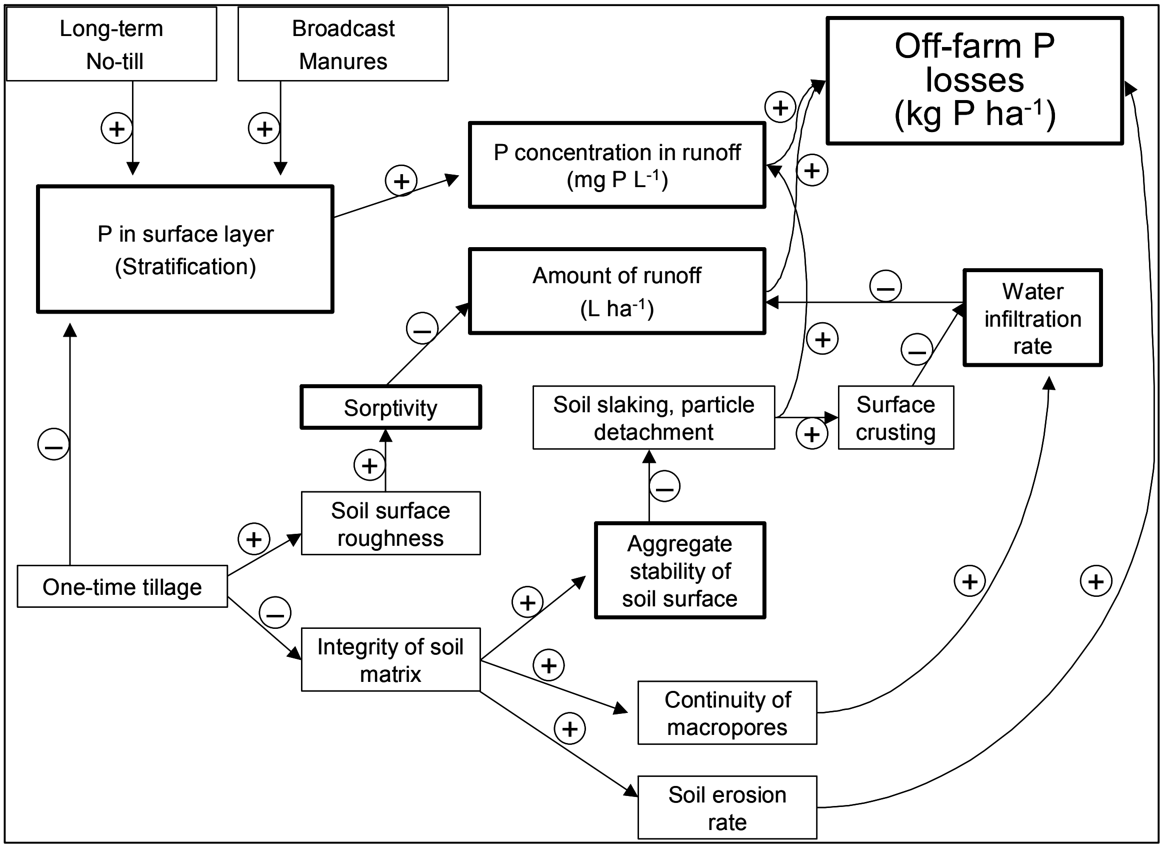

Figure 3.1. Conceptual framework of factors and mechanisms influencing off-farm phosphorus losses in the context of a one-time tillage in long-term no-till systems. (**+**) denotes influence in same direction (i.e. *higher* rates of broadcast manures *causes* higher P in surface layer; (**–**) denotes influence in opposite direction (i.e. *higher* soil sorptivity causes *lower* amount of runoff).

Soil *sorptivity* describes the early, unsteady infiltration of the soil. In the field, *sorptivity* relates to the capacity of a soil to absorb water and to prevent water from running off the soil surface. Since high-intensity rainfall events are generally of short duration, sorptivity is of special interest in runoff studies. Depressions of rough soil surfaces serve as micro-catchments of water and increase soil sorptivity. Tillage can temporarily reduce the risk of runoff through increased sorptivity owing to increased surface roughness.

The vertical flow of water through the soil matrix is best described by the soil hydraulic parameter *infiltrability*. Since it can only be measured in the laboratory using undisturbed soil cores, non-destructive field techniques have become widely accepted (for example Reynolds and Elrick, 1990; Lowery et al., 1996). A number of methods use a single ring inserted in the soil and determine the infiltration rate after steady-state conditions are reached. The single-ring method does not prevent three-dimensional flow from occurring at the bottom of the ring. Consequently, infiltration rate can be considerably higher than infiltrability (which quantifies flow in the vertical dimension only). Therefore, *field-saturated infiltration rate* is an accepted proxy to infiltrability.

The degree of soil aggregation controls runoff losses of P because it determines the amount of soil that can be detached from the soil surface. Therefore, aggregation is negatively correlated with sediment loss (McDowell and Sharpley, 2003). In addition, soils with weak structure are more prone to the surface crusting, which in turn reduces infiltration rate.

We hypothesized that occasional tillage will result in decreased P loads in runoff water, even though water infiltration of tilled soils may decrease because of macro-pore disruption. Therefore, the objectives of this study were (1) to determine tillage effects on soil aggregation, sorptivity, and infiltration rate, (2) to measure P loads in runoff under simulated rainfall as affected by occasional tillage, and (3) to determine how P loads in runoff are controlled by infiltration rate, soil sorptivity and aggregation of the surface soil.

3.2. MATERIALS AND METHODS

3.2.1. Site descriptions and management

Field research was conducted at two sites in eastern Nebraska with long-term, dryland no-till history (Table 3.1.). Both soils are deep, well or moderately well drained, formed in loess on uplands, with a moderately slow permeability. For both sites, mean annual precipitation is 737 mm (29 in) and mean annual temperature is 11 ºC. The sites differ for soil type and crop management history.

Table 3.1. General description of experimental sites.

Experimental Site	Continuous No-Till before start of experiment	Date of Occasional Tillage	Crops that followed occasional tillage	Soil Series	Soil Classification
Rogers Memorial Farm	11 years	Spring 2003 (Mar-26)	Sorghum – Soybeans – <u>Sorghum †</u>	Sharpsburg Si-Cl-Loam	Typic Argiudoll
Agric. R&D Center	7 years	Fall 2003 (Nov-26)	Soybeans – <u>Maize</u>	Yutan Si-Cl-Loam	Mollic Hapludalf

† Underlined crop is the <u>current crop</u> during the present study.

The site at the Rogers Memorial Farm (RMF) of the University of Nebraska–Lincoln (UN–L) was located approximately 16 km east of Lincoln, NE

(40º50’44” N lat, 96º28’18” W long, 380 m altitude). The soil was a Sharpsburg silty clay loam (fine, smectitic, mesic Typic Argiudolls). The site occupied the area between two parallel steep-back sloped terraces that were established in the mid-1960s. Conversion to NT occurred in 1992 with a soybean crop. The NT rotation included small grain cereals and corn rotated with soybeans. Controlled traffic was practiced to minimize soil compaction. Lime was broadcast applied without incorporation in the fall of 1997, 1999 and 2001 at a rate of 4.5 Mg ha^{-1} to correct pH problems. At this site, the crops that followed the application of occasional tillage were sorghum (2003 and 2005) and soybeans (2004).

The site at UN–L’s Agricultural Research and Development Center (ARDC) was located near Mead, NE, about 48 km north of Lincoln (41º10’48” N lat, 96º28’40” W long, 358 m altitude). The soil was a Yutan silty clay loam (fine-silty, mixed, superactive, mesic Mollic Hapludalfs). This soil series was originally classified as fine, montmorillonitic, mesic Typic Argiudolls but reclassified due to loss of the thick dark mollic surface horizon due to severe erosion. The site occupied the dryland corner of a center pivot-irrigated field under a corn – soybean rotation, which was completely converted to NT in 1996. Since 1988 however, most but not all crops were also under NT. No manure had been applied, but cattle grazed on corn stalks. The only fertilizer applied in the system was anhydrous ammonia knifed in between rows, at an average rate of 146 kg N ha^{-1}. At this site, the crops that followed the application of occasional tillage were soybeans (2004) and corn (2005).

3.2.2. Tillage treatments

For this specific study, the following subset of tillage treatments was selected: moldboard plowing (MP); tandem disking (Disk); and continuous no-till (NT) as the control. A broadcast application of 87.4 kg P ha^{-1} as composted feedlot manure was done one day before tillage operations. No-till treatments received the same rate of manure P as the tilled counterparts. Tillage operations were done in spring (26 March 2003) at RMF, and in fall (26 Nov. 2003) at ARDC. More detail regarding the design of the experiments was given in Chapter 1 and summarized in Table 1.1.

3.2.3. Field and laboratory procedures

Available and total phosphorus in the soil surface: Soil samples were collected from the 0- to 2.5-cm depth before planting in each site by taking ten cores of 2 cm diameter. Samples were air-dried and ground to pass a 2-mm sieve. Available soil P was assessed by testing for Bray-P1 (Bray and Kurtz, 1945) and total P was assessed by perchloric acid digestion (Olsen and Sommers, 1982).

Aggregation of surface soil: Soil samples were taken from the 0- to 5-cm depth by means of a shovel. At four random locations between the rows in each plot, a layer of soil was carefully removed to collect approximately 600 g dry soil per sample. Percent water stable aggregates was assessed by a wet-sieving method (Cambardella and Elliott, 1994). Field-moist soil was gently crumbled, air-dried, and passed through an 8-mm sieve. Material retained on the sieve was discarded, and visible pieces of crop residues and roots were removed. A dry 100 g sub-sample of soil was distributed on a 2-mm sieve (20 cm diameter) and immersed in about 3 cm of water for 5 minutes. Wet sieving was achieved by manually moving the sieve up and down 3 cm with 50 cycles during a 2-min period. This step was sequentially repeated with the 0.250-mm and the 0.053-mm sieves. Material retained in each sieve was washed separately into a 150-ml beaker and allowed to settle for about 20 min. Supernatant water was carefully poured from the beaker and discarded, while water-stable aggregates were transferred into a pre-weighed aluminum tin, oven dried at 50ºC, and weighed. Classes of water stable aggregates were large macro-aggregates (>2.0 mm), small macro-aggregates (0.250 – 2.0 mm) and micro-aggregates (0.053 – 0.250).

Field-estimations of soil hydraulic properties and collection of runoff: A Cornell Sprinkle Infiltrometer was used to deliver a simulated rainfall of constant intensity and measure infiltration rate (Ogden et al., 1997). This infiltrometer consisted of a 20-L vessel equipped with about 130 capillary tubes on its bottom. The intensity of the rainfall could be adjusted by varying the height of an air-entry tube. The simulated rain was delivered within a single 241-mm inner diameter ring, which was previously inserted into the soil to a depth of 7.5 cm. This ring had a perforation that had to be flush with the soil surface and allowed for drainage of ponded water from the ring. This runoff was conducted to a beaker by means of an overflow tube connected to the perforation and sloping away from the ring. Therefore, the overflow beaker had to be placed in a small hole.

Two infiltrometers were used simultaneously to conduct two infiltration tests and collect two runoff samples for each plot. Once the equipment was set up in the field, a typical routine of operation and data collection was as follows.

1) Record the height of the water level in the vessel using a ruler that was glued vertically on the inner side of the container's wall.
2) Open the air-entry tube to start the simulated rainfall and simultaneously start a stopwatch.
3) Rotate the vessel slightly every 2- to 3 minutes to avoid drops from falling on the same spots. Watch the overflow tube and record the time when runoff started (this corresponded to the time to water ponding). Runoff collection times were pre-established at every 3 min (i.e. at 3, 6, 9, 12 minutes since initiation of rainfall).
4) On each collection time, replace the runoff beaker by an empty one, read the height of the water level in the vessel and measure the runoff volume using a graduated cylinder. Therefore, each collection time had a corresponding water height in the vessel and a runoff volume.

5) Empty the cylinder into an 8-L bucket to temporarily contain the collected runoff. Runoff collected and measured at minute 12 was the last to be added to the bucket.
6) Transfer a 500-mL sample into a plastic bottle after thoroughly stirring the content of the bucket.
7) Tighten the screw cap and label for storing in a refrigerator by the end of the day.
8) Continue collecting and measuring runoff at 3-minute intervals until minute 36 or less if steady-state conditions were reached earlier.

Phosphorus tests on runoff samples: Dissolved P and total P were analytically determined on each runoff sample, according to Pote and Daniel (2000). Total P (dissolved + particulate) was determined from a subsample taken after shaking the runoff sample to suspend particulate matter. The dissolved fraction was obtained by filtering the sample through a 0.45 μm pore diameter membrane filter. The particulate P fraction was determined as the difference between total and dissolved P. In both fractions, P was determined colorimetrically after hydrolyzing P to orthophosphate using a sulfuric acid – nitric acid digestion (Clesceri et al., 1998).

3.2.4. Calculations and Statistical Analyses

Soil aggregation from the surface 0- to 5-cm layer was calculated from the mass of water-stable soil aggregates in each of the three size classes (> 2mm, 2-0.250mm, and 0.250 – 0.053mm). These classes were termed respectively 'large macro-', 'small macro-' and 'micro-aggregates', and expressed as g aggregates per 100 g air-dried soil. The summation of the three classes was the percent of total soil mass contained as water-stable aggregates.

Data collection during the rainfall simulation comprised: height of the water level (*H,* cm) in the vessel; time to runoff (t_{RO}, minutes); runoff volume (*V*, cm^3); and (4) time (*t*, minutes). These records allowed for the following calculations in order to estimate soil hydraulic properties.

The simulated rainfall rate (*r*, cm min^{-1}) was calculated for each time interval:

$$r = \frac{\Delta H}{\Delta t} \qquad \text{[Eq. 1]}$$

where ΔH is the height of water lost from the vessel as rain during the time interval Δt (usually 3 minutes).

The runoff rate (RO, cm min^{-1}) was also calculated for each time interval:

$$RO = \frac{V}{457.3} * \Delta t \qquad \text{[Eq. 2]}$$

where V is the runoff volume collected during the time interval and 457.3 is the area of the ring (cm^2).

Sorptivity has the dimensions of [L/T$^{1/2}$] and was estimated according to Kutilek (1980):

$$S = r * \sqrt{2 * t_{RO}} \qquad \text{[Eq. 3]}$$

where S is sorptivity (in cm min$^{-0.5}$), t_{RO} is time to runoff (in minutes) and r is the measured rainfall rate (cm min^{-1}).

