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ECOSYSTEM CONSEQUENCES OF AGGREGATION FOLLOWING SOIL DISTURBANCE

BY

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ABSTRACT

ECOSYSTEM CONSEQUENCES OF AGGREGATION FOLLOWING SOIL DISTURBANCE

BY

ANDREW STUART GRANDY

Carbon sequestration and greenhouse gas abatement in soils are two of a limited number of rapidly-deployable, high impact CO_2 stabilization options now available to policy makers. The long-term persistence of stored C, however, remains a major uncertainty in C sequestration forecasts. In this dissertation, I examine the ecological processes underlying soil organic matter permanence and the ecosystem and agronomic consequences of long-term no-till.

Our experimental site is a series of ecosystems that differ in management intensity located at the Kellogg Biological Station (KBS) Long-Term Ecological Research (LTER) site. KBS is located in SW Michigan and receives ca. 90 cm of precipitation annually, about half as snow, and has a mean annual temperature of 9 °C. All ecosystems are in close proximity to each other on the same or very similar soil series, mainly Kalamazoo (Fine-loamy) and Oshtemo (Coarse-loamy) mixed, mesic, Typic Hapludalfs developed on glacial outwash.

In the first series of experiments, reported in Chapter 2, I determined aggregate-associated soil C pools in ten ecosystems on the same soil series along a management intensity gradient. I also quantified the degree to which C is protected by aggregates using size and density fractionation techniques coupled with long-term mineralization assays of crushed and intact aggregates. Enhanced C storage relative to conventional

agriculture principally occurred in macroaggregate (>250 μm) size classes. Increases in active pool C when 2000-8000 μm aggregates were broken into microaggregates (<250 μm) ranged from 18% in conventional agriculture to 59% in alfalfa.

In the second set of experiments, reported in Chapters 3-5, I cultivated a never-previously cultivated field and minimized plant community changes to look at soil disturbance free from the influence of other agricultural management practices. I infer soil C permanence from responses of aggregate-protected soil organic matter, enzyme activities that reflect soil microbial activity, and trace gas fluxes. Cultivation immediately reduced 2000-8000 μm aggregates to levels commonly found in agricultural soils tilled for > than 50 years. The destruction of aggregates released particulate C from protected microsites and limited the incorporation of aboveground C into new aggregates. This lead to a series of biogeochemical transformations due to greater soil organic matter availability I term an aggregate cascade: microbial activity and N cycling increase, substantially increasing fluxes of both nitrous oxide and carbon dioxide. Growing plants can modify the effects of tillage on N availability and N_2O flux but have little effect on aggregation or CO_2 emissions.

In chapter 6, I report on an analysis of data from the till and no-till corn-soybean-wheat treatments of the KBS LTER and conclude that over 12 years there were no yield declines or increases in N_2O emissions associated with no-till. This work was performed in collaboration with classmates in a graduate seminar. Together, my results demonstrate the importance and feasibility of protecting no-till soils from even occasional cultivation.

To my parents, Jeffrey and Mary Grandy, who courageously engaged challenges and inspired critical thinking, curiosity, and open mindedness.

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regarding the long-term mineralization assays and modeling in chapter two and assistance with analyzing samples for trace gases.

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CHAPTER 1

Dissertation Overview

Radiative forcing of the Earth's atmosphere is increasing at unprecedented rates due to human activities. Since 1750, atmospheric concentrations of CO_2, N_2O, and CH_4 have increased by more than 30, 150, and 17%, respectively (IPCC, 2001). Agriculture has contributed substantially to global fluxes of these gases and now represents an important mitigation option as carbon sequestration and greenhouse gas abatement in soils are two of a limited number of rapidly-deployable, high impact CO_2 stabilization options now available to policy makers (Kauppi et al., 2001; Caldeira et al., 2004). Although management strategies such as cover-cropping and improved N fertilizer management potentially reduce the global warming potential of agricultural ecosystems, eliminating tillage may have the broadest impact because of its potential to alter emissions of all three biogenic greenhouse gases.

The area of cropland under no-till management in the U.S. between 1994 and 2004 rose from 14 to 23%, but only a fraction of this is in continuous no-till (CTIC, 2004). This raises questions about the permanence of stored C, which Marland et al. (2001) identified as the fundamental challenge to terrestrial C sequestration. Further, no-till deployment has been limited in part because it may accelerate N leaching (Martens, 2001) and N_2O emissions (MacKenzie et al., 1997; Ball et al., 1999; Baggs et al., 2003) but decrease N availability and yields (Rice et al., 1986; Niehues et al., 2004). In this dissertation I address the ecological processes underlying soil organic matter permanence and the ecosystem and agronomic consequences of long-term no-till.

Short-term total soil C changes are notoriously difficult to detect because of high background C concentrations in most arable soils, spatial variability, and the redistribution of above-ground pools upon cultivation (Sollins et al., 1999). The few studies in temperate ecosystems on SOM permanence have nonetheless focussed on short-term total soil C change (e.g. Tiessen and Stewart, 1983; Bowman et al., 1990; Pierce et al., 1994; VandenBygaart and Kay, 2004). Because of this, I believe they do not adequately predict the long-term effects of converting a long-term no-till field or successional community to conventional tillage; these studies also do not predict the effects of periodic tillage of no-till soils. The consequences of both these strategies on C storage and trace gas fluxes may take decades to fully understand. Here I take a different approach: I infer permanence from short-term responses to tillage of aggregate-protected soil organic matter, enzyme activities that reflect soil microbial activity, and trace gas fluxes. I, therefore, attempt to advance our ability to predict tillage effects on soils by understanding the basic ecological processes controlling soil's response to disturbance.

In Chapter 2, I determine aggregate-associated soil C pools in ten ecosystems on the same two co-occurring soil series along a management intensity gradient. I also assess the degree to which C is protected within aggregates using size and density fractionation techniques coupled with long-term mineralization assays of crushed and intact aggregates. I found that relative to conventional agriculture, enhanced C storage primarily occurred in 2000-8000 μm aggregate size classes. Increases in active pool C when 2000-8000 μm aggregates were broken into microaggregates (<250 μm) ranged from 18% in conventional agriculture to 59% in alfalfa. Potential release of whole-soil

labile C from physical protection following macroaggregate destruction was seven to nine-fold greater in successional systems than conventional agriculture.

These C losses following aggregate destruction in successional systems led me to question the persistence of soil organic matter in long-term no-till soils. How fast do aggregates break down following tillage and what are the biogeochemical consequences of aggregate destruction following tillage? Many studies have shown that long-term tillage depletes aggregation (e.g. Six et al. 2000) but the immediate effects of soil disturbance are not as well known. Further, authors have speculated that a substantial portion of structural breakdown following cultivation is due to changes in plant communities, organic inputs and long-term C losses (Paustian et al. 1997; 2000). I hypothesize that immediate and persistent reductions in soil aggregation occur after cultivation in conjunction with changes in soil temperature and other environmental controls over decomposition and that rapid changes in SOM turnover, microbial activity and trace gas fluxes will occur after a single plowing. These changes can be conceptualized as an "aggregate cascade" (Figure 1) whereby changes in aggregation occur immediately and have far-reaching effects that include changes in microbial communities and nitrous oxide fluxes – as well as changes in organic C protection.

In Chapters 3, 4, and 5 I examine the effects of tillage (moldboard plowing and discing) in a previously undisturbed soil. Tillage in temperate ecosystems has been examined almost exclusively in soils with long histories of agricultural management. The legacies of prior tillage, fertilization and annual cropping effects on soil structure, microbial communities, aggregation, and other factors that control trace gas fluxes may last for more than a century, confounding the influence of recent tillage. Because of this,

our basic understanding of tillage effects on soils and, in particular, our ability to predict future effects of tillage in long-term no-till soils is limited.

In Chapter 3, I report that cultivation reduced aggregate mean size by 35% within 60 d and that these effects were persistent. Mean additional daily CO_2-C losses due to cultivation ranged between 1 and 2 g C m^{-2} d^{-1} and N_2O fluxes increased from 2 to 6-fold over the three years following initial tillage and N2O fluxes were associated with increased soil NO_3^- concentrations, which approached concentrations found in fertilized fields. In Chapter 4 I focus on the changes in aggregation following cultivation and its relationship to SOM availability and microbial processes. In Chapter 5, I look at the ability of plant litter and growing plants to modify the effects of tillage on aggregation, microbial activity and fluxes of CO_2, N_2O and CH_4.

The work supports the idea of an aggregate cascade (Figure 1). The destruction of aggregates releases particulate C from protected microsites and also limits the incorporation of aboveground C into new aggregates. Instead, C increases in unprotected inter-aggregate pools where it is quickly oxidized. This stimulates microbial activity and N cycling, substantially increasing fluxes of both nitrous oxide and carbon dioxide. Growing plants can modify the effects of tillage on N availability and N_2O flux but have little effect on aggregation or CO_2 emissions. This cascade is accelerated by increased soil temperature and other environmental changes following decomposition that promote decomposition.

In the final, sixth chapter I perform an analysis of data from the till and no-till treatments of the KBS LTER and conclude that over 12 years there were no yield declines or increases in N_2O emissions associated with no-till. This work was performed

in collaboration with classmates in a graduate seminar class using data from the KBS LTER data catalogue.

Results show that cultivation immediately destabilizes physical and microbial processes related to C and N retention in soils. We also demonstrate that no-till cropping can be practiced with no yield or environmental trade-offs. Together, our results demonstrate the importance and feasibility of protecting no-till soils from periodic cultivation. This information will become increasingly valuable as greenhouse gas mitigation policies are further developed and deployed.

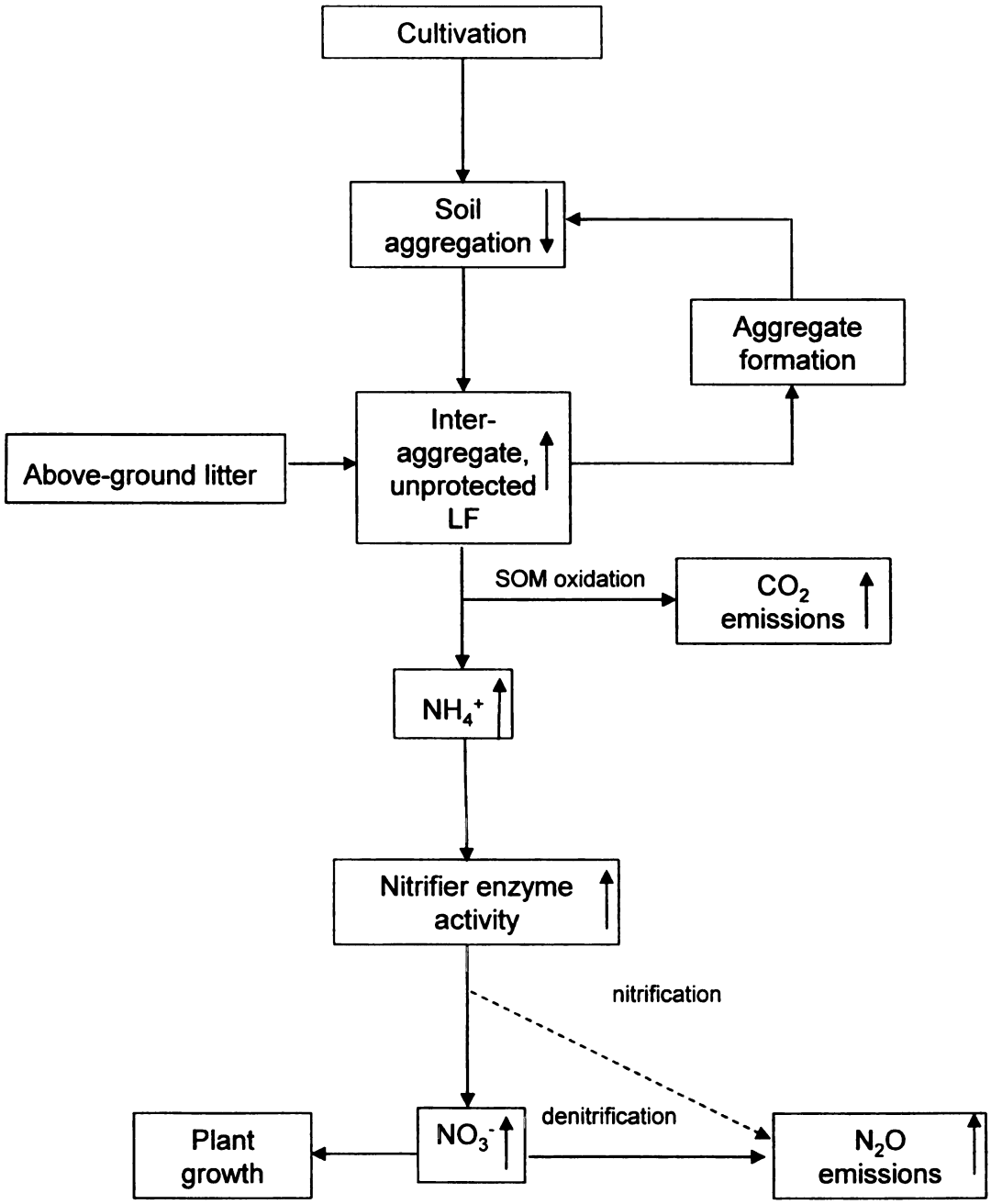

Figure 1.1. Description of the potential effects of tillage in soils conceptualized as an aggregate cascade. In the model, tillage immediately reduces soil aggregation and prevents the long-term stabilization of newly formed aggregates. Reductions in the physical protection of organic matter coupled with incorporation of aboveground C increases unprotected light fraction (LF) soil organic matter (SOM). This increases CO_2 emissions and N mineralization. Increased NH_4^+ concentrations increase nitrifier enzyme activity, NO_3^- production and N_2O emissions via nitrification and denitrification. Plant growth can modify NO_3^- fluxes and N_2O emissions. This cascade is accelerated by increased soil temperature and other environmental changes following tillage that promote decomposition and can be reversed by regeneration of aggregates following conversion to no-till.

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CHAPTER 2

Carbon Storage in Soil Aggregates along a Management Intensity Gradient: Implications for Soil Carbon Persistence

ABSTRACT

The recovery of aggregate-associated organic C pools is an important mechanism for increased soil C storage following reductions in land use disturbance. The persistence of this stored C, however, remains a major uncertainty in C sequestration forecasts. We determined aggregate-associated soil C pools in ten ecosystems on the same soil series along a management intensity gradient in southern Michigan (USA). We also assessed the degree to which C is protected by aggregation using size and density fractionation techniques coupled with long-term mineralization assays of crushed and intact aggregates. Ecosystems included four annual row-crop systems (conventional, no-till, low input, and organic), two perennial cropping systems (alfalfa and poplar), and four native communities (early successional, midsuccessional historically tilled, midsuccessional never-tilled, and late successional forest). Enhanced C storage relative to conventional agriculture ranged from 150 in low input row crops to 1890 g C m^{-2} in late successional forest and principally occurred in heavy-fraction C pools (SOM >1.6 g cm^{-3} plus SOM <53 μm) and macroaggregate (>250 μm) size classes. In the 2000-8000 μm size class, no-till cropping and early successional communities increased C from 4.1 to 8.5 and 15.7 g C kg^{-1} sand-free whole soil, respectively. Active pool C increases when 2000-8000 μm aggregates were broken into microaggregates (<250 μm) ranged from 18% in conventional agriculture to 59% in alfalfa. Potential release of whole-soil labile

C from physical protection following macroaggregate destruction was seven to nine-fold greater in successional systems than conventional agriculture. Our results suggest that conventional agriculture releases microaggregates and light fraction organic matter from within macroaggregates and C in these pools is then rapidly oxidized. Legume cover crops, no-till, perennial crops and unmanaged successional ecosystems can enhance soil C storage but the potentially rapid destruction of macroaggregates following tillage raises concerns about the long-term persistence of these C pools.

INTRODUCTION

Restoring some fraction of terrestrial soil C pools through changes in agricultural management is a high impact, rapidly deployable strategy for partially mitigating increases in atmospheric CO_2 (Caldeira et al. 2004, CAST 2004, Pacala and Socolow 2004). Management strategies that increase soil C storage include reducing tillage intensity and increasing residue inputs with cover crops, green manures, or perennial crops (West and Post 2002, Lal et al. 2004). These practices modify decomposition rates by changing soil aeration, water dynamics, and aggregation as well as the biochemistry and quantity of crop residues (Angers and Caron 1998, Martens 2000).

The quantity and turnover rates of soil aggregates may be a particularly important control over soil organic matter (SOM) protection following changes in cultivation or cropping intensity (Cambardella and Elliott 1994, Jastrow et al. 1996, Paustian et al. 2000). Evidence for protection of SOM by aggregates includes low interior-aggregate oxygen concentrations that limit respiration rates, small intra-aggregate pore spaces that limit SOM access to decomposers, and increases in soil respiration rates when SOM is

released from within aggregates (Sexstone et al. 1985; Mikha and Rice 2004). Field studies have demonstrated that C sequestration in afforested soils and restored grassland ecosystems principally occurs within soil aggregates (Six et al. 1998; Jastrow et al. 1996; DeGryze et al. 2004).

The mechanical impact of tillage can immediately destroy a substantial proportion of macroaggregates while simultaneously exposing soil surfaces to the impacts of rain and freeze-thaw and wet-dry cycles. In a recent study, Grandy and Robertson (2005, *in review*) found that soil in the 2000-8000 μm aggregate size class declined from 0.34 to 0.19 g g^{-1} after plowing once a previously uncultivated field, levels identical to those in adjacent agricultural fields on the same soil type continuously tilled for >50 y. This immediate effect of cultivation on soil structure raises concerns about the persistence of stored soil C following periodic cultivation of no-till soils, a common practice in no-till management. The area of cropland under no-till management in the U.S. between 1994 and 2004 rose from 14 to 23%, for example, but only a fraction of this is in continuous no-till (CTIC 2004). Stored C might also be lost rapidly from sites in the USDA Conservation Reserve Program and other set-aside programs upon cultivation.

Robertson et al. (2000) demonstrated the potential for cropping system management and succession to modify total soil C storage in 10 ecosystems on the same soil type between 1989 and 1999 at a site in the northern U.S. corn belt. Here, we investigate the microsite distribution of C in these ten ecosystems and the susceptibility of physically-protected C to loss following aggregate destruction. We combine physical SOM separation techniques (e.g. Elliott 1986, Conant et al. 2004, DeGryze et al. 2004) with direct estimates of the effects of soil structure on long-term C mineralization rates

(e.g. Plante and McGill 2002, Mikha and Rice 2004) to provide additional insights into the nature of C accumulation in different size fractions and the vulnerability of C in these size fractions to aggregate destruction. Our specific objectives were to determine the effects of cropping system management (conventional, low input, organic, no-till, and perennial) and ecological succession (early, mid, and late old-field succession) on: 1) stabilizing water-stable soil aggregates in different size classes; 2) enhancing C storage in physically protected aggregate-associated C pools; and 3) controlling changes to the size and decay rates of physically-protected C pools following soil structural disturbance. We hypothesize that soil aggregation and total soil C storage will increase in agricultural systems where legume cover-crops, no-till, or perennial crops are used and that late successional ecosystems will have the greatest structural stability and C storage. Further, we hypothesize that C preferentially accumulates in intra-aggregate C pools, particularly in macroaggregate size classes that are especially susceptible to loss upon tillage.

MATERIALS AND METHODS

Experimental Site and Approach

Our experimental site is a series of ecosystems that differ in management intensity located at the Kellogg Biological Station (KBS) Long-Term Ecological Research (LTER) site (Table 2.1). KBS is located in SW Michigan and receives ca. 90 cm of precipitation annually, about half as snow and a mean annual temperature of 9 °C. All ecosystems are in close proximity to each other on the same or very similar soil series; mainly Kalamazoo (Fine-loamy) and Oshtemo (Coarse-loamy) mixed, mesic, Typic Hapludalfs developed on glacial outwash. These two series co-occur in all ecosystems and differ

mainly in their Ap horizon texture, although variation within a series can be as great as variation between series (Crum and Collins 1995, Robertson et al. 1997).