Infiltration rate (i) was calculated based on the data collected at the end of the measurement period, assuming that steady-state conditions were reached. Infiltration rate was the difference between rainfall (r) and runoff (RO) rates, with units of cm min^{-1}:

$$i = r - RO \qquad \text{[Eq. 4]}$$

Although the rainfall simulators were calibrated for an intensity of 30 cm h^{-1}, actual intensity varied. During the first 12 minutes of rain, measured intensity had an overall coefficient of variation of 24%. This can cause the volume of collected runoff to vary because of factors other than soil differences. In order to control this variability and make the volume of runoff (V_{RO}) independent of rainfall intensity, V_{RO} was computed as a fraction of the rainfall during the first 12 minutes, according to the following:

$$V_{RO} = \frac{V_{12}}{\Delta H12 * 457.3} \qquad \text{[Eq. 5]}$$

where V_{12} is the volume of runoff collected during the first 12 min of rain (cm^3) and ΔH_{12} is the amount of rain delivered during first 12 min (cm). The units of V_{RO}

are cm cm^{-1}. Alternatively, multiplying V_{RO} by 100 allowed for runoff volume to be computed as percent fraction ($\%V_{RO}$) of the total rainfall.

Total losses of P in runoff can then be calculated independent of rainfall intensity, by using V_{RO} and normalizing the results to a uniform intensity of 30 cm h^{-1} (or 6 cm / 12 min):

$$\frac{MassP}{Area} = Pconcentration * VRO * 6 * 100 \qquad \text{[Eq.6]}$$

where *Pconcentration* (mg P cm^{-3}) is analytically determined for each P fraction and 6 cm is the rain accumulated during 12 min if the intensity was 30 cm h^{-1}. The partial result was multiplied by 100 (cm^{2} ha^{-1} kg mg^{-1}), and the resulting units for $MassP/Area$ were kg ha^{-1}.

Data for surface soil phosphorus and aggregation, soil sorptivity, infiltration rate, concentration of P fractions in runoff and mass of P lost to runoff per unit area were analyzed using analysis of variance (ANOVA) and mixed model procedures in SAS (SAS Inst., 1989). First the ANOVA for each variable was done on a dataset that had both sites combined. Tillage and site were treated as fixed effects and replication as random. Where the site-by-tillage interaction was significant (α=0.05), the effect of tillage was tested by conducting ANOVAs by site. Each site had a randomized complete block design with four replications. Means were separated using the LSD option at P = 0.05 if a significant ANOVA occurred.

3.3. RESULTS

The Rogers Memorial Farm (RMF) site had significantly greater available P and soil organic matter in the surface soil (0–2.5 cm depth) than at UN–L's Agricultural Research and Development Center (ARDC) (Table 3.2). The two sites did not differ in total water-stable aggregates, except that RMF had about 10% more soil than ARDC in the large macro-aggregate class (>2mm). ARDC had 10% more soil than RMF in the small macro-aggregates class (2 – 0.250mm).

Tillage did not reduce total soil in aggregates (micro and macro-aggregates) (Table 3.2). However, moldboard plowing (MP) had more soil in stable micro-aggregates than disking (Disk), while more soil was in the macro-aggregate fractions with Disk than MP tillage or no-till (NT).

At RMF, soil sorptivity of NT was higher than MP tillage at RMF, indicating that continuous no-till may be more effective in retaining rainfall water from short but intense precipitations (Table 3.3). Infiltration rate and runoff volume were not different among tillage treatments at RMF. By contrast at the ARDC-site, tillage resulted in a greater infiltration rate, and tended to have greater sorptivity than continuous no-till. As a result, the runoff volume was reduced by a mean of 34% with Disk and MP compared to NT at ARDC, but was increased by 28% with MP at RMF; although these effects on runoff volume were not statistically significant for either site.

Table 3.2. Available soil phosphorus and water stable aggregates in the surface soil after a one-time Disk or plow tillage operation.

	Available soil P (Bray-1)	Soil organic matter	Water-stable aggregates‡			
Site / Tillage†			Micro (0.053-0.250 mm)	Small macro (0.250 – 2.0 mm)	Large macro (> 2 mm)	Micro + macro
	mg kg^{-1}	g kg^{-1}		g aggregates / 100 g soil		
RMF-site	103 a§	41.3 a	34.4	31.2 b	17.4 a	82.9
ARDC-site	40.5 b	32.3 b	34.5	41.2 a	7.6 b	83.4
No-till	97.9 a	38.9 a	34.9 ab	36.1	11.9	82.9
Disk	97.8 a	40.9 a	28.9 b	40.6	14.5	84.0
Moldboard plow	19.6 b	30.6 b	39.6 a	32.0	11.0	82.6

† Dates of tillage were respectively Mar. 26 2003 at Rogers Memorial Farm (RMF) and Nov. 26 2003 at UN–L's Agricultural Research & Development Center (ARDC).

‡ Water-stable aggregates were assessed on soil from the 0- to 5cm layer sampled in September 2005, during 3rd and 2nd crops post-tillage at RMF and ARDC, respectively.

§ Letters denote significant differences between sites or tillage (α=0.05).

Moldboard plowing reduced the *concentration of dissolved P* in runoff at both sites, when compared to runoff samples from Disk or NT (Figure 3.2). Tillage did not affect *concentration of particulate P* in runoff, but *total P concentration* was reduced with MP tillage at ARDC (Figure 3.2.b).

Table 3.3. The effect of tillage operations on soil hydraulic properties†.

Tillage‡	Sorptivity	Infiltration rate	Runoff volume
	cm min$^{-1/2}$	cm h^{-1}	%
		-------------------- RMF-site ‡ --------------------	

No-till	1.39 a	4.0	43.9
Disk	1.21 ab	4.8	44.3
Moldboard plow	0.95 b	3.0	60.8
p-value	0.04	0.56	0.07
LSD	0.34	3.5	16.1
	--------------------	ARDC-site ‡	--------------------
No-till	1.13	4.7 b	46.8
Disk	1.44	15.8 a	34.4
Moldboard plow	1.33	16.7 a	29.7
p-value	0.45	< .01	0.13
LSD	0.51	5.1	17.3

† Soil hydraulic properties were assessed *in situ* in September 2005, during 3rd and 2nd crops post-tillage at RMF and ARDC, respectively.

‡ Dates of tillage were respectively Mar. 26 2003 at Rogers Memorial Farm (RMF) and Nov. 26 2003 at UN–L's Agricultural Research & Development Center (ARDC).

§ Letters denote significant differences between tillage treatments within sites (α=0.05).

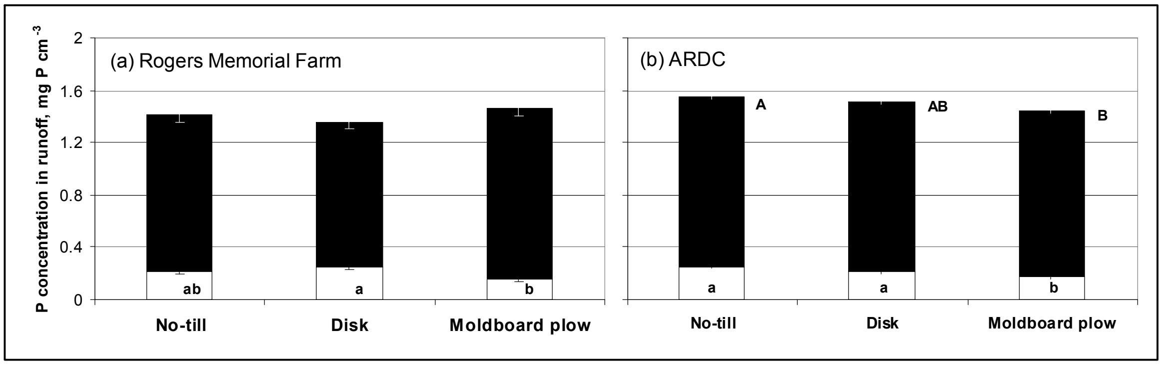

Figure 3.2. P concentration in runoff samples collected during a 12-minute simulated rain. P fractions are d*issolved P* (white bars) and *particulate P* (black bars). The site at Rogers Memorial Farm (a) was tilled in the spring 2003, while the site at ARDC (b) was tilled in the fall 2003. In some cases, standard error bars are too small to visualize. Differences between tillage treatments within sites are denoted by lower case letters for *dissolved* or *particulate P* and by upper case letters for total P (α=0.05).

Phosphorus loss in runoff (kg P ha^{-1}) was calculated from P concentration *and* runoff volume (Eq. [6]). At RMF, moldboard plowing increased loss of *particulate P* compared to Disk or NT, resulting in higher *total P loss* (Figure 3.3). In contrast, moldboard plowing at ARDC lessened both runoff volume and dissolved P concentration, and resulted in lower *dissolved P loss* than Disk or NT.

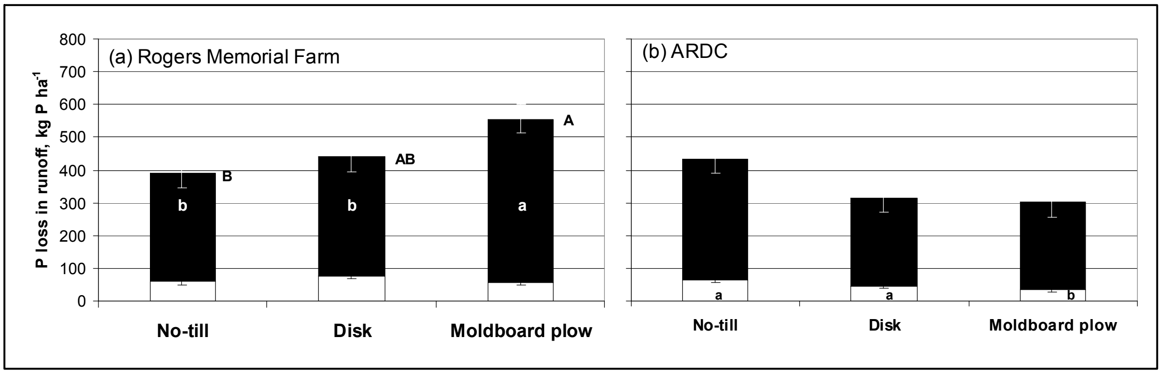

Figure 3.3. Mass P lost to runoff during a 12-minute simulated rain. P fractions are d*issolved P* (white bars) and *particulate P* (black bars). The site at Rogers Memorial Farm (a) received tillage in spring 2003, while the site at ARDC (b) was tilled in the fall 2003. In some cases standard error bars are too small to visualize. Differences between tillage treatments within sites are denoted by lower case letters for *dissolved* or *particulate P* and by upper case letters for total P (α=0.05).

3.4. DISCUSSION

A one-time tillage operation with moldboard plow (MP) mixed the surface soil with deeper layers as found for P and other nutrients (Garcia, 2005), and as verified in previous chapters in this thesis for various pools of soil organic matter. The overall conclusion was that a one-time MP tillage in early spring or late fall effectively re-distributes nutrients and soil organic matter without increasing decomposition of soil organic matter. It was hypothesized that soil disturbance by tillage was not severe enough to elicit major alterations of the soil structure, which partially controls availability of soil organic matter.

Results from the present study support this hypothesis because tillage did not reduce total soil in water-stable aggregates. More than 80% of soil was in water stable aggregates. Stable aggregates physically protect soil organic matter to reduce the rate of decomposition (Six et al., 2000).

Aggregate stability may account for the lack of tillage effect on particulate P concentration in runoff (Figure 3.2) with much particulate P bound within aggregates and not readily detached during simulated rain. In contrast, dissolved P in runoff was effectively reduced with MP compared with NT or Disk tillage. Even though dissolved P was on average less than 15% of total P, MP reduced total P in runoff at the ARDC site. These results indicate a positive correlation between dissolved P in runoff and available P in the soil surface at both sites

(Figure 3.4a). This relationship was not consistent for total P concentration in runoff across sites (Figure 3.4b).

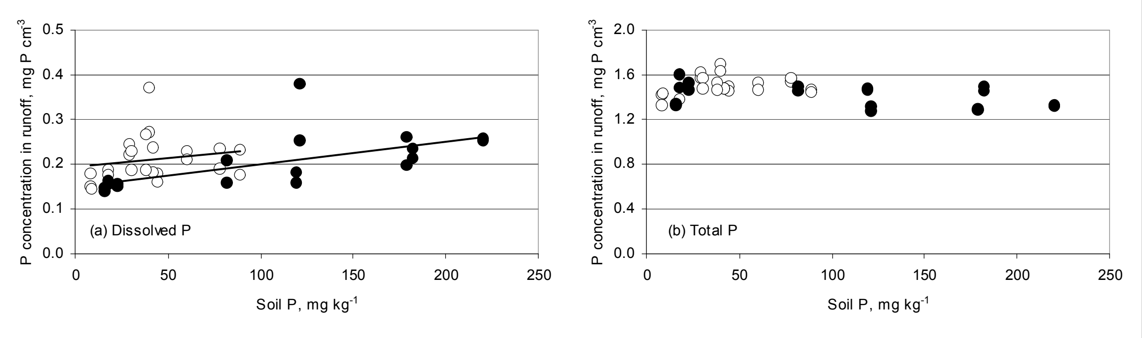

Figure 3.4. Phosphorus concentration in runoff collected during a 12-min simulated rainfall in relation to soil P test. P fractions are (a) dissolved and (b) total. Average rainfall intensity was 32 cm h^{-1} at Rogers Memorial Farm (closed symbols), and 38 cm h^{-1} at ARDC (open symbols). The resulting equation of a linear regression analysis on the pooled data is: dissolved P = 0.0003*Bray-P1 + 0.1844 (r^2= 0.1374).

Runoff volume was not statistically affected by tillage, but it was a critical variable in determining P losses (Figure 3.5). Soil hydraulic properties assessed in this study were useful to understand differences in runoff volume.

At Rogers Memorial Farm, the lower sorptivity of MP compared to NT, and the trend to lower infiltration rate with MP, resulted in increased runoff volume (Table 3.3). The lower sorptivity can be explained by the fact that MP removed the mulch of residues typical of NT systems. The study at RMF was done in the third year after tillage and enough time had elapsed to allow the soil to resettle and recover the smoothness of non-tilled soils. However, the disrupted macropores and channels were probably not reestablished, which hindered the vertical flow of water through the soil profile. This could explain why the infiltration rate in MP tended to be lower than NT.

At ARDC, the higher infiltration rate for MP and Disk, and the trend to higher sorptivity, for MP and Disk reduced the runoff volume compared to no-till. The study at ARDC was done in the second (rather than the third) year after tillage and the soil surface likely had roughness features from the tillage operation. This could explain the higher sorptivity in MP and Disk. It was not possible to support the 3-fold increase in infiltration from available data at this site. It has to be noted that the runoff- and infiltration tests were done on rows that were trafficked only during harvest operations, which happened once at ARDC but twice at RMF after occasional tillage. Therefore, soils may not have completely resettled after tillage, so that water could have preferential flow through large voids and internal fissures created by tillage.