The experimental ecosystems include four annual cropping systems, two perennial cropping systems, and four successional plant communities (Table 2.1). The annual cropping systems are corn-soybean-wheat rotations and include four management regimes: 1) conventional chemical management with tillage; 2) tilled, low chemical input; 3) tilled organic; and 4) no-till. Both low input and organic management systems have a leguminous winter cover crop (*Trifolium pretense* L.) to provide nitrogen in two out of every three years. No systems receive compost or manure and the standard input systems receive inorganic N fertilizers according to regional best management practices. The perennial crops include poplar trees (*Populus* sp.) on a 10-year rotation cycle and alfalfa (*Medicago sativa*) on a 6-8 year rotation. The successional communities include recently abandoned, 12 yr-old early successional old-fields, historically tilled 50-year-old midsuccessional communities, a never-tilled 50-year old midsuccessional community, and a set of late successional oak-hickory forests that were never cleared or plowed. The annual and perennial cropping systems as well as the early successional community are replicated in six one ha plots within the 60 ha KBS LTER main experimental site. These treatments were all established in 1989 in a previously conventionally managed row-crop field. Midsuccessional historically tilled, midsuccessional never-tilled, and late successional ecosystems are replicated at three different locations within a 2 km radius of the main site on the same soil series. These ecosystems have been organized along a management intensity gradient based on tillage, external inputs, and above-ground net primary productivity (Table 2.1).

Our overall approach was to sieve soils from each site into 4 aggregate size classes and characterize the total organic C associated with each aggregate size fraction using size and density fractionation techniques, as well as to assess the potential for aggregate structure to control the distribution of C in biologically-defined pools by comparing long-term mineralization dynamics of crushed and intact aggregates.

Soil Sampling and Storage

Previous research at KBS (De Gryze et al. 2004) and other studies (e.g. West and Post 2002) have shown that C accumulates primarily near the soil surface following cessation of tillage. To better understand the mechanisms underlying this accumulation and the persistence of accumulated C we sampled to 5 cm. Soil samples were collected from five locations within each plot in June and July, 2001. At each of the five sample locations, two subsamples with a diameter of 7.6 cm to a soil depth of 5 cm were taken by gently hammering a PVC core into the ground to minimize compression and slicing of aggregates. In row-crop ecosystems, one of the subsamples at each location was taken in the row and the other between rows. All ten subsamples from each plot were combined to produce one representative sample for each of the 52 plots. Four separate samples for bulk density analysis were taken at the same time as those for aggregate analysis, using an 8 cm diameter root corer.

Field-moist soil samples were put into a cooler (4 °C) prior to being broken along natural fracture planes and passed through an 8 mm sieve within 72 h of the sampling time. Rock fragments > 8 mm were discarded. After sieving, soils were dried at room

temperature in paper bags prior to storage in plastic bags. Care was taken throughout the study to minimize disturbance of the samples that might influence aggregate structure.

Water-Stable Aggregate Distribution

Aggregate distribution was determined on four replicate 100 g air-dried soil samples by wet-sieving in water through a series of 2000 μm, 250 μm, and 53 μm sieves (Cambardella and Elliott 1993, Six et al. 1998). Soil was submerged for 5 min on the surface of the 2000 μm sieve which was then moved up and down for two minutes with a stroke length of 3 cm for 50 strokes. Sieving was repeated on the 250 μm (50 strokes) and 53 μm (30 strokes) sieves using the soil plus water that passed through the next larger sieve. Aggregates remaining on each sieve were dried at 60 °C. Sand content was determined on an aggregate subsample after dispersing soil in sodium hexametaphosphate (0.5%) for 48 h on a rotary shaker at 190 rpm.

Aggregate-Associated Light Fraction Organic Matter

The method we used to separate inter- and intra-aggregate light fraction (LF; organic matter of relatively low density) is based on previously published protocols (Six et al. 1998, Gale et al. 2000). Aggregate subsamples were pre-wet prior to LF analysis in order to minimize aggregate destruction during LF separation. An 8 g subsample of aggregates was divided in half and placed on two membrane filters (47 mm diameter; Pall Supor-450) overlaying two paper filters (70 mm diameter; Whatman 42) in a 10 cm Petri dish. Four mL of deionized (DI) water were trickled onto the paper filters in order to

slowly wet all of the aggregates by capillarity. Aggregates were transferred from the membrane filters to 100 mL beakers after 16 h with 5 mL aliquots of sodium polytungstate (NAPT) at a density of 1.62 g cm^{-3}. A total of 55 mL NAPT was used for each sample. A preliminary test showed that the final density of NAPT was about 1.60 g cm^{-3} following equilibration with the water contained in aggregates.

After 24 h, LF was aspirated from the surface of the NAPT and then rinsed on a hardened, ashless filter paper with at least 600 mL DI H_2O. We refer to this pool as inter-aggregate LF. After removal of this pool, we aspirated the remaining NAPT. Aggregates were then dispersed to release the intra-aggregate LF using sodium hexametaphosphate as described previously and resuspended in NAPT (d=1.62 g cm^{-3}). The intra-aggregate LF was collected from the surface. The mineral-associated aggregate C plus POM with a density >1.6 was determined by difference and is referred to as heavy-fraction C (HF). Organic C and total N concentrations of organic matter and whole soil samples were determined by dry combustion and gas chromatography in a CHNS analyzer (Costech ECS 4010, Costech Analytical Technologies, Valencia CA.). Inorganic carbonates were not a significant interference in these soils to organic C measurement (S.K. Hamilton, pers. comm.)

Laboratory Incubations

A subsample of aggregates (15-20 g) was transferred into 60 mL glass serum vials with a 13 mm diameter opening. We estimated the bulk density of each of our samples after tamping down the serum vials 10 times on a laboratory bench and from this determined the amount of water needed to bring them to 55% water-filled pore space

(WFPS). It has been shown that microbial activity is highest at this moisture content across a range of soil types (Linn and Doran, 1984; A.J. Franzluebbers, personal communication). Water applications were made slowly via 5 mL pipettes to minimize breakdown of aggregate structure following rewetting.

After wetting the samples, the serum vials were placed in a ½ pint jar with ca. 60 mL of water in the bottom. These jars were covered with polyfilm that permits relatively free O_2 and CO_2 exchange but retains water. Jars were put into boxes and then into dark incubation chambers maintained at 25 °C. Samples were periodically checked for water loss by weighing them and rewetted, as necessary.

We added additional sand to the <53 μm size class to minimize O_2 depletion. The 2000-8000 μm size class contained an average of 45% sand and the 250-2000 μm size class an average of 60% sand while the <53 μm size class contained an average of 33%. Sand additions consisted of particles with a diameter between 250-1000 μm and brought the average sand content in this size class up to 48%.

Respiration in all size classes was measured a minimum of 12 times over 205 d with greater sampling intensity early in the incubation. At each sampling date serum vials were flushed for 45 s with a humidified air stream. After flushing, bottles were sequentially capped with a rubber septum. A 0.5 mL sample of headspace was immediately drawn with a syringe and then two additional samples were taken over a 90 min sampling interval. CO_2 content of each gas sample was analyzed using an infrared gas absorption (IRGA) analyzer, followed by calculation of the respiration potential for the time interval (Robertson et al. 1999).

Active, slow and passive pool C associated with different aggregate size fractions were determined by modelling the long-term respiration data (Paul et al. 2001). We used a differentiated version of a standard three-pool first order model to accommodate discontinuous sampling:

$$dC/dt = C_a * k_a e^{(-ka*days)} + (C_{soc} - C_r - C_a) * k_s e^{(-ks*days)} + C_r * k_r e^{(-kr*days)}$$

where C_a and k_a are the active C pool size and decay rate constant, C_s and k_s are the slow pool size and decay constant, and C_r and k_r are the resistant pool C size and decay rate constant. C_{soc} is total soil C. C_a, k_a and k_s were determined by modelling; slow pool C (C_s) was determined by difference and C_r was determined by acid hydrolysis. All detectable plant residues and POM were removed with tweezers prior to hydrolysis because of the potential for relatively young lignin to resist hydrolysis and inflate C_r estimates (Paul et al. 2001). After removal of these materials soil samples were ground with a mortar and pestle. Hydrolysis was carried out for 16 h at 110 °C in 110 mL test tubes containing 2 g soil and 20 mL 6N HCl. The k_r was assumed to be $8.3 \cdot 10^{-6}$ d^{-1} (Paul et al. 2001). Carbon dating of C_r in previous studies at our site has demonstrated that that this pool ranges from hundreds to thousands of years old (Paul et al. 1997), resulting in a mean residence time so large and a k_r so small that deviations of the assumed value from the actual decay rate constant have little affect on the other parameters (Paul et al. 2001; Fortuna et al. 2003).

To determine the potential for aggregate structure to control the distribution of C, an additional 15 - 20 g subsample of aggregates was crushed prior to carrying out the

long-term mineralization assays. Aggregates ranging in size from 2000-8000 and 250-2000 μm were fractured to create microaggregates by passing them through a 250 μm sieve. The structure of aggregates in the 53-250 μm size class was destroyed by crushing aggregates in a mortar and pestle. Total potential physical protection of C by aggregates was estimated from increases in C_a associated with aggregate destruction where the difference in C_a between crushed and intact aggregates was positive. Samples where differences were negative were analyzed separately.

Statistical Analysis

Statistical analysis was carried out using a completely random-design analysis of variance (ANOVA) with the Proc Mixed procedure in SAS (SAS Version 8.2, SAS Institute 1999). Data were analyzed by considering ecosystem and aggregate size class as fixed effects after log transformation where necessary to improve homogeneity of variance. Single degree of freedom comparisons were made using the LSD statistic to calculate a 95% confidence interval around the differences between means generated using the diff option in Proc Mixed. The LSD was carried out using the PDMIX800 algorithm (Saxton 1998). Distribution of C in different size fractions as well as potential increases in C_a due to aggregate breakdown were analyzed per gram aggregate and also on a whole soil basis after making adjustments for the percentage of soil in a particular size class. Aggregates and aggregate-associated SOM pools were corrected for sand >53 μm.

RESULTS

Total Soil C and N

Relative to conventional agriculture, increases in soil C concentration from 0 to 5 cm occurred with no-till (48%), low input (24%) and organic (32%) treatments (Table 2.1). Perennial crops increased soil C concentrations relative to conventional by 63% in alfalfa and 68% in poplar stands. The early successional community increased soil C concentrations by >100% relative to conventional agriculture and contained C concentrations similar to the historically tilled midsuccessional community, although soil C concentrations were substantially less than those in the never-tilled midsuccessional soil and deciduous forest. Organic N concentrations also increased across the management intensity gradient. C/N ratios were similar among agricultural systems but increased in perennial and successional communities (Table 2.1).

Aggregate C Distribution

All ecosystems except for low input had an increased mass of soil in the 2000-8000 μm size class aggregates relative to conventional (Figure 2.2). No-till increased the mass of soil in the 250-2000 μm aggregate class and decreased the proportion of soil in smaller size fractions relative to conventional (Figure 2.2). Alfalfa and poplar ecosystems and the successional communities increased the mass of soil in the 250-2000 μm class relative to conventional.

All ecosystems except for zero-input increased total C concentrations in the 2000-8000 μm aggregate size class (Figure 2.3). In the 250-2000 μm size class, low input, perennial and successional ecosystems increased total C relative to conventional agriculture. Poplar, early successional communities, and the mid and late successional communities increased C in the 53-250 μm size class relative to the annual agricultural

treatments. In the <53 μm size class the successional treatments increased total C relative to the annual agricultural treatments (Figure 2.3). C concentrations in macro- and micro-aggregates were similar in mid and late successional communities but in other ecosystems C concentrations were lower in microaggregates. Expressed on a whole soil basis after correcting for the amount of soil in a particular size class, increased C storage relative to conventional agriculture primarily occurred in the two macroaggregate (>250 μm) size fractions (Figure 2.4). Heavy fraction organic matter accounted for most of the C difference between ecosystems (Figure 2.5)

In the 2000-8000 μm aggregate size class, the inter-aggregate LF concentrations were indistinguishable among annual cropping systems and early successional communities (Figure 2.6). Midsuccessional historically tilled, midsuccessional never-tilled, and late successional ecosystems increased inter-aggregate LF by 301, 247, and 503%, respectively, compared to conventional agriculture (Figure 2.6). In the 250-2000 μm aggregate size class, inter-aggregate LF was generally similar among ecosystems (Figure 2.6). In the 53-250 μm size class, inter-aggregate LF was similar among annual cropping systems. All four successional communities had higher inter-aggregate LF than the agricultural treatments.

Intra-aggregate LF in the 2000-8000 μm size class was similar among agricultural treatments; alfalfa, poplar, and early successional ecosystems all increased LF relative to conventional agriculture. Poplar increased intra-aggregate LF relative to conventional, organic, and no-till agriculture. The mid and late successional communities had similar intra-aggregate LF but early successional ecosystems had lower intra-aggregate LF than did midsuccessional historically tilled and late successional systems. Intra-aggregate LF

was higher in organic than in no-till or conventional ecosystems. Intra-aggregate LF concentrations in poplar and early successional ecosystems were greater than those in conventional and alfalfa systems. Early and late successional systems had greater intra-aggregate LF than midsuccessional historically tilled ones.

Active, Slow and Passive C

Annual and perennial cropping systems and early successional communities generally had similar active C pool sizes while the mid and late successional communities had greater active pool C than the other ecosystems (Table 2.2). Low input, organic and no-till organic management increased slow pool C relative to conventional agriculture. Alfalfa and early successional systems had greater slow pool carbon (C_s) than annual cropping systems other than no-till. The mid and late successional communities had greater C_s than other treatments. Annual crop management had no effect on resistant pool C (C_r). Alfalfa increased C_r relative to conventional and organic agriculture; poplar and early successional systems increased C_r relative to annual row crop systems. Alfalfa had the lowest k_s; the other perennial systems and successional communities had higher k_s than the annual systems. Total C, C_a and C_s were greater in large and medium sized aggregates than small ones while k_a and k_s were greater in small and medium aggregate sizes (Table 2.2).

Physical Protection of C

In the 2000-8000 μm size class, no-till, perennial crops, and successional treatments had greater transfer of C from C_s to C_a following aggregate breakdown than

did conventional agriculture. Increases in C_a ranged from 98 mg C kg^{-1} in conventional agriculture to 626 mg C kg^{-1} aggregated soil in midsuccessional historically tilled communities (Figure 2.7). The potential transfer of C from slow into active pools following aggregate destruction represents a large proportion of the active pool C of intact aggregates (Table 2.2; Figure 2.7): conventional (18%); low input (50%); organic (26%); no-till (29%); alfalfa (59%); poplar (46%); early successional (52%); midsuccessional historically tilled (29%); midsuccessional never tilled (43%); and late successional (32%). Crushing aggregates had no effect on k_a and effects on k_s were generally small or nonsignificant (data not shown).

Differences in aggregate distribution and potential transfer of C_s to C_a led to large differences in the effects of aggregate breakdown expressed on a whole soil basis (Figure 2.8). All treatments except low input increased potential C losses from 2000-8000 μm aggregate size classes. The increase in C_a following breakdown of 2000-8000 μm aggregates was 4-fold greater in no-till than conventional cropping systems (86.1 vs. 20.4 mg C kg^{-1} whole soil). Differences were particularly great in successional communities where increases in C_a on a whole soil basis were 7-fold greater in the two midsuccessional communities and 9 times greater in late successional systems. Breakdown of 250-2000 μm aggregates increased C transfer from C_s to C_a in the successional communities while destruction of microaggregates generally had less effect (Figure 2.8).

In each aggregate size class there was a total of 55 comparisons between crushed and intact aggregates. In the 2000-8000 μm size class three of these comparisons were negative, indicating a transfer of C from C_a to C_s following aggregate crushing (data not

shown); in the 250-2000 μm size class five comparisons were negative (data not shown); and in the 53-250 μm size class 29 comparisons were negative.

DISCUSSION

Soil C Storage

Our results demonstrate the potential for no-till soil management, cropping intensity and successional age to enhance total soil C storage and that this is related to changes in aggregation and the distribution of C in different size fractions (Table 2.1). Increases in soil C over 12 yr in no-till are equivalent to an annual C increase in the top 5 cm of 26 g C m^{-2} y^{-1}. These rates are similar to those reported by Robertson et al. 2000 (30 g C m^{-2} y^{-1}) for this no-till system between 1989 and 1999. Although these rates are lower than those reported in some studies (e.g. West and Post 2002) they are consistent with results presented in recent review papers (Davidson and Ackerman 1993; Six et al. 2004) and are similar to reported average C accumulation rates for the Midwest U.S. (Franzluebbers and Steiner 2002).

Increases in C with organic and low input systems demonstrate the potential for legume cover crops to increase soil C pools, although at lower rates than no-till cropping. Some studies have reported that C additions from legume cover crops are relatively small and therefore insufficient to increase total soil C (MacRae and Mehuys 1985). However, long-term use of legume cover crops can increase SOM relative to rotations that consist exclusively of residues with higher C:N ratios, perhaps because of effects on aggregation and/or microbial communities (Drinkwater et al. 1998, Grandy et al. 2002). The potential effects of plant communities on soil C were further demonstrated by the increased C

measured in perennial cropping systems and early successional communities relative to no-till since these communities were converted from conventional agriculture in 1989; SOM differences between these treatments are likely a function primarily of plant community structure and productivity and their effects on residue quality and quantity, aggregation, and microbial communities.

Aggregate Stability

Our results show potential for tillage and plant communities to influence soil aggregate size distributions. It is widely recognized that tillage decreases macroaggregation (e.g. Six et al. 2000, Mikha and Rice 2004, Wright and Hons 2004). The destructive effects of tillage on soil structure begin to occur immediately after cultivation and are persistent (Grandy and Robertson 2005a, *in review*). Opinions regarding the effects of plant communities on soil structure, however, remain varied as residue quality and quantity, as well as root growth, may influence aggregation (Angers and Caron 1998, Kavdir and Smucker 2005). Aboveground biomass inputs from the organic and low input systems are similar to those from conventional crops (KBS LTER, 2005) suggesting that root dynamics or the quality of residues may be increasing aggregation.

Legume cover crop decomposition may rapidly stimulate production of polysaccharides and fungal hyphae capable of stabilizing soil aggregates (Haynes and Beare 1996). Legumes grown in crop rotations can increase aggregation but in monoculture legumes may decrease aggregation because their stabilizing effects are short-lived relative to residues with higher C/N ratios (Haynes and Beare 1996, Wright

and Hons, 2004). Greater macroaggregation in poplar and early successional communities than in alfalfa highlights the potential for soil structural declines in legume monocultures. Additional evidence that residue quality differences and/or belowground dynamics are controlling aggregate stabilization is that poplar produces similar aboveground biomass to the agricultural row-crop systems while early successional communities produce less (DeGryze et al. 2004).

Aggregate Associated C Pools

Heavy-fraction (HF) organic matter, rather than light fraction (LF), accounted for the largest proportion of C increases across ecosystems. For example, in the no-till system differences in HF accounted for 82% of the C increase in 2000-8000 μm aggregates and in midsuccessional never-tilled systems HF accounted for 80% of the difference in total C. Six et al. (1999) found that between 50 and 66% of new C associated with macroaggregates was in the form of mineral-associated C, and Jastrow et al. (1996) similarly found that C deposition in macroaggregates occurs principally in mineral-associated pools and that particulate soil organic matter accounted for only 20% of C gain.