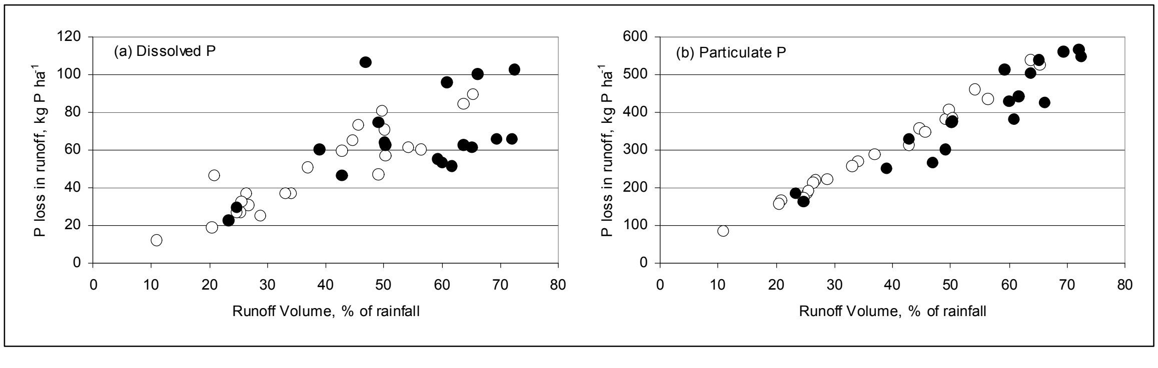

Figure 3.5. Phosphorus losses in (a) dissolved and (b) particulate fractions vs. runoff volume as a ratio of total rainfall. A 12-minute storm was simulated that delivered on average 6.4 cm at Rogers Memorial Farm (closed symbols), and 7.6 cm at ARDC (open symbols).

The value of rainfall simulation methods has been questioned if simulated rain did not closely approximate natural conditions. In this study, rainfall simulators allowed for subjecting soils to a rainfall of controlled intensity, duration and energy. Additionally, runoff rate could be monitored to infer soil hydraulic properties that were critical to understand qualitative differences between tillage that affect potential P losses due to runoff. Rainfall was set at an intensity of 30 cm h-1, which doubles the intensity of an event with a return period of 25 or 50 years in Lincoln, Nebraska (U.S. Weather Bureau, 1955). Therefore, resulting values have to be treated as relative, rather than absolute.

3.5. CONCLUSIONS

A broadcast manure application of 87.4 kg P ha^{-1} increased the Bray-P1 test in the surface soil by about 40 mg P kg soil, unless it was adequately incorporated by means of tillage. This was best achieved with a moldboard plow, which reduced the Bray-P1 test by 78 mg P kg^{-1} on average in the 0- to 2.5 cm layer. The concentration of dissolved P in runoff was reduced by moldboard plowing. Tillage did not affect soil aggregate properties at either location. Tillage affected sorptivity and infiltration differently at the two locations with more and less runoff with tillage at RMF and ARDC, respectively. Moldboard plowing resulted in less dissolved P loss at both sites and less total P loss at ARDC, although the reductions were small relative to absolute P loss. The benefit of one-time moldboard plow tillage in terms of reduced P loss in runoff was generally positive and presumably would be greater if surface soil P levels were extremely high.

3.6. REFERENCES

Behn, E.E. 1977. More profit with less tillage. Wallace-Homestead Book Co. Des Moines, Iowa, U.S.A.

Bray, R.H., and L.T. Kurtz. 1945. Determination of total, organic and available forms of phosphorus in soils. Soil Sci. 59:39-45.

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.

Clesceri, L.S., A.E. Greenberg, and A.D. Eaton (Eds.). 1998. Standard Methods for the Examination of water and wastewater. 20th Edition. American Public Health Association, Washington, DC. USA.

Daverede, I.C., A.N. Kravchenko, R.G. Hoeft, E.D. Nafziger, D.G. Bullock, J.J. Warren, and L.C. Gonzini. 2003. Phosphorus runoff: effect of tillage and soil phosphorus levels. J. Environ. Qual. 32:1436-1444.

Doran, J.W. 1987. Microbial biomass and mineralizable nitrogen distributions in no-tillage and plowed soils. Biol. Fertil. Soils 5:68-75.

García, J.P. 2005. The effects of occasional tillage on no-till systems on nutrient distribution and uptake and on vesicular arbuscular mycorrhizal (VAM) colonization. MS Thesis. University of Nebraska–Lincoln.

Gaynor, J.D., and W.I. Findlay. 1995. Soil and phosphorus loss from conservation and conventional tillage in corn production. J. Environ. Qual. 24:734-741.

Griffin, T.S., C.W. Honeycutt, and Z. He. 2003. Changes in soil phosphorus from manure application. Soil Sci. Soc. Am. J. 67:645-653.

http://www.sera17.ext.vt.edu/Documents/Methods_of_P_Analysis_2000.pdf

Kutilek, M. 1980. Constant rainfall infiltration. J. Hydrol. 45:289-303.

Laboski, C.A.M., J.A. Lamb. 2003. Changes in soil test phosphorus concentration after application of manure or fertilizer. Soil Sci. Soc. Am. J. 67:544-554.

Lowery, B., W.J. Hickey, M.A. Arshad, and R. Lal. 1996. Soil Water Parameters and Soil Quality. *In:* Doran, J.W., and A.J. Jones (Eds.). Methods for assessing soil quality. SSSA Special Publication Number 49. Soil Science Society of America, Inc. Madison, Wisconsin, USA.

McDowell, R.W., and A.N. Sharpley. 2003. The effect of soil carbon on phosphorus and sediment loss from soil trays by overland flow. J. Environ. Qual. 32:207-214.

Ogden, C.B., H.M. van Es, and R.R. Schindelbeck. 1997. Miniature rain simulator for measurement of infiltration and runoff. Soil Sci. Soc. Am. J. 61:1041-1043.

Olsen, S.R., and L.E. Sommers. 1982. Phosphorus. P. 403-430. IN: A.L. Page. R.H. Miller, and D.R. Keeney (eds), Methods of Soil Analysis. 2nd ed. Agronomy Series No. 9, Part 2. SSSA, Inc., Madison, WI.

Pote, D.H., and T.C. Daniel. 2000. Analyzing for total phosphorus and total dissolved phosphorus in water samples. p. 94. In: G.M. Pierzynski (Ed.) Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters. Southern Cooperative Series Bulletin No. 396.

Reynolds, W.D. and D.E. Elrick. 1990. Ponded infiltration from a single ring: I. Analysis of steady flow. Soil Sci. Soc. Am. J. 54:1233-1241.

SAS Institute Inc., 1989. SAS/STAT® User's Guide, Version 6, 4th ed., vol. 1 & 2. SAS Institute Inc. Cary, NC.

Schreiber, J.D., and L.L. McDowell. 1985. Leaching of nitrogen, phosphorus and organic carbon from wheat straw residues: I. Rainfall intensity. J. Environ. Qual. 14:251-256.

Sharpley, A.N. 2003. Soil mixing to decrease surface stratification of phosphorus in manured soils. J. Environ. Qual. 32:1375-1384.

Sharpley, A.N., and S.J. Smith. 1994. Wheat tillage and water quality in the Southern Plains. Soil Tillage Res. 30:33-48.

Sims, J.T., R.R. Simard, and B.C. Joern. 1998. Phosphorus loss in agricultural drainage: Historical perspective and current research. J. Environ. Qual. 27:277-293.

Six, J., E.T. Elliot, and K. Paustian, 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biology & Biochemistry 32:2099-2103.

U.S. Weather Bureau, “Rainfall Intensity-Duration-Frequency Curves”, Technical Paper No. 25, Washington, D.C., December 1955.

Chapter 4

Soil microbial community change and recovery after one-time tillage of a long-term no-till system

Abstract: The stratification of soil properties with long-term no-till results in soil quality improvements primarily in the surface 5 cm of the soil profile. Occasional tillage for no-till systems is proposed as a single, one-time tillage, conducted once in 12 or more years, to invert the highly improved surface layer with less improved deeper soil. This presents an opportunity to further improve agronomic and environmental performance of no-till systems. We hypothesize that occasional tillage will result in more soil microbial biomass, even though significant short-term losses of these microbes may occur in the surface 5 cm following tillage. Objectives were (1) to determine the change in the soil microbial biomass and microbial community composition after a one-time tillage of a long-term no-till system, and (2) to establish the recovery dynamics of the soil microbial communities over one or two cropping seasons following tillage. Two experiments in long-term NT fields were installed under rainfed corn or sorghum rotated with soybeans in eastern Nebraska. Fatty acid methyl ester (FAME) profiles were used to 'fingerprint' soil microbial communities in response to the following tillage treatments: continuous no-till, moldboard plow, and mini-moldboard plow. At both sites the continuous no-till soil had the highest microbial biomass at 0–5 cm depth, dropped to about half at 5-20 cm, and further decreased in the 20-30 cm depth. The ARDC site had higher contents of C16:1(c11), which was linked to the corn crop in the rotation at this site, compared to sorghum at RMF. On an equivalent mass basis, total microbial biomass in the first year after one-time tillage was on average 3% lower than continuous no-till. In the second year after tillage, the reduction in total microbial biomass was 7% on average. However, the mycorrhizal biomarker C16:1(c11) in the first year after tillage decreased on average 7% with respect to continuous no-till, while in the second year after tillage the reduction from no-till was 22% on average. In contrast, the fungal biomarker C18:2(c9,12) showed an average increase of 15% in the first sampling after tillage, and of 6% in the second sampling. It was shown that different microbial groups have different sensitivity to tillage, and that effects may not be highest in the first sampling date after tillage.

Abbreviations: ARDC, Agricultural Research & Development Center; FAMEs, fatty acid methyl esters; miniMP, mini-moldboard plow; MP, moldboard plow; NT, continuous no-till; RMF, Rogers Memorial Farm; VAM, vesicular arbuscular mycorrhizae.

Keywords: no-till, one-time tillage, FAMEs, lipid biomarkers.

4.1. INTRODUCTION

Managing the stratification of soil properties may be important to the agronomic and ecological performance of long term NT systems. We use the term ‘occasional tillage’ to refer to a single, one-time tillage operation in a system that is otherwise maintained without tillage. With the right type of tillage, the high stratification of phosphorus and carbon can be reduced through mixing and dilution with deeper, less improved soil.

Soil microbes are important to plant growth. Beneficial activities of soil bacteria include mineralization of nutrients, fixation of nitrogen, production of growth-promoting hormones, competitive suppression of pathogens (Gaskins et al., 1985), and facilitation of soil aggregation. Symbiosis with vesicular arbuscular mycorrhyzae (VAM) enables increased supply of P, Zn, and Cu to the host plant (Miller, 2000; Jeffries et al., 2003). Under no-till (NT) conditions, distinct soil microbial communities develop at different depths. Within the surface soil of a typical long-term NT, saprophytic fungi are key decomposers of plant residues (Hendrix et al., 1986) as these organisms can translocate nutrients from the soil into surface residues and tolerate the low water potentials often occurring in surface residues. Rhizospheric microbes, such as VAM and gram-negative bacteria are also often more numerous in the surface of no-till soils because of the high root biomass in this soil below the residue litter (Doran et al., 1998;

Mozafar et al., 2000). Conversely, soil disturbance due to tillage can be detrimental to the soil microbial community. Fungal mycelia networks are disrupted, while opportunistic decomposers can accelerate depletion of available C sources and suppress the formerly stabilized microbial community. The change in microbial community composition and biomass with tillage of NT systems, and the re-establishment of equilibrium, has not been well studied.

Traditional approaches used to study soil microbiology rely on recovery of viable cells from soil that can be cultured. It has been shown that culturing microbes on artificial media recover only 0.01-1% of the total microbes present in a given sample (Perfilev and Gabe, 1969). Analysis of fatty acid methyl esters (FAMEs) recovered from microbial cells within the soil can largely overcome this limitation. FAME analysis is based on a major constituent of living cells, the phospholipids, which can be efficiently extracted from soil. FAME analysis has been proposed as a method for ‘fingerprinting’ soil microbial communities (Zelles et al, 1995), since FAME profiles reveal the relative abundance of certain types of microbes and allow changes to be detected in soil microbial community composition. Certain fatty acids can be used as biomarkers for specific microbial types (Vestal and White, 1989) or individual taxa (Zelles et al., 1995; Olsson and Persson, 1999). Inferences about the overall physiological status of the bacterial community have been possible as well (Vestal and White, 1989; Petersen et al., 2002).

Several studies have been conducted in which phospholipid-linked fatty acid (PLFA) analysis proved to be sensitive in detecting soil microbial community changes. Steer and Harris (2000) grew *Agrostis stolonifera* plants in laminar pots and sampled bulk and rhizosphere soils separately to follow temporal shifts in soil microbial community structure. Evidence of rhizosphere development came from increases in fatty acids common to gram-negative bacteria (mainly mono-unsaturated fatty acids of 16 or 18 carbon-chains) and fungi (C18:2(c9,12)), while decreases in branched fatty acids indicated a decline in gram-positive bacteria.

Calderón et al. (2001) used PLFA profiles to show that roto-tillage significantly altered the composition of soil microbial communities without a concomitant change in microbial biomass carbon. Increases in microbial biomass nitrogen, soil nitrate content and denitrification rates indicated that the microbial community responded to newly available N after tillage.

Drijber et al. (2000) used FAMEs to ‘fingerprint’ soil microbial communities of dryland cropping systems in western Nebraska. Microbial communities under native sod were clearly distinct from those under 25-year-old wheat-fallow. The FAME C16:1(c11), a biomarker for arbuscular mycorrhizal fungi, was high in sod compared to cropped soil, and higher with long-term NT than with plowing. In a later study, Drijber (2002) examined the soil microbial recovery of no-till soil following a one-time tillage at the site in western Nebraska. Five years later, the microbial community structure in soil that received the one-time moldboard plow tillage still differed from soil of long term NT. The soil microbial communities of the one-time plowed and the long term plowed plots were not significantly different at depth under wheat. The mycorrhizal marker, C16:1(c11) became the dominant fatty acid at depth, and accounted for much of the difference between the continuous NT and the tilled treatments.

We use the term ‘occasional tillage’ to refer to the practice of a single, one-time tillage operation, conducted once in maybe 10-15 years, in a system that is otherwise maintained without tillage. We hypothesized that one-time tillage can effectively reallocate SOC from the surface into deeper soil layers to improve the deeper soil while increasing potential for C sequestration in the surface soil. In Chapter 1 of this dissertation, it was shown that soil organic matter in the surface 2.5 cm of soil was significantly less after moldboard plowing compared to continuous no-till, but total soil organic matter loss was negligible. If the enrichment of deeper soil layers can be maintained despite of soil disturbance, the C-sequestration capacity of these soils can be increased.

Under no-till, distinct FAME profiles are observed among strata, implying that soil microbial communities display a clear stratification in such conditions. The first hypothesis of this study is that the disturbance effect of occasional tillage will result in an overall loss of certain microbes in the entire sampling profile, while others will be more resilient and will not show overall losses. The second hypothesis is that the soil microbial community will show a recovery trend in subsequent years after tillage as a result of resuming no-tillage.

Therefore, the objective of this research was to determine the change in the soil microbial community after a one-time tillage of a long-term NT system, and to establish the recovery dynamics of the soil microbial communities over one or two cropping seasons following tillage.