Differences in HF among size classes within ecosystems were proportionally smaller than LF differences, and HF differences explained a smaller proportion of the variation in total C among size classes. In the no-till system the difference in C concentration between the 2000-8000 μm and 53-250 μm size classes was 8.1 g kg^{-1} and 47% of this was accounted for by differences in HF. In early successional systems, HF was similar in the 2000-8000 (24.7 g kg^{-1}) and 53-250 (24.4 g kg^{-1}) μm size classes so the

differences between size classes in total C concentration could be accounted for entirely by changes in LF. In midsuccessional historically-tilled, midsuccessional never-tilled, and late successional systems there were no differences in HF between size classes. There were differences in LF, although they were small relative to total pool sizes and did not result in a change in total C across aggregate size classes. These results suggest that LF and HF C pools are an important component of C differences between size classes but that between ecosystems HF C accounts for most of the total C gain. Further, succession reduces differences in total C and heavy C between size classes.

The lack of agricultural management effects on inter-aggregate LF is similar to other reports that this pool is highly sensitive to any kind of agricultural management and that differences primarily occur between managed and unmanaged ecosystems (Arrouays and Pelissier 1994, Six et al. 1999); its accumulation may occur slowly, driven primarily by plant inputs, root dynamics, soil disturbance, and other activities that influence the decomposition environment (DeGryze et al. 2004).

SOM Mineralization

When aggregate structure was left intact there were no differences in active pool C (C_a) among the annual or perennial agricultural treatments or early successional communities (Table 2.2). There were differences, however, in the concentration of C in the slow (C_s) and resistant pools (C_r) of intact aggregates, demonstrating that C accumulation occurred principally in SOM pools that are chemically or physically protected. Those ecosystems with the greatest inter-aggregate POM were also the ones with the greatest C_a, suggesting that some portion of the free, unprotected LF pool

contributes to C_a.

Increases in C_a following the breakdown of large aggregates in no-till, alfalfa, poplar and early successional systems suggest that physical mechanisms make important contributions to C accumulation in the 2000-8000 μm size class and that the incorporation of LF and mineral-associated C into aggregates will decrease its decomposition rate. Fewer differences among treatments in the 250-2000 μm size class and no differences when microaggregates were crushed are likely due to greater chemical protection of SOM in these size classes. Microaggregates contain higher concentrations of humic materials and other degraded and chemically complex compounds than do macroaggregates (Tisdall and Oades, 1982; Haynes and Beare, 1996; .

The potential transfer of C from slow into active pools following aggregate destruction is within range of those from other comparisons of C mineralization rates from crushed and intact aggregates (Balesdent et al 2000). Elliott (1986) found in a cultivated soil that breaking macroaggregates down to microaggregates increased C mineralization 19% in a cultivated soil and 4% in a previously uncultivated field. In a review of experimental comparisons of crushed and intact aggregates, Balesdent et al. (2000) concluded that the protective capacity of aggregates increases with SOM concentration, clay content, and the absence of tillage. Our results support the hypothesis that protective capacity is related to SOM concentration and tillage intensity.

Potential whole soil additional C losses with soil disturbance (Figure 2.8) are a function of aggregate C content, tillage intensity, and also aggregate size distribution. Successional plant communities should thus be highly susceptible to C losses following cultivation. They have high SOM concentrations within macroaggregates and also

contain >30% soil in macroaggregate size classes that break down immediately following cultivation. In a recent study, Grandy and Robertson (2005a, *in review*) demonstrated that initial cultivation of the midsuccessional never-tilled communities reduced 2000-8000 μm aggregates within 60 d to levels indistinguishable from those in conventional agriculture. Concomitantly, there were significant decreases in the proportion of LF protected within aggregates and CO_2 fluxes increased an average of 1.0 to 1.9 g C m^{-2} d^{-1} over three years, likely as a result of decreased aggregate protection of LF.

The potential for oxidation rates to decrease following aggregate destruction was demonstrated when C_a in crushed aggregates was smaller than that in intact aggregates. This principally occurred in 53-250 μm aggregates. Other researchers have also reported decreases in respiration rates when soil structure is destroyed (Balesdent et al. 2000). Although we added supplemental sand to this size class it is possible that anaerobic sites reduced respiration rates or that crushing facilitated organo-mineral interaction that protected C.

Management Implications

SOM losses following a decade or more of cultivation range from 30-60% of original surface soil carbon (Davidson and Ackerman 1993). Restoring a portion of this oxidized C is one of a limited number of rapidly-deployable, high impact CO_2 stabilization options now available to policy makers (Caldeira et al. 2004). Widespread adoption of no-till cropping and other soil C conservation strategies could re-sequester up to 0.5 - 1.0 Pg soil C y^{-1} and contribute significantly to stabilizing global CO_2 levels (Caldeira et al. 2004;

CAST 2004). Our results demonstrate that physical protection of C by aggregates is an important mechanism controlling SOM turnover in managed ecosystems and that 2000-8000 μm aggregates may reduce active pool C turnover by as much as 50%. Ultimately, however, however, net greenhouse gas mitigation by due to soil C storage depends on the long-term persistence of stored soil organic matter (Paustian et al. 2000; Pacala and Socolow 2004). No-till soil management in the U.S., although increasing, continues to be rotated with tillage because of perceptions that no-till limits nitrogen availability and decreases yields (Martens 2001). This practice increases aggregate turnover rates, oxidizes C, and reduces the potential for agricultural land to sequester C. In a recent analysis of long-term data from the W.K. Kellogg Biological Station, Grandy et al. (2005b, *in review*) demonstrated no negative effects of continuous no-till on yields, inorganic N availability, or N_2O fluxes over 12 yr. Further, Six et al. (2004) demonstrate that long-term no-till may decrease N_2O fluxes and that this may be due to long-term soil structural improvements in no-till. Practices such as no-till and legume cropping and set-aside programs that increase soil C storage within aggregates should thus be continuously maintained long-term to maximize potential ecosystem benefits.

CONCLUSIONS

Our results support theories that agricultural soil C losses near the soil surface can be partially reversed by using less intensive cultivation and enhancing plant community complexity. We found that the highest C accumulation rates occur in perennial cropping systems and early successional communities. Across ecosystems, C accumulation relative to conventional agriculture principally occurred in heavy-fraction (d >1.6 g cm^{-3}

or particle sizes <53 μm), slow, and acid-resistant pools of macroaggregates >250 μm. The 2000-8000 μm size range demonstrated the greatest increases in active pool C following aggregate destruction. This suggests that macroaggregates (and their constituent microaggregates) have the greatest protective capacity across a range of ecosystems with contrasting histories of plant and soil management but raises concerns about the persistence of sequestered C. The vulnerability of macroaggregates to destruction following tillage intensification and substantial shifts of C from physically protected slow pools into active pools following aggregate destruction demonstrates the need to protect stabilized SOM from increases in tillage intensity. Greatly increased macroaggregation and enhanced protective capacity of macroaggregates in perennial crops and successional ecosystems underscores the need to protect these systems, in particular, from even occasional tillage and other increases in management intensity.

Table 2.1. Cropping system and successional vegetation effects on soil C and N to 5 cm soil depth at the Kellogg Biological Station Long-Term Ecological Research Project in 2001[†].

	Total C	Total N	Bulk Density	Total C	Total N	C/N Ratio	pH
	%	%	g cm^{-3}	g m^{-2}	g m^{-2}		
Annual Crops (corn-soybean-wheat rotation)							
Conventional	1.01 (0.07)	0.10 (0.01)	1.37 (0.01)	687 (42.7)	70.8 (3.64)	9.69 (0.19)	6.26 (0.04)
Low input w/ legume cover	1.25 (0.05)	0.13 (0.00)	1.34 (0.03)	837 (40.8)	86.7 (2.69)	9.65 (0.24)	6.25 (0.05)
Organic w/ legume cover	1.33 (0.07)	0.13 (0.01)	1.36 (0.04)	907 (64.2)	91.5 (4.75)	9.88 (0.24)	6.18 (0.04)
No till	1.48 (0.08)	0.15 (0.01)	1.36 (0.03)	1000 (42.7)	102 (6.12)	9.92 (0.37)	6.40 (0.05)
Perennial Crops							
Alfalfa	1.64 (0.07)	0.16 (0.01)	1.35 (0.01)	1110 (38.6)	110 (3.90)	10.1 (0.07)	6.63 (0.05)
Poplar	1.69 (0.10)	0.14 (0.01)	1.27 (0.03)	1060 (43.0)	90.7 (5.64)	11.8 (0.34)	6.51 (0.10)
Successional Communities							
Early	2.08 (0.10)	0.18 (0.01)	1.21 (0.02)	1260 (66.4)	111 (4.19)	11.3 (0.22)	6.39 (0.03)
Mid (HT)	2.38 (0.19)	0.20 (0.01)	1.16 (0.02)	1390 (123)	115 (8.74)	12.0 (0.42)	5.46 (0.12)
Mid (NT)	4.32 (0.05)	0.35 (0.00)	0.93 (0.03)	2010 (60.3)	161 (3.75)	12.4 (0.18)	5.93 (0.11)
Deciduous Forest	4.65 (0.46)	0.32 (0.02)	1.11 (0.01)	2580 (266)	175 (13.2)	14.6 (0.43)	5.33 (0.06)

[†]Means with standard errors in parentheses.

Table 2.2. Acive (C_a), Slow (C_s)and Passive (C_r) Pool C associated with ecosystems and intact aggregate size classes[†‡]

	C Pool								
	Active				Slow				Resistant
	C_a mg g^{-1}	k_a d^{-1} ($\cdot 10^{-2}$)	Lmrt d^{-1}	Fmrt d^{-1}	C_s mg g^{-1}	k_s d^{-1} ($\cdot 10^{-4}$)	Lmrt y^{-1}	Fmrt y^{-1}	C_r mg g^{-1}
2000-8000 µm size class									
Conventional	0.545Ac	4.91	20.7	62.8	11.4Ac	3.06Bbc	10.3	31.3	7.36e
Low input	0.593Ac	5.43	18.6	56.3	15.8Abc	2.56bc	11.7	35.4	9.66de
Zero input	0.568Ac	5.77	17.4	52.8	13.4Abc	3.60ABab	8.29	25.1	9.67de
No till	0.585Ac	5.22	19.5	59.2	14.1Abc	3.78ab	8.38	25.4	11.2cd
Alfalfa	0.577Ac	4.34B	23.2	70.4	17.5Ab	1.73c	16.8	50.9	11.4cd
Poplar	0.447c	5.01	20.2	61.2	14.8bc	4.70Ba	6.12	18.5	11.4cd
Early	0.576Ac	5.05	20.6	62.3	15.9Abc	4.00ab	7.05	21.4	14.8c
Mid-HT	2.17ABa	3.19B	32.1	97.2	47.0Aa	3.00B	9.42	28.6	28.8b
Mid-NT	1.39ABb	3.36B	32.9	99.6	30.6ABa	3.95	7.72	23.4	28.6ABb
Late	1.32ABb	3.07	33.0	100	28.1ABa	3.20	9.27	28.1	4.94a
250-2000 µm size class									
Conventional	0.353Bbc	5.24	23.1	70.0	8.16Bc	4.63Ab	9.49	28.8	9.67d
Low input	0.372Abc	6.21	17.1	51.8	16.9Ab	3.98b	7.33	22.2	11.1cd
Zero input	0.330Abc	6.46	16.6	50.5	13.9Ab	4.60b	6.11	18.5	11.9cd
No till	0.386Abc	6.22	16.5	49.9	14.6Ab	3.82b	7.51	22.8	12.9cd
Alfalfa	0.352Abc	5.29B	20.9	63.3	17.5Ab	1.50Ac	19.2	58.1	11.9cd
Poplar	0.251c	7.60	15.7	47.7	13.1b	6.78Aa	4.20	12.7	15.3bc
Early	0.356Abc	6.14	17.2	52.2	18.1Ab	5.00ab	5.73	17.4	15.7b
Mid-HT	1.26Aa	4.36AB	32.8	99.5	34.1ABa	6.77a	4.54	13.7	24.6ab
Mid-NT	0.405Abc	4.94AB	22.8	69.0	44.2Aa	5.12ab	6.58	20.0	19.3Ccd
Late	0.686Ab	4.37	23.5	71.2	40.4Aa	3.90b	7.53	22.8	39.6a
53-250 µm size class									
Conventioanl	0.177c	5.14bc	20.9	63.5	5.31Cc	3.93AB	7.20	21.8	9.64d
Low input	0.230Bbc	6.63ab	17.5	53.1	7.94Bb	3.27	9.17	27.8	10.2d
Zero input	0.191Bc	5.01bc	20.4	61.7	7.53Bb	3.08	10.7	32.6	9.46d
No till	0.245Bbc	4.26c	40.7	123	7.52Bb	3.22	12.6	38.1	10.1d
Alfalfa	0.142bc	8.29Aa	15.1	45.7	9.54Bb	2.82B	12.1	36.8	12.4cd
Poplar	0.189bc	6.60abc	16.8	51.0	10.5b	4.66B	6.51	19.7	16.5bc
Early	0.243Bbc	6.18abc	17.8	53.9	10.1Bb	4.80	6.49	19.7	15.6c
Mid-HT	0.588Bab	6.51A	15.4	46.6	24.7Aa	4.47AB	6.18	18.7	27.1ab
Mid-NT	0.476Babc	6.26A	18.2	55.1	21.5Ba	6.33	4.73	14.3	38.6Aa
Late	0.867Ba	5.57abc	20.0	60.7	24.7Ba	5.77	4.79	14.5	42.5a

[†] Within an aggregate size class, ecosystems with different lowercase letters are significantly different ($p<0.05$). Within an ecosystem, size classes followed by different uppercase letters are significantly different.

[‡] C_a and k_a represent active pool C and kinetics, k_s represents slow pool kinetics, and C_r and k_r represent resistant pool C and kinetics. C_a, k_a and k_s were determined by modeling; slow pool C (C_s) was determined by difference. k_r was assumed to be $8.3 \cdot 10^{-06}$ d^{-1}.

[§] Laboratory mean residence time (Lmrt) was calculated as 1/k. Field mean residence time (Fmrt) was determined after doing a Q_{10} correction for the difference in lab temperature (25°C) and field mean temperature at KBS (9.0°C).

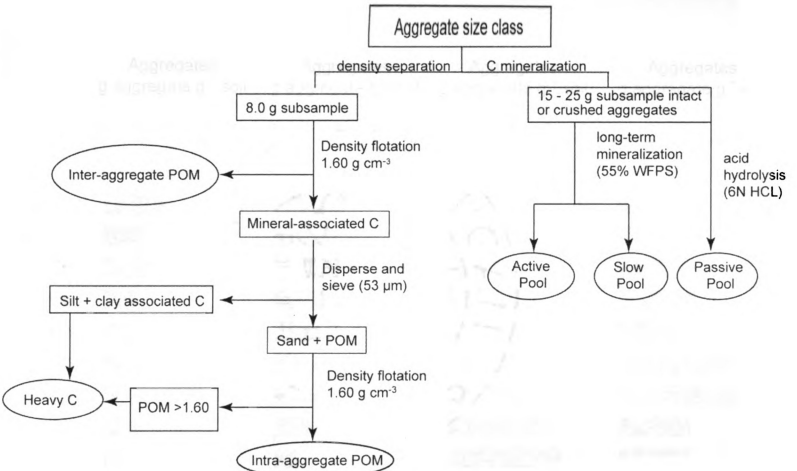

Figure 2.1. Outline of the soil organic matter pools (SOM) produced by density separation and long-term mineralization of different aggregate size classes. SOM pools that we quantify in this study are shown within

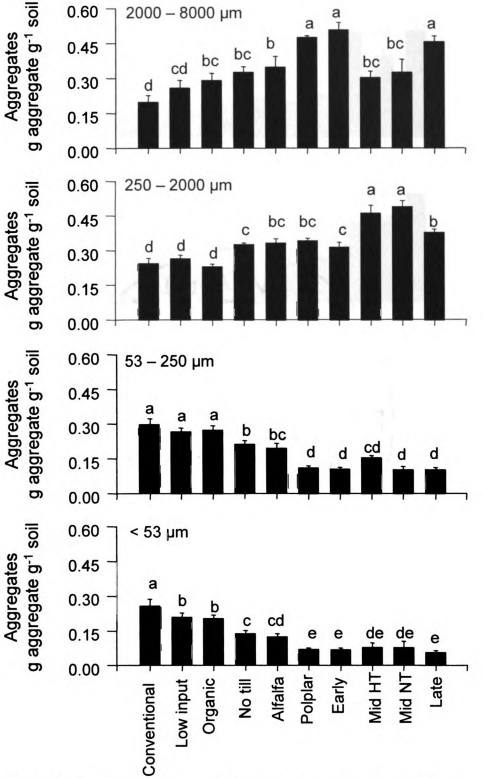

Figure 2.2. Ecosystem effects on total sand-free C concentrations in different aggregate size fractions at the KBS LTER. Within an aggregate size class, ecosystems with different lowercase letters are significantly different ($p<0.05$). Within an ecosystem, size classes with different uppercase letters are significantly different. Bars are means ± S.E.

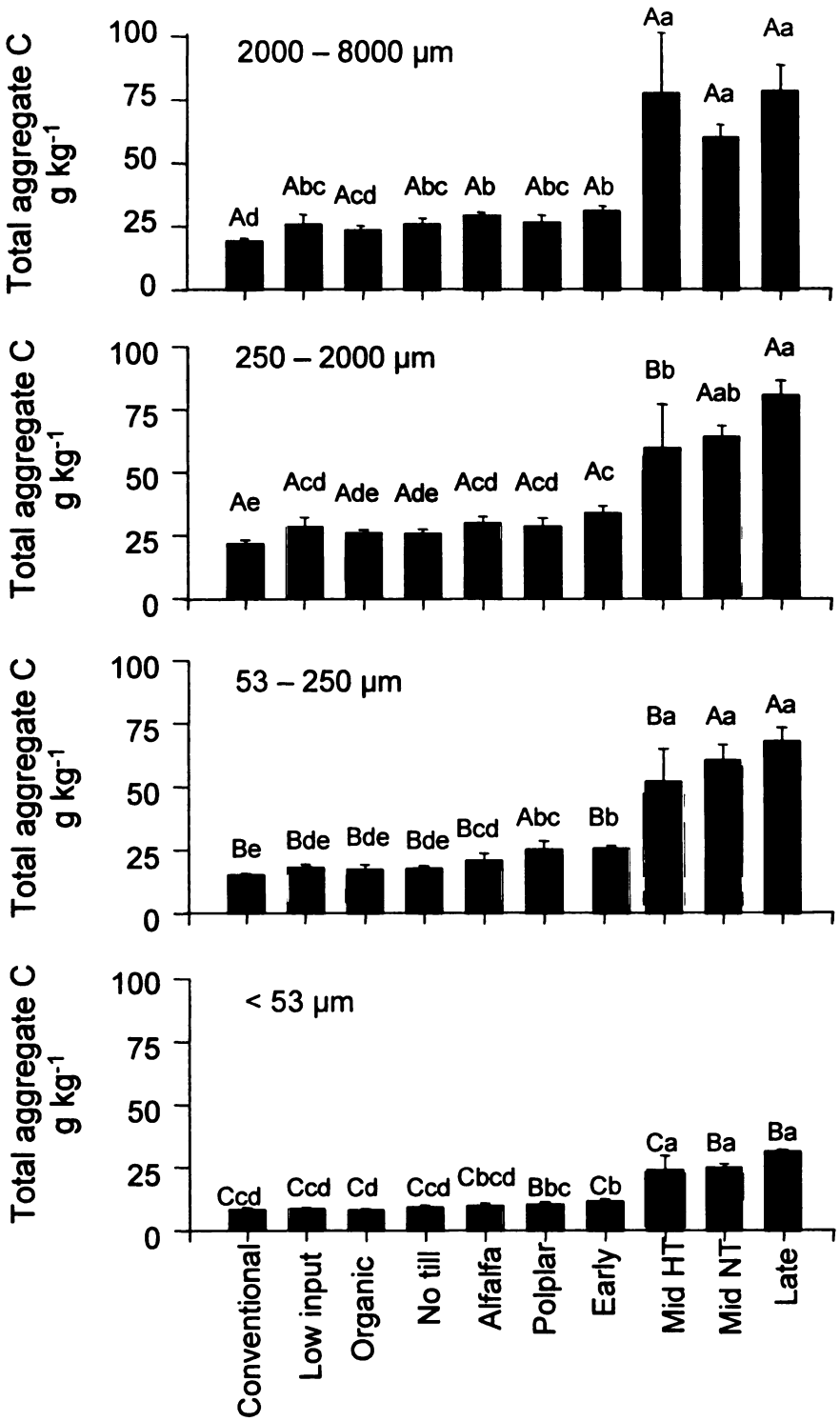

Figure 2.3. Ecosystem effects on total sand-free C concentrations in different aggregate size fractions at the KBS LTER. Within an aggregate size class, ecosystems with different lowercase letters are significantly different ($p<0.05$). Within an ecosystem, size classes with different uppercase letters are significantly different. Bars are means ± S.E.