4.2. MATERIALS AND METHODS

4.2.1. Site descriptions and management

Field research was conducted at two sites in eastern Nebraska with long-term, dryland no-till history (Table 4.1). Both soils are deep, well or moderately well drained, formed in loess on uplands, with a moderately slow permeability. For both sites, mean annual precipitation is 737 mm (29 in) and mean annual temperature is 11ºC. The sites differ in soil type and crop management history.

Table 4.1. General description of experimental sites for the study of the effects of one-time tillage in no-till systems in eastern Nebraska.

Experimental Site	**Continuous no-till history**	**Date of one-time tillage**	**Crops that followed tillage**	**Soil series**	**Soil classifi-cation**
Rogers Memorial Farm	12 years	Spring 2003 (Mar-26)	<u>Sorghum</u>† - Soybeans - Sorghum	Sharpsburg Si-Cl-Loam	Typic Argiudoll
Rogers Memorial Farm	13 years	Fall 2003 (Oct 24)	<u>Soybeans</u> - Sorghum	Sharpsburg Si-Cl-Loam	Typic Argiudoll
Agric. R&D Center	7 years	Fall 2003 (Nov-26)	<u>Soybeans</u> - Maize	Yutan Si-Cl-Loam	Mollic Hapludalf

† Underlined crop is the <u>first crop</u> after occasional tillage.

The site at the Rogers Memorial Farm (RMF) of the University of Nebraska–Lincoln (UN–L) was located approximately 16 km east of Lincoln, NE (40º50’44” N lat, 96º28’18” W long, 380 m altitude). The soil was a Sharpsburg silty clay loam (fine, smectitic, mesic Typic Argiudolls). The site occupied the area between two parallel steep-back sloped terraces that had been established in the mid-1960s. Conversion to NT occurred in 1991 with a soybean crop. The NT rotation included small grain cereals and corn rotated with soybeans. Controlled traffic has been practiced in order to minimize soil compaction. Lime was broadcast applied in the fall of 1997, 1999 and 2001 at a rate of 4.5 Mg ha^{-1} to correct soil pH without incorporation. At this site, the first crop following the one-time tillage was sorghum (2003, for spring-tillage) and soybeans (2004, for fall-tillage).

The site at UN–L’s Agricultural Research and Development Center (ARDC) was located near Mead, NE, about 48 km north of Lincoln (41º10’48” N lat, 96º28’40” W long, 358 m altitude). The soil was a Yutan silty clay loam (fine-silty, mixed, superactive, mesic Mollic Hapludalfs). This soil series was originally classified as fine, montmorillonitic, mesic Typic Argiudolls but reclassified due to loss of the thick dark mollic surface horizon due to severe erosion. The site occupied an unirrigated corner of a center pivot-irrigated field under a corn – soybean rotation, which was completely converted to NT in 1996. Since 1988 however, most but not all crops had also been under NT. No manure had been applied, but cattle have grazed on corn stalks. The only fertilizer applied in the system was anhydrous ammonia knifed in between rows in corn years, at an average rate of 146 kg N ha^{-1}. At this site, the first crop following the one-time tillage was soybeans (2004).

4.2.2. Experimental design

The experimental design at both sites was a randomized complete block design with four replicates. Tillage treatments at the RMF site were moldboard plowing (MP) in spring or fall 2003, and continuous no-till (NT) as the control. Main plots were 24 m long and 4.6 m wide. At the ARDC site, tillage treatments were moldboard plowing (MP), mini moldboard plowing (miniMP), and continuous no-till (NT). Main plots were 24 m long and 6.1 m wide. A thorough description of tillage treatments in this study is given in Chapter 1 of this dissertation (Table 1.1).

Tillage operations were completed on 26 March 2003 (DOY 85) for the spring tillage (RMF-site only) and a one-pass tandem disk was done on the MP-plots on 23 April 2003. Fall-tillage treatments were performed on 24 Oct. 2003 (DOY 297) and on 26 Nov. 2003 (DOY 330) at the RMF and ARDC sites, respectively.

4.2.3. Soil sampling and sample preparation

Soil samples were collected from three depths (0 - 5, 5 - 20, and 20 - 30 cm) in August 2003, May 2004 and May 2005. Eight cores were taken per plot with a 1.8-cm diameter probe, plus four cores with a 3.2-cm diameter probe for the depth of 0 – 5 cm. The entire sample was put into a plastic zip-lock bag after gently breaking up core segments by hand. Samples were transported to the lab in a cooler with icepacks and then stored in a refrigerator at 4 ºC. Within a few days, field-moist soils were passed through a 4-mm sieve and visible crop residues removed. Each sample was thoroughly mixed by sliding the soil repeatedly in different directions on a large sheet of paper. The entire sample preparation was done relatively quickly to minimize moisture loss. Each sample was sealed in a plastic bag and frozen at –12 ºC.

Soil bulk density was determined twice at both sites. **The first bulk density determination** was in summer 2004 and was described in detail in Chapter 1 of this dissertation. Briefly, for the 0- to 2.5- and 2.5- to 5-cm strata, a cavity was carved with a hand shovel, lined with a fine plastic sheet, and filled with sand measured with a graduated cylinder to determine the volume of the cavity. A push probe with an inserted plastic tube liner was used for the 5- to 10-cm, 10- to 20-cm, and 20- to 30-cm depths. Soil cores were slid out of the tube in the laboratory and cut into segments according to the corresponding depths. The mass of the soil from all five depths was obtained after oven-drying at 105 ºC for 48 h. Measurements and samples were taken at three locations per plot and the arithmetic mean was computed for each plot. Because this study had three, rather than five sampling depths, an arithmetic average was calculated (for the 5 to 20 cm stratum, a weighed average was calculated from bulk densities of the 5- to 10-cm and 10- to 20-cm depths, considering that these contribute respectively 1/3 and 2/3 to the 15-cm depth). A **second bulk density determination** was done in fall 2005 for the RMF site and in spring 2006 for the ARDC site for the three depths of interest. A 3.2 cm diameter push probe was used to collect soil samples for bulk density from the surface stratum (0-5cm). Special care was given while pushing the probe into the soil in order to avoid compressing the surface soil. A known volume of soil was systematically collected by cutting the cores of exact length, using a spatula and reference marks on the probe. For the next two sampling depths (5-20 and 20-30 cm), the push probe with inserted plastic tubes was used as explained above, after removing the surface soil to approximately 5-cm depth. Two blocks in each site were sampled and the average was assumed to represent the bulk density of each tillage treatment across blocks.

Table 4.2. Treatment effects on bulk density† for three depth increments at two research sites in eastern Nebraska.

	Depth increment (cm)		
Tillage, Season	0- 5	5 - 20	20 - 30
	----------- Mg m^{-3} -------------		
	Rogers Memorial Farm, 2004		
No-till	1.03	1.36	1.33
Moldboard plow, spring tillage	–	–	–
Moldboard plow, fall tillage	0.87	1.18	1.30
	Rogers Memorial Farm, 2005		
No-till	1.24	1.30	1.37
Moldboard plow, spring-tillage	1.27	1.27	1.28
Moldboard plow, fall-tillage	1.28	1.28	1.29
	Agric. R&D Center, 2004		
No-till	0.96	1.31	1.31
Mini moldboard plow	0.89	1.22	1.30
Moldboard plow	0.91	1.17	1.36
	Agric. R&D Center, 2005		
No-till	1.17	1.38	1.39
Mini moldboard plow	1.06	1.29	1.37
Moldboard plow	1.08	1.32	1.37

† Bulk density estimations were averages from 2 blocks for each site.

4.2.4. Laboratory procedures

Total ester-linked FAMEs were extracted from the soil by mild alkaline methanolysis (White et al.,1979; Drijber et al., 2000). Samples were hydrolyzed using freshly prepared 0.2 M potassium hydroxide in methanol, and the resulting FAMEs were partitioned into hexane. This procedure does not methylate *free* fatty acids, but only *ester-linked* fatty acids (Kates, 1986; Grogan and Cronan, 1997) contained in the cell membranes of most cells. Lipids from archaebacteria are an exception because these contain *ether-linked* lipids, which cannot be measured by the actual method (Vestal and White, 1989). Released FAMEs were separated by capillary gas chromatography, using helium as a carrier gas, on a Hewlett Packard 5890 Series II gas chromatograph. This instrument contained an Ultra 2 HP capillary column (50 m, 0.2 mm I.D., 0.33 µm film thickness) and was run in split mode (44:1) with a 0.75 min purge time. Injector and flame ionization detectors were maintained at 280 and 300 ºC, respectively, and oven temperature was ramped from 50 ºC to 160 ºC at 40 ºC min^{-1} and held for 2 min, then ramped at 3 ºC min^{-1} to 300 ºC and held for 30 min. An internal standard, methyl-nonadecanoate (0.4 μg C19:0 μl^{-1}) was added to the FAME extract to ensure reproducibility in the amount of lipid entering the capillary column. Identification of the FAMEs was by comparison of retention time and equivalent chain length with C19:0 as known standard (Bacterial Acid Methyl Esters CP Mix, Supelco USA), and confirmed by gas chromatography mass spectrometry (GC-MS). Unsaturated and substituted FAMEs were identified by GC-MS as dimethyloxazoline derivatives (Yu et al., 1989). All solvents used were HPLC grade, glass distilled and filtered.

Fatty acids were designated as the total number of carbon atoms followed by a colon, the number of double bonds followed by the position of the double bond from the carboxyl end of the molecule and its *cis* or *trans* configuration in brackets. The prefixes *a* and *i* indicate *anteiso* and *iso* branching, respectively, *br* indicates an unknown branch position, 10 Me indicates a methyl branch on the 10th carbon atom from the carboxyl end of the molecule, and cy(9,10) refers to cyclopropane ring between the 9th and 10th carbon atom.

4.2.5. Statistical Analyses

FAMEs with retention times less than C14:0 and greater than C20:0 were deleted from the data set. Two statistical approaches described below were used to analyze changes in microbial biomass and microbial composition caused by tillage, sampling year and sampling depth.

FAME indices for microbial groups

FAME indices for various microbial groups were calculated from the summation of individual FAMEs (Table 4.3), reported as 'absolute amounts' (nmol g^{-1} oven dried soil). Concentrations of FAME indicators for the three depths were used to estimate their content in the soil profile per unit area. Data were used to quantify losses or gains in certain microbial groups between the one-time tilled soils and the continuous no-till baseline. These differences in stocks of microbial groups were calculated as % relative to the no-till counterpart for a given sampling year, site and block.

Table 4.3. List of individual FAMEs used in the computation of indicators of soil microbial groups.

Microbial group	**FAME(s) included in the summation**
Bacteria	iC14:0; C15:0; iC15:0; aC15:0; iC16:0; C17:0; iC17:0; aC17:0; cyC17(9,10); C17:1(c9); cyC19(11,12)
Actinomycetes	10MeC18:0; i10MeC18:0; 10MeC19:0
VAM	C16:1(c11)
Fungi	C18:2(c9,12)
Microeukaryotes	C20:4(5,8,11,14)
Total microbial biomass	all 17 above-listed FAMEs

This was done on an equivalent mass basis of 400 kg soil m^{-2} (~30-cm depth), according to equation (1), after Gifford and Roderick (2003).

$$Stock_{400kg} = Stock_{20cm} + \frac{Stock_{30cm} - Stock_{20cm}}{Ms_{30cm} - Ms_{20cm}}(400 - Ms_{20cm}) \qquad \text{[Eq. (1)]}$$

where Stock$_{400kg}$ is the mass of FAMEs contained within a soil mass of 400 kg m^{-2}; Ms$_{20cm}$ and Ms$_{30cm}$ are masses of dry soil to a fixed depth of 20 and 30 cm, respectively (in units of kg m^{-2}); and 400 kg dry soil per m^2 is the reference soil mass chosen for this study and approximately corresponds 30 cm depth in NT. Stock$_{20cm}$ and Stock$_{30cm}$ are amounts of FAMEs stored in the soil at a fixed depth of 20 and 30 cm, and were calculated according to the following general equation:

$$StockD = \sum (conc_i * \rho_i * t_i) \qquad \text{[Eq. (2)]}$$

where each stratum i had a specific FAME concentration (conc$_i$), bulk density (ρ_i) and thickness (t_i). Soil depth (D) was 20 or 30 cm; and thickness of the layer (t_i) was respectively 5, 15 and 10 cm for the 1st, 2nd and 3rd strata, respectively.

Stepwise and canonical discriminant analyses

FAMEs were converted from ‘absolute amounts’ to ‘relative amounts’ (nmol% of total FAMEs) by dividing the nmol value of an individual FAME by the total nmol FAMEs of a given plot. Stepwise and canonical discriminant analyses (SAS, 1989) were used to detect relationships between FAME profiles and tillage treatments, sampling depth, and year after tillage.

Contents of FAME indicators for the three depths, as well as their stocks on an equivalent soil mass basis, were analyzed using analysis of variance (ANOVA) and mixed model procedures in SAS (SAS Inst., 1989) appropriate for a randomized complete block design with four replications. Depth of sampling and sampling time were analyzed as repeated measures. Because the treatment design slightly differed between sites, separate ANOVAs were run by site in order to compare all treatments of the study. An ANOVA for tillage and site effects was done combining the data of both sites, but excluding miniMP and MP_{Spring}, since these were not used at both sites. To test for the season-by-tillage interaction (for the case of RMF only), a subset of data was used that excluded no-till. Replications were always treated as random effects, while tillage treatments were fixed effects. Whenever a significant ANOVA occurred, means were separated using the LSD 0.05 option.

4.3. RESULTS

4.3.1. Continuous no-till

FAME indicators of microbial groups were assessed on a mass-per-area basis for an equivalent dry soil mass of 400 kg m^{-2}, according to equation (2). Table 4.4 shows how the continuous no-till baseline was affected by the sampling year and site. At RMF, the total microbial biomass was more in 2003 and 2005 than 2004, and was related to higher FAME amounts associated with bacteria, actinomycetes, fungi, VAM and microeukaryotes. At ARDC, total microbial biomass was less in 2005 than in 2004 due to less FAME amounts associated with bacteria and actinomycetes, although fungal biomass was more in 2005 than 2004. In 2004, total microbial biomass was greater at ARDC than at RMF due to higher FAME amounts for bacteria, actinomycetes, and mycorrhizae. ARDC had significantly higher amounts of the mycorrhizal FAME than RMF in both 2004 and 2005. In 2004, FAMEs indicative of bacteria and actinomycetes were also higher at ARDC, resulting in higher total microbial biomass at ARDC than RMF.

Table 4.4. Effect of year and site on FAME biomass indicators of microbial groups for continuous no-till soil, measured on an equivalent soil mass basis of 400 kg m^{-2} at Rogers Memorial Farm (RMF) and Agricultural Research and Development Center (ARDC) in eastern NE.

	Bacteria		Actinomycetes		Fungi	
	RMF	ARDC	RMF	ARDC	RMF	ARDC
			μmol m^{-2}			
2003	11336 a	-	3061 a	-	1495 a	-
2004	9694 b,B†	10944 a,A	2833 b,B	3699 a,A	1074 b,A	1054 b,A
2005	11114 a,A	9853 b,B	3125 a,A	3274 b,A	1602 a,A	1266 a,A

	Micorrhyzae		Microeukaryotes		Total Microbial Biomass	
	RMF	ARDC	RMF	ARDC	RMF	ARDC
			μmol m^{-2}			
2003	2822 a	-	337 a	-	19050 a	-
2004	1109 b,B	2061 a,A	250 b,A	268 a,A	14959 b,B	18026 a,A
2005	1379 b,B	1821 a,A	344 a,A	298 a,A	17563 a,A	16740 b,A

† Different lower case letters indicate differences between years within a site; and different upper case letters indicate differences between sites within a year.

Canonical discrimination analysis for the continuous no-till soils indicated that significant shifts in FAME profiles occurred among depths and among sites (Fig. 4.1). Eighteen FAMEs were significant in the discrimination of these two factors. The first canonical variable (Can1) discriminated mostly for sampling depth, and accounted for 68% of the variability in FAMEs, while the second canonical variable (Can2) discriminated between sites and accounted for 19% of the variability. Fatty acids iC16:0, aC17:0, and i10MeC17:0 had the highest *positive* correlations with Can1 and their relative amounts consistently increased from the surface to deeper sampling strata. Can1 was negatively correlated with C16:0, C17:1(c9), 10MeC18:0, C18:1(c11), and unkC18:1, which significantly decreased with depth at both sites. C:20:0 had the *highest* correlation with Can2 and was significantly higher in RMF than ARDC, regardless of sampling depth. Fatty acids cyC19(11,12) and unkbrC19 were also *positively* correlated with Can2 and were more dominant in RMF than ARDC in the 5-20 and 20-30 cm depths. In turn, C17:1(c9), 10MeC18:0, and C18:1(c11) were *negatively* correlated with Can2, and were less dominant in RMF than ARDC in the surface 0-5 cm. The mycorrhizal biomarker C16:1(c11) was positively correlated with Can1 and negatively correlated with Can2, and became significantly more abundant in ARDC than RMF in the 5-20 and 20-30 cm strata.

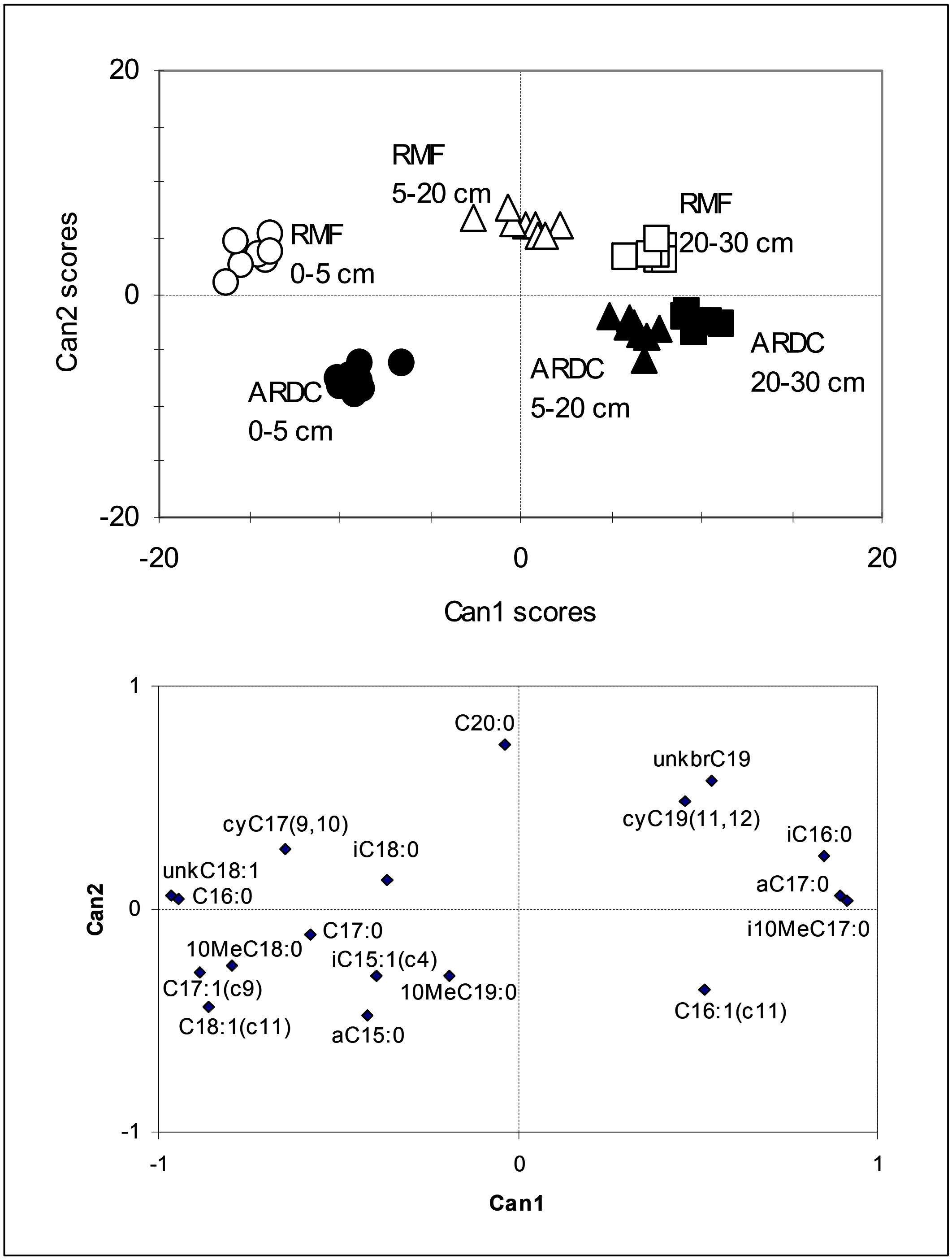

Figure 4.1. Canonical discrimination and correlation analysis of three sampling depths in continuous no-till soils at Rogers Memorial Farm (RMF, open symbols) and Agricultural Research and Development Center (ARDC, closed symbols): (a) discriminant scores of sites and sampling depths; and (b) correlations of FAMEs with the first two significant discriminant functions, Can1 and Can2.

4.3.2. One-time tillage and sampling year

Tillage affected microbial biomass and microbial groups when calculated for the sampled soil profile, compared to the NT baseline. This is shown as relative losses with respect to continuous NT in Fig. 4.2.

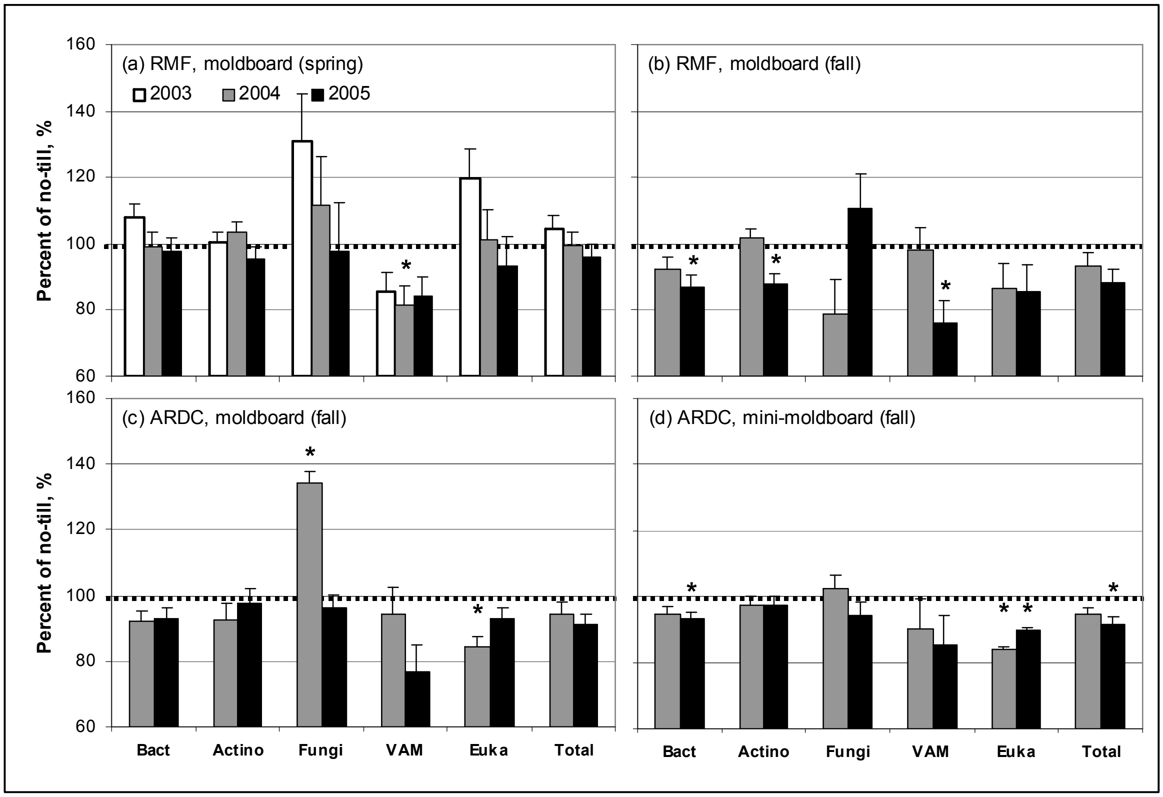

Figure 4.2. Losses or gains in FAME indices between the one-time tilled soils and the continuous no-till baseline at Rogers Memorial Farm (RMF) and Agricultural Research and Development Center (ARDC). In RMF, moldboard plow was used in spring or fall 2003, and in ARDC, moldboard plow and mini-moldboard plow were used in fall 2003. Stocks of microbial groups were calculated on an equivalent mass basis, and the relative difference calculated (continuous no-till = 100%) for each sampling year, site and block. FAME indices were: bacteria (Bact), actinomycetes (Actino), fungi, vesicular arbuscular mycorrhizae (VAM), microeukaryotes (Euka) and total microbial biomass (Total).

Moldboard plowing in the spring (MP-spring) did not cause significant losses in total microbial biomass, but its mass per hectare tended to decline with sampling years, compared to NT (Fig. 4.2.a). The greatest relative losses due to MP-spring occurred for the mycorrhizal FAME, averaging 14, 19 and 16% for 2003, 2004 and 2005, respectively (this loss was statistically significant only in 2004). The means for the other microbial groups were not reduced by spring tillage. However, in the first year after MP-spring, FAME indicative of fungi, microeukaryotes and bacteria increased 31, 20 and 8%, respectively, compared to NT. These FAME indicators declined in subsequent years, and averaged below the NT level in 2005.

Moldboard plowing in the fall (MP-fall) at **RMF** caused a decline in total microbial biomass that averaged 7 and 12% in 2004 and 2005, respectively, compared to NT (Fig. 4.2.b). Although this decline was not statistically significant, FAMEs indicating mycorrhizae, bacteria and actinomycetes were significantly lower than NT in 2005.

Moldboard plowing in the fall at ARDC caused a decline in total microbial biomass that averaged 6 and 9% in 2004 and 2005, respectively, compared to NT (Fig. 4.2.c). The mycorrhizal FAME had an average loss of 6 and 23% compared to NT in 2004 and 2005, but differences were not statistically significant. The fungal FAME significantly increased 34% with respect to NT in 2004. Microeukaryotes significantly decreased 16% compared to NT in 2004.

The **mini-moldboard** (mini-MP) caused a decline in total microbial biomass that averaged 5 and 8% in 2004 and 2005, respectively, and the difference was statistically significant in 2005 (Fig. 4.2.d). Bacterial FAMEs significantly declined 7% with respect to NT in 2005, and microeukaryotes significantly declined 16 and 10% with compared to NT in 2004 and 2005, respectively. The mycorrhizal FAME declined 10 and 15% with respect to NT in 2004 and 2005, although differences were not significant.

4.3.3. Microbial changes within sampling depths

In order to explore how tillage caused FAME profiles to shift within each sampling depth, canonical discrimination analysis was done separately for RMF and ARDC.

Rogers Memorial Farm

FAME profiles were significantly affected by tillage and sampling year in the three sampling depths at RMF (Fig. 4.3 and Table A.1). Canonical discrimination analysis for the 0-5, 5-20 and 20-30 cm sampling depths revealed that 11, 10, and 7 FAMEs, respectively, were significant for each discrimination model. The first and second canonical variables (Can1 and Can2) were highly significant (<0.0001) in all three depths, and explained between 89 and 96% of the variance.

In the 0-5 cm depth, fatty acids iC16:0, aC17:0 and i10MeC17:0 had the highest positive correlations with Can 1 and were relatively more abundant in moldboard plow than NT soils (Fig. 4.3.a). Fatty acids C16:0 and unkC18:1 had high negative correlations with Can1 and were lower in MP than NT, especially in 2003 and 2004.

Moreover, moldboard plow caused a reduction in fungal biomass that was greatest in the first sampling after tillage (Fig. 4.4.d–f). Relative to NT, this reduction averaged more than 60% for spring and fall plowing at RMF. After one crop post-tillage, the fungal biomass increased and averaged respectively 25% and 15% lower than continuous NT. After two crops post-tillage, moldboard plow in the spring averaged 8% lower than continuous NT.

Similar to the fungal biomass, the mycorrhizal biomarker C16:1(c11) had a decrease in the first sampling time post-tillage that averaged 65% and 53% lower than NT for spring and fall tillage, respectively (Fig. 4.4. g–l). After one crop post-tillage, the VAM biomass was respectively 54% and 56% lower, and two crops after tillage, VAM was 40% lower than NT for the moldboard plow in spring.

In the 5-20 cm depth, the fatty acid C16:1(c11) was positively correlated with Can1 and Can2, and was significantly higher in 2003 than 2004 and 2005 (Fig. 4.3.b). Fatty acids C15:0, iC16:0 and i10MeC17:0 were more abundant in 2005. Fatty acids C17:1(c9), C18:0 and unkC18:1 had a negative correlation with Can2 and were more abundant in plowed than NT soil. **In the 20-30 cm depth**, C16:1(c11) had the highest positive correlation with Can1, with higher abundance in 2003 than 2004 and 2005 (Fig. 4.3.c). The FAME a10MeC18:0 had the highest negative correlation with Can1, and had lower abundance in 2003 than 2004 and 2005.