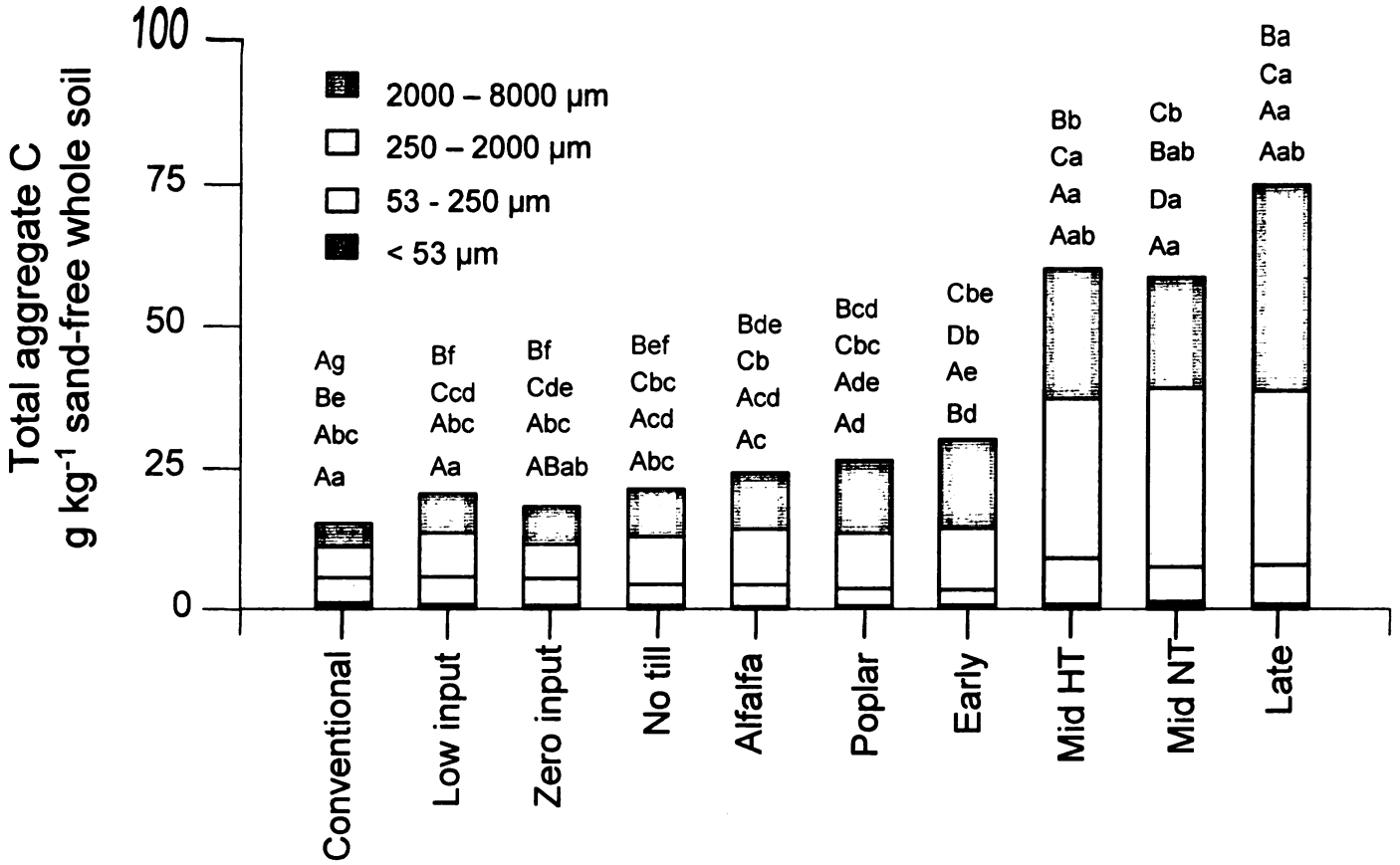

Figure 2.4. Ecosystem effects on the distribution of sand-free C in different aggregate size classes. Results are a presented on a per unit soil basis and are thus a function of C concentration and the proportion of soil within an aggregate size class. Within an aggregate size class, ecosystems with different lowercase letters are significantly different ($p<0.05$). Within an ecosystem, size classes with different uppercase letters are significantly different. Statistical results and stacked bars for size classes are shown from top to bottom in the same order.

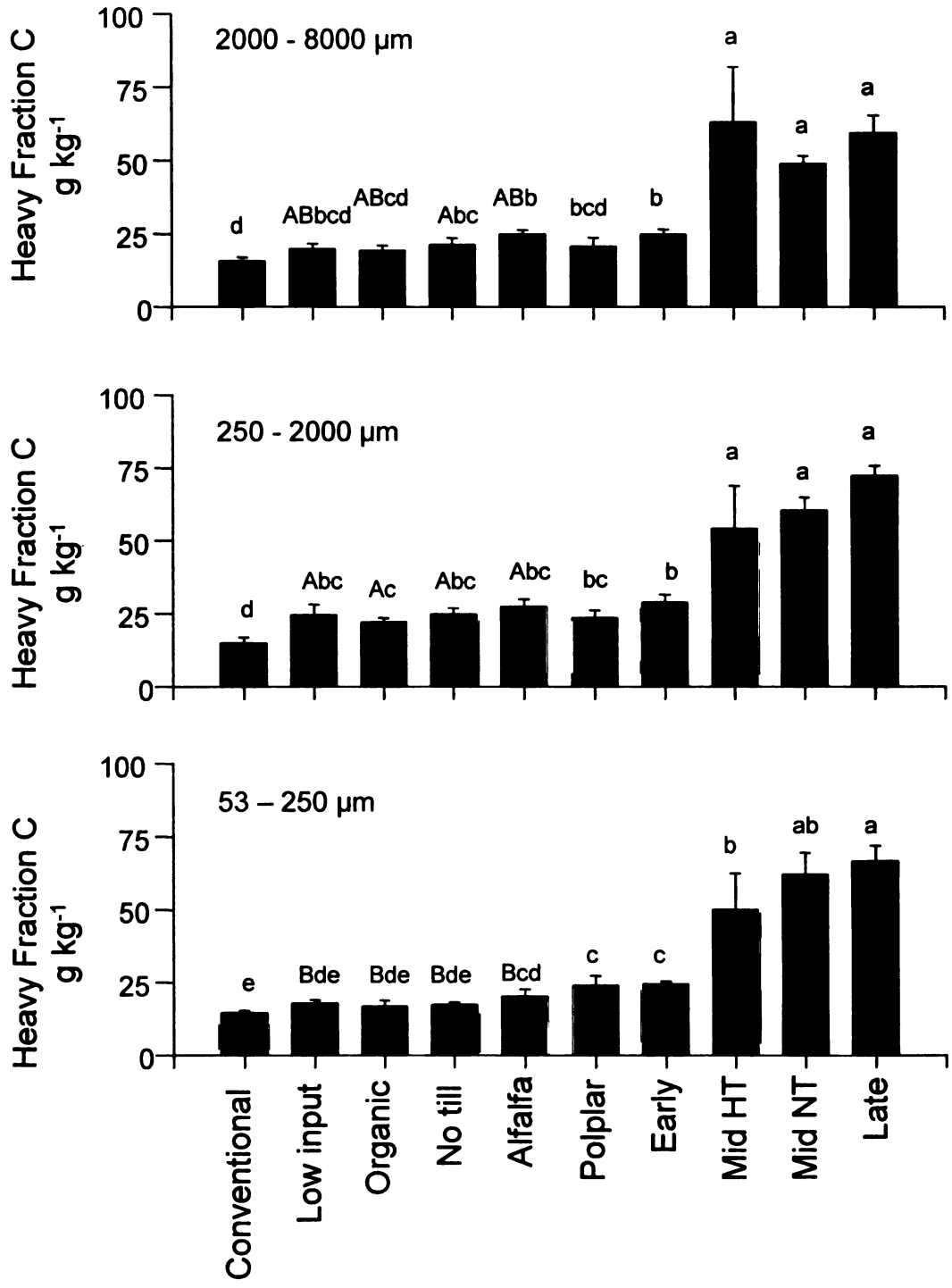

Figure 2.5. Ecosystem effects on the concentration of heavy-fraction C in different aggregate size classes at the KBS LTER. Within an aggregate size class, ecosystems with different lowercase letters are significantly different ($p<0.05$). Within an ecosystem, size classes with different uppercase letters are significantly different. Bars are means ± S.E.