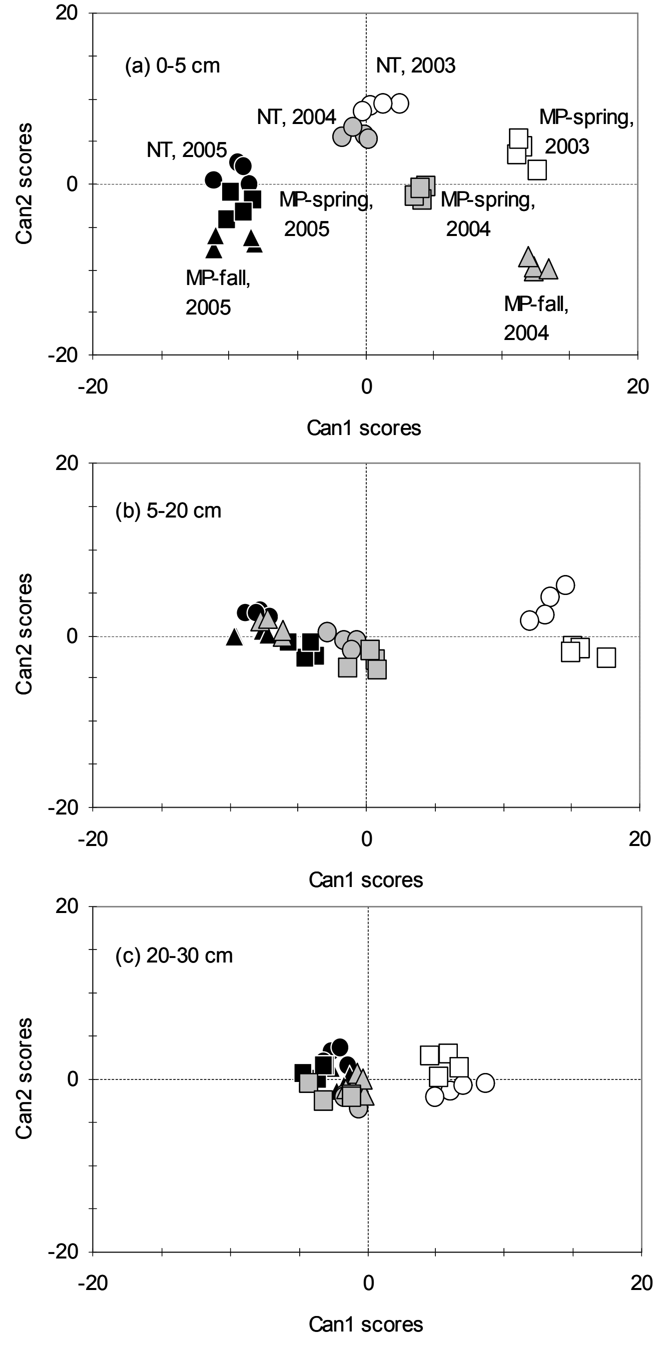

Figure 4.3. Canonical discrimination analysis of FAME profiles for tillage treatments and sampling year at Rogers Memorial Farm (RMF), within each of three sampling depths: (a) 0-5 cm, (b) 5-20 cm, and (c) 20-30 cm. No-till (NT, circles), moldboard plow in spring (MP-speing, squares), and moldboard plow in fall (MP-fall, triangles) were sampled in different years: 2003 (open symbols), 2004 (gray) and 2005 (black). Can1 and Can2 were the first two significant discriminant functions and their correlations with individual FAMEs are shown in Table A.1 in the appendix.

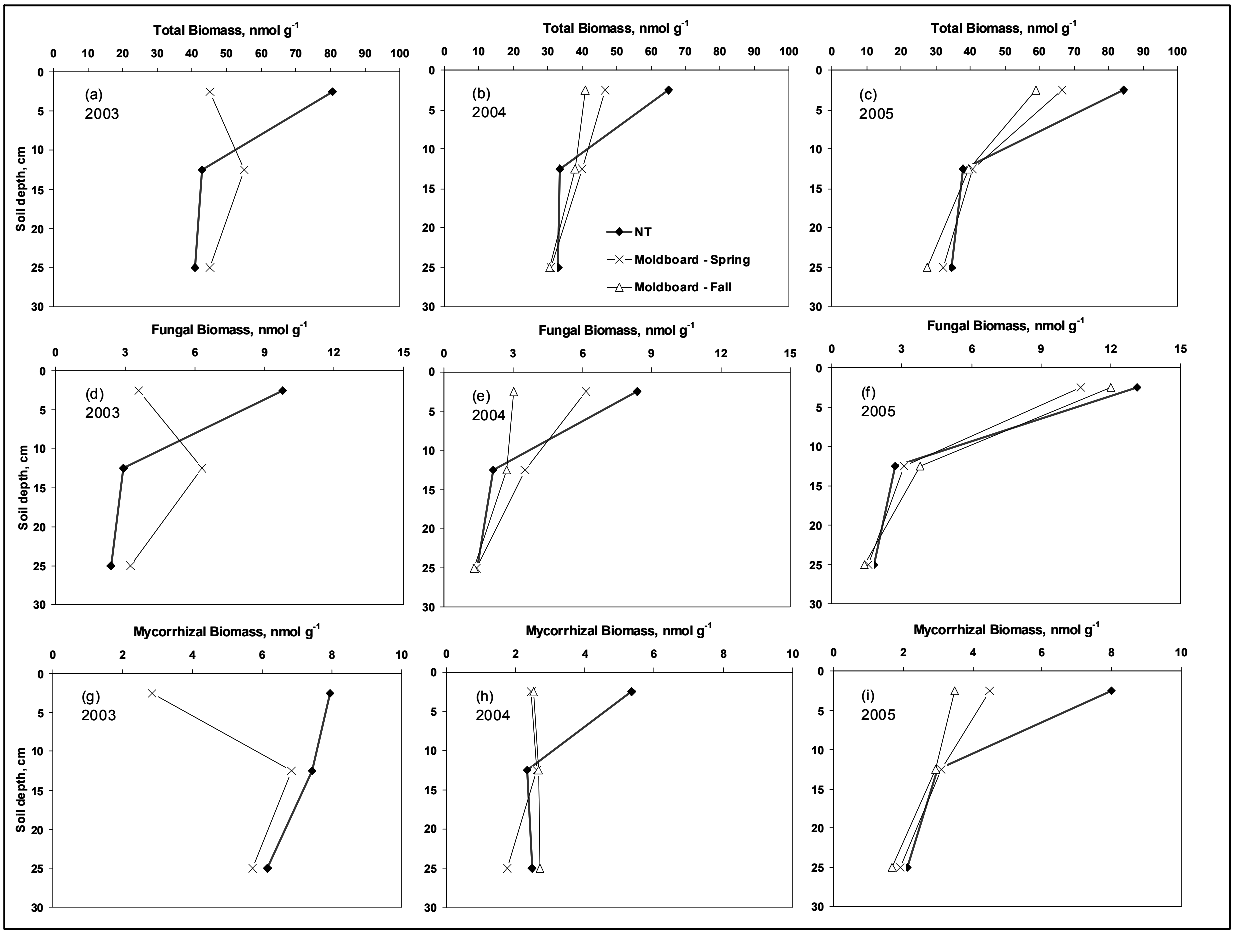

Figure 4.4. Vertical distribution of FAME indicators of microbial biomass (a,b, c), fungal biomass (d, e, f) and mycorrhizal biomass (g, h, I) for tillage treatments and sampling year at Rogers Memorial Farm (RMF), within each of three sampling depths, 0-5 cm, 5-20 cm, and 20-30 cm.

Agricultural Research and Development Center

Similar to RMF, FAME profiles were significantly affected by tillage and sampling year in the three sampling depths at ARDC (Fig. 4.5 and Table A.2). Canonical discrimination analysis for the 0-5, 5-20 and 20-30 cm sampling depths revealed that 7, 11, and 5 FAMEs, respectively, were significant in for each discrimination model. The first and second canonical variables (Can1 and Can2) were highly significant (<0.0001) in all three depths (except for Can2 at 20-30 cm, with $P<0.05$), and explained between 91 and 94% of the variance.

In the 0-5 cm depth, differences in FAME profiles due to tillage were more significant in 2004 than 2005 (Fig. 4.5.a). Fatty acids iC18:0 and i10MeC18:0 were positively correlated with Can1, and were higher for MP and miniMP than NT, especially in 2004. Conversely, fatty acids C18:1(c9) and unkC18:1 were negatively correlated with Can1, and were higher for NT than MP and miniMP. In 2005, FAME profiles from NT and miniMP were not significantly different at the surface depth.

Similar to RMF, moldboard plow caused a reduction in fungal biomass that was greatest in the first sampling after tillage (Fig. 4.6.c and d). Relative to NT, this reduction averaged 28% for moldboard plow, while mini-moldboard plow showed no reduction in fungal biomass. After one crop post-tillage, the fungal biomass was 22% lower than continuous NT.

The biomass of VAM decreased in the first sampling time post-tillage by 34% and 8% for moldboard plow and mini-moldboard plow with respect to NT.

After one crop post-tillage, the VAM biomass was respectively 30% and 43% lower than continuous NT (Fig. 4.6. e and f).

In the 5-20 cm depth, and contrary to the surface depth, FAME profiles of miniMP and NT were not different in 2004, but a significant shift occurred in 2005 (Fig. 4.5.b). FAME profiles from MP were significantly different in 2004, but the difference with NT was greater in 2005, with fatty acids C16:1(c5), C18:1(c11), unkC18:1 and C18:2(c9,12) having higher abundance in MP and miniMP than NT. **In the 20-30 cm depth**, FAME profiles of MP and miniMP were not different from NT in 2004 (Fig. 4.5.c). In 2005 however, a small but significant shift in FAME profiles occurred, with NT having higher abundance of C18:1(c11).

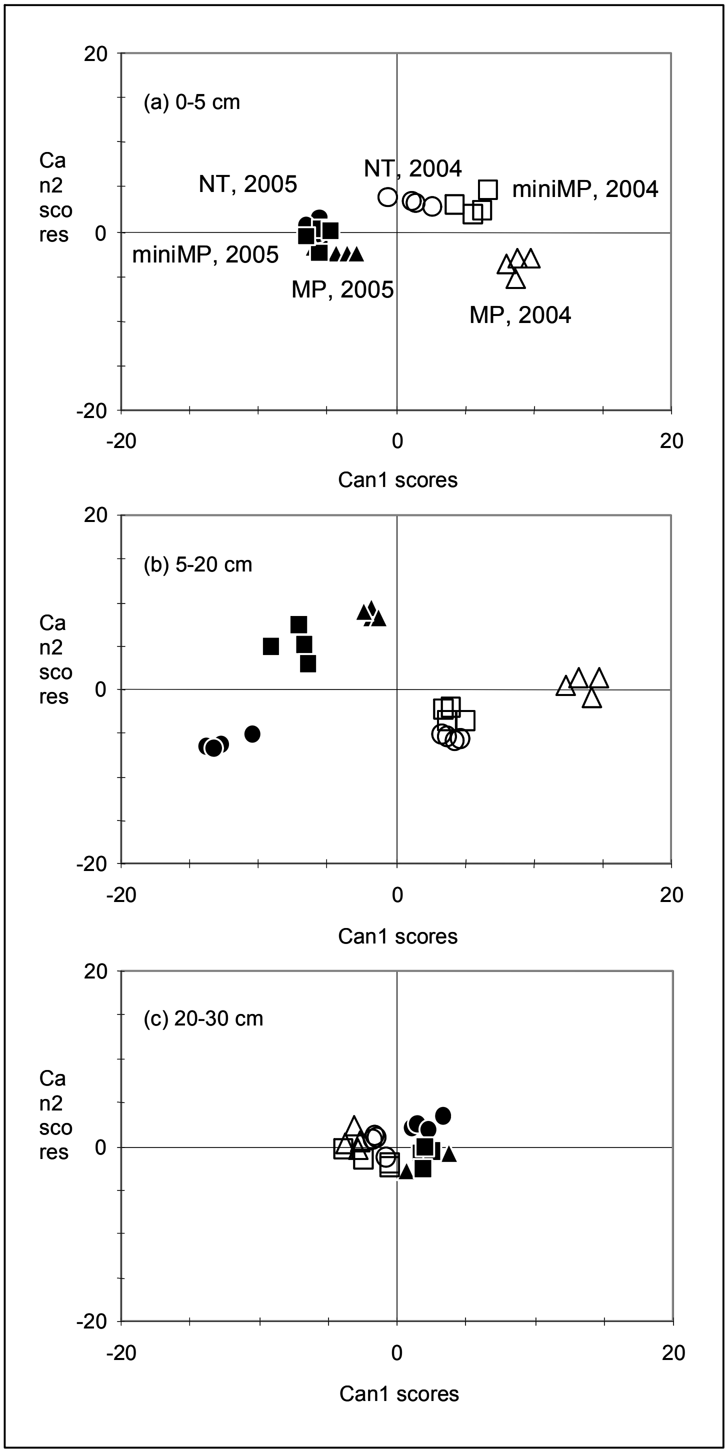

Figure 4.5. Canonical discrimination analysis of FAME profiles for tillage treatments and sampling year at Agricultural Research and Development Center (ARDC), within each of three sampling depths: (a) 0-5 cm, (b) 5-20 cm, and (c) 20-30 cm. No-till (NT, circles), mini-moldboard plow (miniMP, squares), and moldboard plow (MP, triangles) were sampled in different years: 2003 (open symbols), 2004 (gray), and 2005 (black). Can1 and Can2 were the first two significant discriminant functions and their correlations with individual FAMEs are shown in Table A.2 in the appendix.

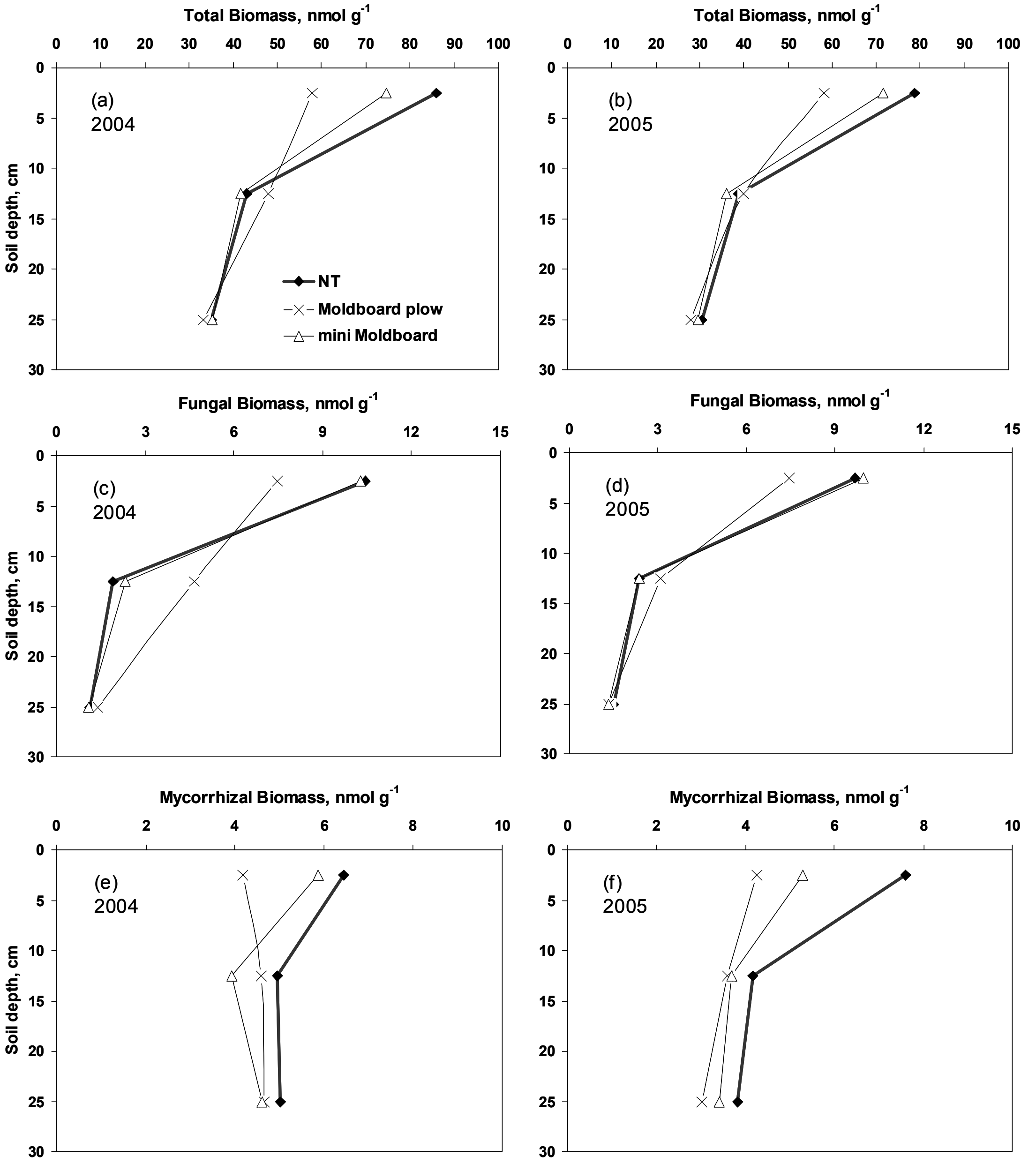

Figure 4.6. Vertical distribution of FAME indicators of microbial biomass (a,b), fungal biomass (c, d) and mycorrhizal biomass (e, f) for tillage treatments and sampling year at Agricultural Research and Development Center (ARDC), within each of three sampling depths, 0-5 cm, 5-20 cm, and 20-30 cm.

4.4. DISCUSSION

The objective of this research was to determine the change in the soil microbial community after a one-time tillage of a long-term NT system, and to establish the recovery dynamics of the soil microbial communities over one or two cropping seasons following tillage.

Continuous no-till

This study supports previous findings that the soil microbial community displays a clear stratification under continuous no-till. Corn generally supports a greater colonization of mycorrhizae than other cereal crops (Mozafar et al., 2000). In the same experimental sites as the present study, García (2005) assessed mycorrhizal colonization of crop roots following occasional tillage and found that corn roots had significantly more C16:1(c11) than sorghum roots. ARDC was under a corn-soybean rotation, while RMF was under a sorghum-soybean rotation since 1991. This supports the finding of the present study that ARDC had higher mycorrhizal FAME than RMF (Table 4.4).

High crop residue inputs on the soil surface are inherent to no-till systems (Doran et al., 1998). Consequently, total soil microbial biomass is highest just beneath the mulch of crop residues. In particular, the fatty acid C18:2(c9,12), indicative of saprophytic fungi had remarkably higher concentrations in the 0 – 5 cm soil than in deeper soil.

As opposed to saprophytic fungi, the presence of mycorrhizal fungi is intimately associated with the root system and the specific crops used in the cropping system (Harrier and Watson, 2003). In long-term NT soils in western Nebraska, Drijber et al. (2000) found that the mycorrhizal FAME became dominant with depth, and was a highly significant FAME to explain profile shifts due to sampling depth. This is in agreement with findings in the present study that the mycorrhizal biomarker C16:1(c11) was significant in the discrimination model and became relatively more abundant with depth compared with other FAMEs. The relatively great biomass of this FAME in the 5-20 and 20-30 cm strata was more pronounced at ARDC where corn was part of the rotation, than at RMF where the cereal crop was sorghum.

One-time tillage

Assessing mass per hectare of FAME indicators (of total microbial biomass or microbial groups) for continuous NT compared with one-time tillage, requires accounting for changes in soil bulk density. The equivalent soil mass approach has proven valid for calculating stocks of nutrients and organic C in the soil, as discussed in Chapter 2 of this dissertation. Amounts of FAMEs per unit area, on an equivalent soil mass basis have not been reported to the knowledge of the present author. However, a number of studies have reported that cropping systems based on tillage generally sustain a smaller microbial biomass, compared to no-till systems (Drijber et al., 2000). The present study support this general finding, even though decreases from the NT baseline were relatively small (<12%) and rarely significant.

The canonical discrimination analysis to detect significant differences in FAME profiles (Figs. 4.3 and 4.5) showed that differences between tilled and NT were greatest in the soil surface and lowest in the deepest sampling layer (20-30 cm). The finding that MP increased iC16:0 and aC17:0 may indicate an increase in bacteria in the 0 – 5 cm soil depth at RMF. Fatty acid C16:0 is regarded as primarily of bacterial origin, but has been found as a constituent of saprophytic fungi, as has C18:1(c9) (Stahl and Klug, 1996). These fatty acids were found relatively abundant in the surface of NT soil, and were significantly reduced by moldboard plowing.

Do FAME profiles shift towards the NT baseline after one-time tillage?

To better explore the dynamics of the soil microbial community shifts with respect to the no-till baseline in each sampling depth, the matrix of Mahalanobis distances between clusters was examined, in association with the probability of significance. Figure 4.7 shows that in RMF, the biggest shifts in FAME profiles occurred in the soil surface in the first sampling after tillage (i.e. 2003 for MP-spring and 2004 for MP-fall). However, the differences between profiles with respect to NT became smaller in the second sampling and indicates that the biological environment in the surface layer returns to that of continuous NT soil (Fig. 4.7.a). At ARDC, distances between clusters of MP and miniMP with respect to NT were less with the second compared with the first sampling year.

The miniMP was clearly intermediate between NT and MP, indicating that miniMP caused a smaller shift in the soil microbial community (Fig. 4.8.a). For the deeper sampling strata (5–20 and 20–30 cm), there was no clear trend among sites towards reducing differences in FAME profiles between tilled and NT soil. In the case of ARDC, the Mahalanobis distances became greater in the second year post-tillage and FAME profiles following tillage were significantly different from NT (Figs. 4.8.b-c).

Changes in fungal biomass in the surface soil with time after tillage indicated that at least the fungal component of the soil microbial community recovers after tillage to return to NT conditions quickly. However, VAM biomass did not show such a response and indicates that effect of tillage on VAM persists for at least two cropping seasons. Reduced mycorrhizal colonization in crops following occasional tillage (García, 2005) confirmed this observation, and supports that lower VAM biomass can be found in deeper soil layers. Moreover, similar results have been reported for a one-time tillage in western Nebraska (Drijber, 2002) in that soil microbial community after 5 years of resumed no-till was still significantly different than continuous NT, mostly due to VAM.

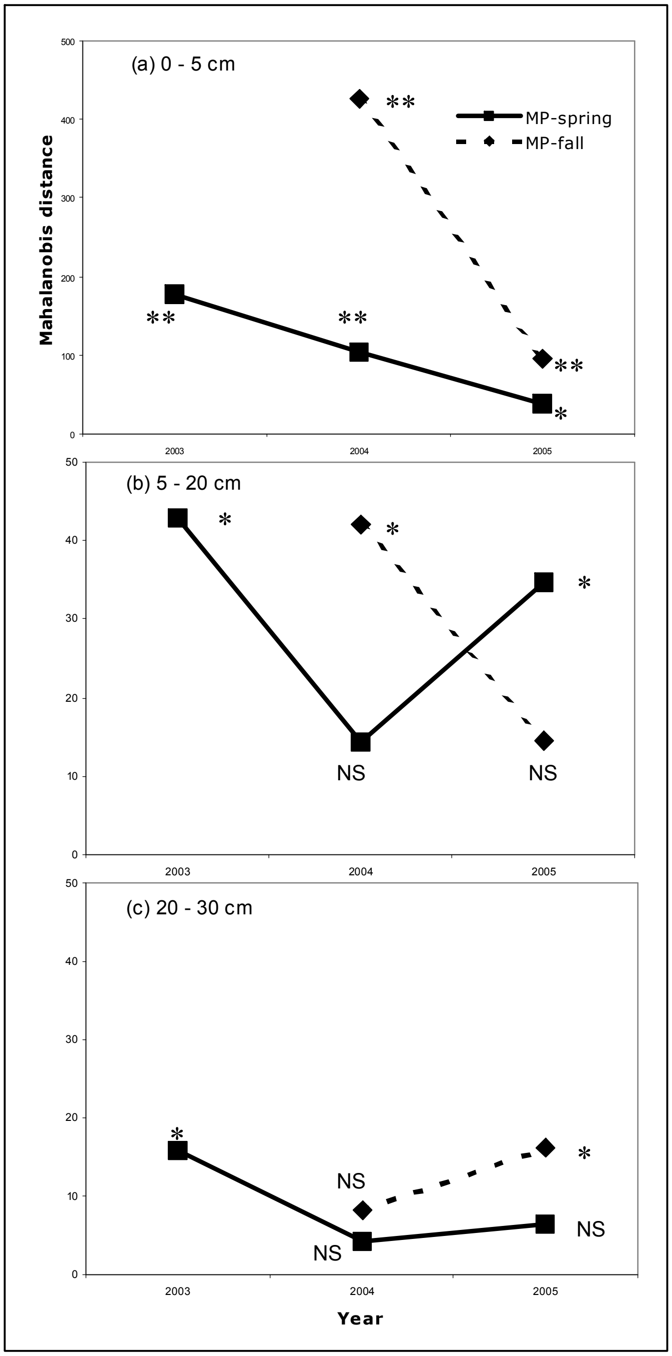

Figure 4.7. Differences in FAME profiles as a function of sampling year in Rogers Memorial Farm, measured as 'Mahalanobis distances' between cluster centroids of moldboard plow in spring or fall (MP-spring and MP-fall, respectively) and continuous no-till counterparts. Results of the corresponding canonical discrimination analysis is shown in Fig. 4.3 and Table A.1. ** and * denote a significant difference with the FAME profile of NT, with a=0.05 and 0.001, respectively. NS = not significant.

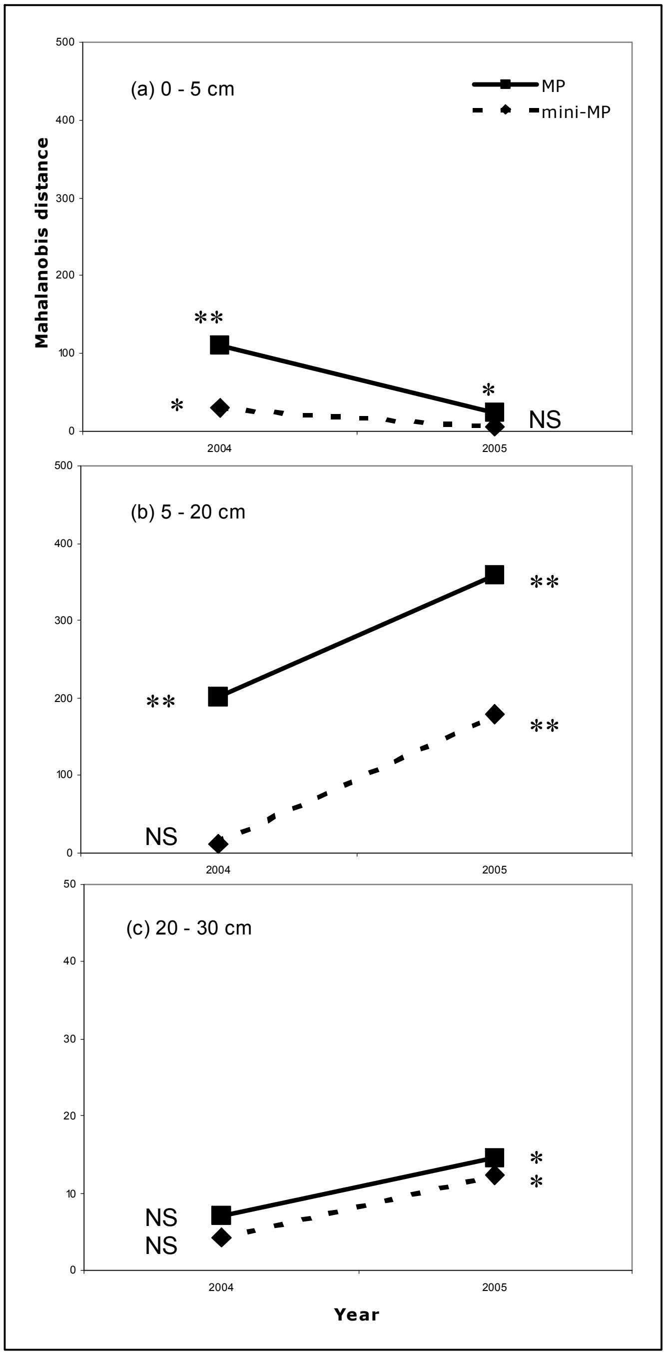

Figure 4.8. Differences in FAME profiles as a function of sampling year in Agricultural Research and Development Center, measured as 'Mahalanobis distances' between cluster centroids of moldboard plow or mini-moldboard plow (MP and mini-MP, respectively) and continuous no-till counterparts. Results of the corresponding canonical discrimination analysis is shown in Fig. 4.5 and Table A.2. ** and * denote a significant difference with the FAME profile of NT, with a=0.05 and 0.001, respectively. NS = not significant.

4.5. CONCLUSIONS

A one-time tillage significantly reduced the stratification of microbial biomass and of microbial groups, especially fungi and mycorrhizae, without producing a significant overall loss of microbial biomass.

The greatest changes caused by moldboard plowing occurred in the surface 0 – 5 cm layer. The return of the soil microbial community following tillage to that of continuous no-till is expected to be quicker for the surface soil layer than for deeper soil layers. Mycorrhizae are highly sensitive to tillage throughout the profile and recovers very slowly compared to the general microbial biomass. In short, one-time tillage affected the microbial community but with no evidence of a negative effect except for situations where optimal crop growth is dependent on a high level of mutualistic symbiosis with VAM.

4.6. REFERENCES

Calderón, F.J., L.E. Jackson, K.M. Scow, and D.E. Rolston. 2001. Short-term dynamics of nitrogen, microbial activity, and phospholipid fatty acids after tillage. Soil Sci. Soc. Am. J. 65:118-126.

Dembitsky, V.M., T. Rezanka, and E.E. Shubina. 1993. Chemical composition of fatty acids from some fungi. Cryptogamic Botany 3:383-386.

Doran, J.W., E.T. Elliot, and K. Paustian. 1998. Soil microbial activity, nitrogen cycling, and long-term changes in organic carbon pools as related to fallow tillage management. Soil Tillage Res. 49:3-18.

Drijber, R.A. 2002. Microbial Signatures for Crop Production Systems. Proceedings of the OECD Workshop on Innovative Soil-Plant Systems for Sustainable Agricultural Practices, Izmir, June 3-7, 2002. J.M. Lynch, J.S. Schepers and I. Ünver (Eds). Tübitak, Turkey. pp. 132-146.

Drijber, R.A., J.W. Doran, A.M. Parkhurst, and D.J. Lyon. 2000. Changes in soil microbial community structure with tillage under long-term wheat-fallow management. Soil Biol. Biochem. 32:1419-1430.

Frostegård, A., E. Bååth, E. and A. Tunlio. 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biol. Biochem. 25:723-730.

García, J.P. 2005. The effects of occasional tillage on no-till systems on nutrient distribution and uptake and on vesicular arbuscular mycorrhizal (VAM) colonization. MS Thesis. University of Nebraska–Lincoln.

Gaskins, M.H., S.L. Albrecht, and D.H. Hubbell. 1985. Rhizosphere bacteria and their use to increase plant productivity: a review. Agric. Ecosystems and Environ. 12:99-116.

Gifford, R.M., and M.L. Roderick. 2003. Soil carbon stocks and bulk density: spatial or cumulative mass coordinates as a basis of expression? Global Change Biology 9:1507-1514.

Grogan, D.W. and J.E. Cronan, Jr. 1997. Cyclopropane ring formation in membrane lipids of bacteria. Microbiology and Molecular Biology Reviews 61:429-441.

Harrier L.A. and C.A. Watson. 2003. The role of arbuscular mycorrhizal fungi in sustainable cropping systems. Adv. Agron. 79:185-225.

Hendrix, P.F., R.W. Parmelee, D.A. Crossley, Jr., D.C. Coleman, E.P. Odum, and P.M. Groffman. 1987. Detritus food webs in conventional and no-tillage agroecosystems. BioScience 36:374-380.

Jansa, J., M. Gryndler, and M. Matucha. 1999. Comparison of the lipid profiles of Arbuscular Mycorrhizal (AM) fungi and soil saprophytic fungi. Symbiosis 26:247-264.

Jeffries, P., S. Gianinazzi, S. Perotto, K. Turnau, and J.M. Barea. 2003. The contribution of arbuscular mycorrhizal fungi in sustainable mantainance of plant health and soil fertility. Biol Fertil Soils 37:1-16.

Kates, M., 1986. Techniques of lipidology: isolation, analysis and identification of lipids. In: Burdon, R.H., van Kippenberg, P.H. (Eds.), Laboratory Techniques in Biochemistry and Molecular Biology, vol. 3, Part 2. Elsevier, NY.

Miller, M.H. 2000. Arbuscular mycorrhizar and the phosphorus nutrition of maize: A review of Guelph studies. Can J. Plant Sci. 80:47-52.

Mozafar, A., T. Anken, R. Ruh, and E. Frossard. 2000. Tillage intensity, mycorrhizal and nonmycorrhizal fungi, and nutrient concentrations in maize, wheat, and canola. Agron. J. 92:1117-1124.

Olsson, S., and P. Persson, P. 1999. The composition of bacterial populations in soil fractions differing in their degree of adherence to barley roots. Appl. Soil Ecol. 12:205-215.

Perfilev, B.V., and D.R. Gabe. 1969. Capillary methods of investigating microorganisms. Oliver and Boyd, Edinburgh, UK.

Petersen, S.O., P.S. Frohne, and A.C. Kennedy. 2002. Dynamics of a soil microbial community under spring wheat. Soil Sci. Soc. Am. J. 66:826-833.

SAS Institute Inc., 1989. SAS/STAT® User's Guide, Version 6, 4th ed., vol. 1 & 2. SAS Institute Inc. Cary, NC.

Stahl, P.D., and D.J. Klug. 1996. Characterization and differentiation of filamentous fungi based on fatty acid composition. Applied and Environmental Microbiology 62:4136-4146.

Steer, J., and J.A. Harris. 2000. Shifts in the microbial community in rhizosphere and non-rhizosphere soils during the growth of *Agrostis stolonifera*. Soil Biol Biochem. 32:869-878.

Vestal, J.R. and D.C. White. 1989. Lipid analysis in microbial ecology. BioScience 39:535-541.

White, D.C., W.M. Davis, J.S. Nickels, J.D. King, and R.J. Bobbie. 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40:51-62.

Yu, Q.T., B.N. Liu, J.Y. Zhang, and Z.H. Huang. 1989. Location of double bonds in fatty acids of fish oil and rat testis lipids. Gas chromatography-mass spectrometry of the oxazoline derivatives. Lipids 24:79-83.

Zelles, L., Q.Y. Bai, R. Rackwitz, D. Chadwick, and F. Beese. 1995. Determination of phospholipid- and lipopolysaccharide-derived fatty acids as an estimate of microbial biomass and community structures in soils. Biol. Fertil. Soils 19:115-123.

4.7. APPENDIX

Table A.1. Correlations of FAMEs with the first two significant discriminant functions (Can1 and Can2) for the canonical discrimination analysis of tillage treatments and sampling year at Rogers Memorial Farm (RMF) in each of three sampling depths (0-5 cm; 5-20 cm; and 20-30 cm).

RMF, 0-5 cm			RMF, 5-20 cm			RMF, 20-30 cm		
FAME	**Can1**	**Can2**	**FAME**	**Can1**	**Can2**	**FAME**	**Can1**	**Can2**
aC15:0	-0.42	-0.48	C15:0	-0.82	0.11	iC14:0	-0.28	0.34
iC15:1(c4)	-0.40	-0.30	iC16:0	-0.54	0.06	iC15:1(c4)	-0.48	0.59
C16:0	-0.95	0.05	C16:1(c11)	0.74	0.54	C16:1(c5)	-0.50	0.37
iC16:0	0.85	0.24	i10MeC17:0	-0.62	0.17	iC16:1(c9)	-0.51	-0.34
C16:1(c11)	0.52	-0.36	C17:1(c9)	0.01	-0.77	C16:1(c11)	0.92	-0.05
C17:0	-0.58	-0.11	C18:0	-0.24	-0.55	iC18:0	0.43	0.20
aC17:0	0.89	0.06	iC18:0	0.42	-0.16	a10MeC18:0	-0.83	0.07
i10MeC17:0	0.92	0.04	unkC18:1	0.07	-0.74			
C17:1(c9)	-0.89	-0.28	cyC19(11,12)	-0.18	0.17			
cyC17(9,10)	-0.65	0.27	C20:4	0.10	-0.09			
iC18:0	-0.37	0.13						
10MeC18:0	-0.80	-0.25						
C18:1(c11)	-0.86	-0.44						
unkC18:1	-0.97	0.06						
10MeC19:0	-0.19	-0.30						
unkbrC19	0.54	0.58						
cyC19(11,12)	0.46	0.48						
C20:0	-0.04	0.74						

Table A.2. Correlations of FAMEs with the first two significant discriminant functions (Can1 and Can2) for the canonical discrimination analysis of tillage treatments and sampling year at the Agricultural Research and Development Center (ARDC) in each of three sampling depths (0-5 cm; 5-20 cm; and 20-30 cm).

ARDC, 0-5 cm			ARDC, 5-20 cm			ARDC, 20-30 cm		
FAME	**Can1**	**Can2**	**FAME**	**Can1**	**Can2**	**FAME**	**Can1**	**Can2**
C16:0	-0.21	0.63	C15:0	-0.49	-0.05	iC15:1(c4)	-0.52	-0.17
C17:0	0.63	0.62	iC15:0	0.26	-0.04	iC16:1(c9)	0.02	-0.02
iC18:0	0.78	-0.02	C16:1(c5)	0.10	0.71	C18:1(c11)	-0.19	0.71
i10MeC18:0	0.46	-0.77	iC16:1(c9)	0.27	-0.13	unkC18:1	0.86	0.17
C18:1(c9)	-0.62	0.37	cyC17(9,10)	0.45	-0.22	10MeC19:0	0.51	-0.19
unkC18:1	-0.64	0.70	a10MeC18:0	0.02	-0.11			
C20:3	-0.72	-0.31	C18:1(c11)	0.23	0.17			
			unkC18:1	-0.09	0.56			
			C18:2(c9,12)	0.40	0.45			
			cyC19(11,12)	0.10	-0.49			
			C20:0	-0.19	-0.22			

Synthesis

Established no-till systems present a challenging limitation because soil organic carbon (SOC) accumulates mostly in the top 5 cm and most of the improvement of soil chemical, physical and microbial properties occurs in this surface soil layer. In this dissertation, occasional tillage of no-till systems is proposed as a single, one-time tillage, conducted once in 12 or more years, to mix the high SOC surface layer with less improved deeper soil. The comprehensive hypothesis was that occasional tillage in NT can be conducted with little degradation of soil quality and mineralization of soil organic matter and that it will eventually result in net gains in soil quality and C sequestration.

The first study in this dissertation (**Chapter 1**) was on short-term losses of CO_2 and changes in labile carbon pools in the surface soil. Two experiments in long-term NT fields were installed under rainfed corn or sorghum rotated with soybeans in Eastern Nebraska. Tillage treatments were done in spring or fall and included: continuous no-till, tandem disk, 10-cm wide twisted shank-chisel, moldboard plow and mini-moldboard plow. A portable infrared gas analyzer (Li-Cor 6200) with a 980-cm^3 chamber was used to monitor carbon dioxide (CO_2) efflux before and immediately after tillage and continuing until planting. Profile distribution of SOC, particulate organic C (POC) and permanganate-oxidizable C were measured on samples taken at planting time at the depths of 0-2.5, 2.5-5.0, 5.0-10, 10-20, and 20-30 cm. Some tillage operations effectively redistributed total and labile organic C with negligible CO_2 losses when compared to the undisturbed continuous no-till. Total and labile organic matter pools were reduced by 24 to 88% in the 0- to 2.5-cm depth and increased by 13 to 381% for the 5 to 10-cm depth for the various tillage operations, with moldboard plowing having the greatest effect. On an equivalent soil mass basis, tillage did not cause significant losses of organic matter or labile pools between tillage and planting of the next crop. It was concluded that the pronounced stratification of soil organic matter in long-term NT soil could be reduced most effectively by means of occasional moldboard plow tillage without loss of labile organic matter pools relative to continuous no-till.

The second study (**Chapter 2**) was on potential gains in SOC after NT had been resumed following occasional tillage. After harvest of the second or third crop post-tillage, concentration of SOC was assessed for three sampling depths: 0-5, 5-20, and 20-30 cm. Total stocks of SOC were calculated on an equivalent soil mass basis for 60, 250, and 400 kg soil m^{-2}. Moldboard plowing reduced the concentration of SOC compared to no-till in the 0-5 cm soil depth, but SOC was similar for the other tillage options. At a soil depth of 5-20 cm, moldboard plowing increased SOC concentration relative to no-till, while SOC stocks in the entire soil profile were not different from no-till. A one-time moldboard plowing effectively redistributed SOC throughout the soil profile by incorporating surface SOC to lower depths without causing losses of SOC due to increased decomposition. A less C-saturated soil surface resulted from moldboard plowing, and supported the potential for increasing the C-sequestration rate under continuous no-till.

The third study (**Chapter 3**) addressed one-time tillage effects on soil hydraulic properties and phosphorus run-off. It was hypothesized that occasional tillage resulted in decreased P loads in runoff water, even though tillage may disrupt macro-pores and reduce the rate of water infiltration. Sorptivity, infiltration rate, runoff, and P loads were measured under simulated rainfall, using a Cornell

Sprinkle Infiltrometer. during the second or third growing seasons post-tillage. Composted feedlot manure was applied before tillage at a rate of 87.4 kg ha^{-1} of phosphorus. Occasional tillage with a moldboard plow reduced the concentration of available P in the surface soil, and lessened the concentration of dissolved P in runoff. It was concluded that occasional tillage could alter soil hydraulic properties and increase runoff volume. In consequence, this can counterbalance the benefits from reducing soil P availability at the surface, and occasional tillage can result in higher losses of P related to runoff.

Finally, the fourth study (**Chapter 4**) was conducted to (1) determine the change in the soil microbial biomass and microbial community composition after one-time tillage, and (2) establish the recovery dynamics of the soil microbial communities over one or two cropping seasons following tillage. Fatty acid methyl ester (FAME) profiles were used to ‘fingerprint’ soil microbial communities in response to moldboard plow or mini-moldboard plow tillage, compared to continuous no-till. On an equivalent mass basis, total microbial biomass had a small, mostly non significant reduction when compared to continuous no-till. In the surface 0 – 5 cm depth, moldboard plow caused a reduction in fungal biomass that was greatest in the first sampling after tillage. Relative to NT, this reduction averaged 60% for spring and fall plowing at RMF, and 30% at ARDC. After one or two crops post-tillage, the fungal biomass gradually increased, and averaged slightly lower than continuous NT. The mycorrhizal biomarker C16:1(c11) had a decrease in the first sampling time post-tillage that was similar to fungal biomass. However, there was no evidence that VAM in the surface soil layer increases after one or two crops post-tillage. On an equivalent mass basis, VAM decreased on average 7% with respect to continuous no-till in the first year after tillage, while in the second year after tillage the average reduction from no-till was 22%. Different microbial groups have different sensitivity to tillage, and effects may not be highest in the first sampling date after tillage. The return of the soil microbial community following tillage to that of continuous no-till is expected to be quicker for the surface soil layer than for deeper soil layers.

FUTURE RESEARCH NEEDS

The experimental field plots of the present study are of special value for more comprehensive study of research questions about occasional tillage of long-term no-till systems. In this final section, needs of future research are identified.

- **Crop performance during a longer term.** One-time tillage effects on crop yield need to be determined for a longer term, rather than two or three crops post-tillage. To understand possible yield responses, determination of yield components is highly recommended. This information will be highly valuable and likely will be among the determinant factors in recommending or not occasional tillage among commercial growers.
- **Soil organic carbon and bulk density.** The present dissertation was not conclusive with respect to the potential increase in C sequestration rate in response to occasional tillage. One or more soil samplings in alternate years are warranted to monitor possible changes in C stocks (expressed on an equivalent soil mass basis). Three sampling depths are considered appropriate and should be the same as used in Chapter 2 for consistency of data collection procedures. Soil sampling in the fall of odd-numbered years (i.e. 2007, 2009) would allow minimizing a source of error due to crops, as this would result in sampling systematically after a sorghum and a corn crop, at the RMF and ARDC sites, respectively.

- **Soil microbial community shifts.** Data collected on soil microbial changes in the present study indicated that occasional tillage can have a considerable impact on the biomass of mycorrhizal fungi. These rhizospheric soil microbes are known to be beneficial to most crops. Therefore, it is considered agronomically relevant to continue monitoring the recovery of the soil microbial community in a longer term beyond this dissertation. Spring sampling is preferred over fall to reduce the effects of the previously grown crop and to allow better detection of the tillage effects.