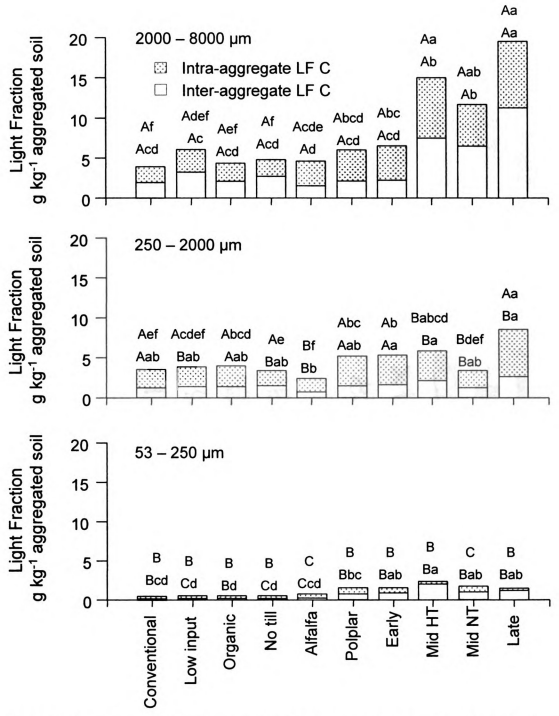

Figure 2.6. Ecosystem effects on the concentration of inter- and intra-aggregate light fraction (LF) C in aggregate size classes. Within a column, the results on top are from intra-aggregate LF and those on the bottom are for inter-aggregate LF. Within an aggregate size class, ecosystems with different lowercase letters are significantly different ($p<0.05$). Within an ecosystem, size classes with different uppercase letters are significantly different. Statistical results and stacked bars for light fraction location are shown from top to bottom in the same order.

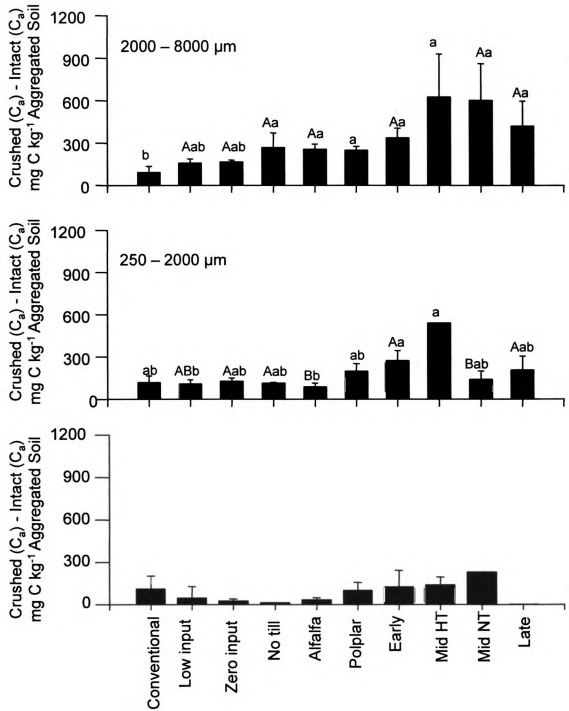

Figure 2.7. Ecosystem effects on the potential release of labile C from physical protection following aggregate destruction. Results are presented as the difference between crushed and intact aggregates. The larger the difference the greater the transition of C from slow to active pools. Within an aggregate size class, ecosystems with different lowercase letters are significantly different ($p<0.05$). Within an ecosystem, size classes with different uppercase letters are significantly different. Bars are means ± S.E.

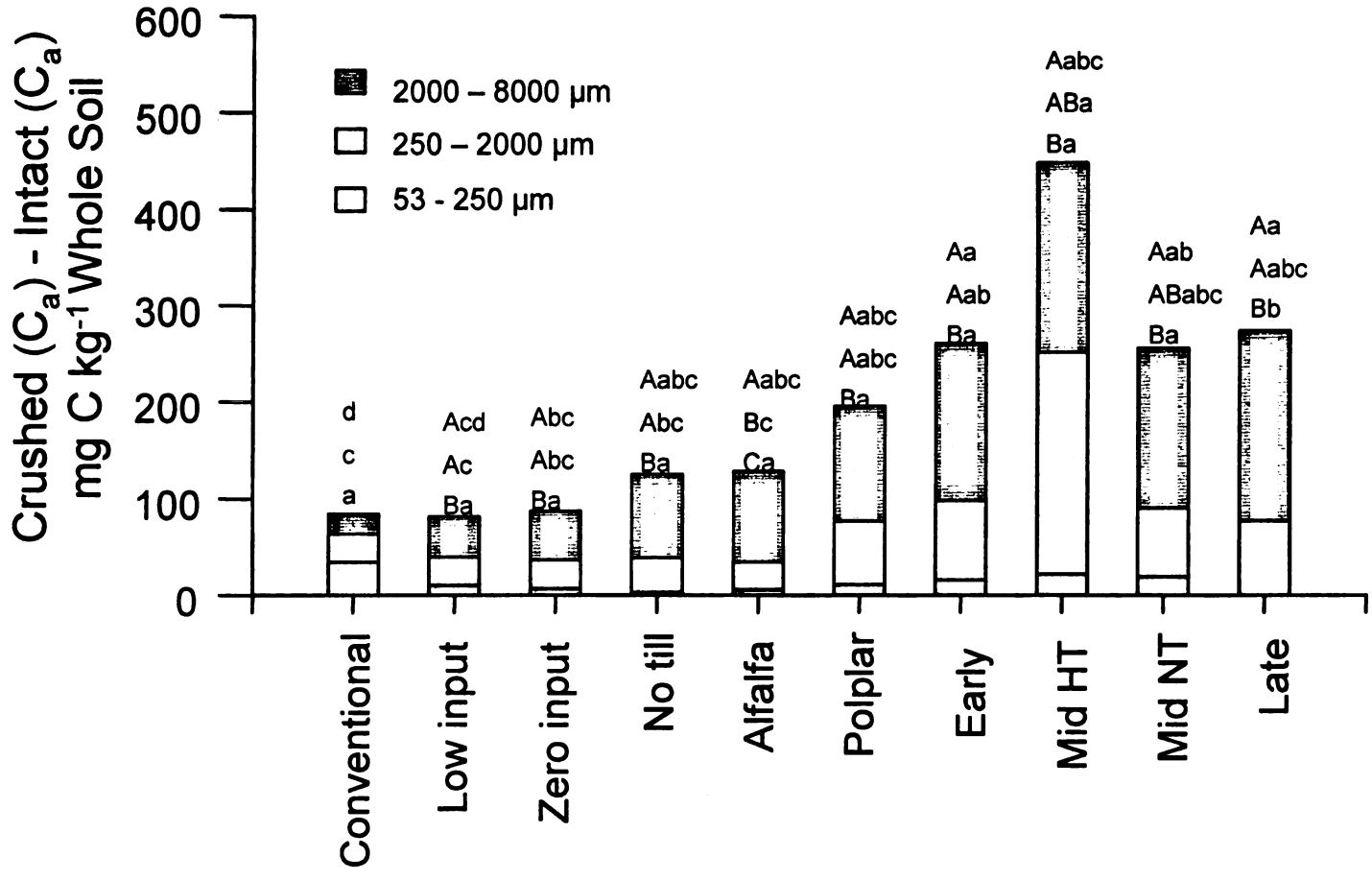

Figure 2.8. Ecosystem effects on the total potential release of active C (C_a) from physical protection following destruction of aggregates in different size classes. Results are presented on a sand-free whole soil basis and are thus a function of the increase in active pool C due to aggregate destruction and the proportion of soil within an aggregate size class. Within an aggregate size class, ecosystems with different lowercase letters are significantly different ($p<0.05$). Within an ecosystem, size classes with different uppercase letters are significantly different. Statistical results and stacked bars for size classes are shown from top to bottom in the same order.

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CHAPTER 3

Initial Cultivation of a Temperate-Region Soil Immediately Accelerates Aggregate Turnover and CO_2 and N_2O Fluxes

ABSTRACT

The immediate effects of tillage on protected soil C and N pools and on trace gas emissions from soils at pre-cultivation levels of native C remain largely unknown. We measured the response of CO_2 and N_2O emissions and associated environmental controls to cultivation in a previously uncultivated U.S. Midwest Alfisol with organic C concentrations indistinguishable from those in adjacent old-growth forests on the same soil type (3.2%). Within 2 days of initial cultivation, tillage significantly ($P < 0.001$, $n = 4$) increased CO_2 fluxes from 91 to 196 mg CO_2-C m^{-2} hr^{-1} and within the first 30 d higher fluxes due to cultivation were responsible for losses of an additional 85 g CO_2-C m^{-2}. Additional daily C losses were sustained during a second and third year of cultivation at rates of 1.9 and 1.0 g C m^{-2} d^{-1}, respectively. Associated with the CO_2 responses were increased soil temperature, substantially reduced soil aggregate size (mean weight diameter decreased 35% within 60 d), and a reduction in the proportion of intra-aggregate, physically protected light fraction organic matter. N_2O fluxes increased 6-fold in 2002, 2-fold in 2003 and 6-fold in 2004 and were associated with increased soil NO_3^- concentrations, which approached 15 μg N g^{-1}. Decreased plant N uptake immediately after tillage, plus increased mineralization and nitrification rates (nitrifier enzyme activity increased 5-fold), contributed to increased NO_3^- concentrations. Our results demonstrate that initial cultivation of a soil at pre-cultivation levels of native soil C immediately destabilizes physical and microbial processes related to C and N retention

in soils and accelerates trace gas fluxes. Policies designed to promote long-term C sequestration may thus need to protect soils from even periodic cultivation in order to preserve sequestered C.

INTRODUCTION

Carbon sequestration and greenhouse gas abatement in soils are two of a limited number of rapidly-deployable, high impact CO_2 stabilization options now available to policy makers (Kauppi et al., 2001; Caldeira et al., 2004). Converting agricultural land to perennial crops or to successional communities has the potential to sequester ca. 60 g soil C m^{-2} y^{-1} (CAST, 2004) and conversion to no-till annual crops has the potential to sequester C at about half this rate (West and Post, 2002; Lal, 2003). Ultimately, however, net greenhouse gas mitigation by soil C storage depends on the persistence of stored soil organic matter (SOM) and an important challenge to persistent C and N is the potential reversibility of C sequestration (Paustian et al., 2000; Lal, 2004; Pacala and Socolow, 2004). Some models predict that soils with sequestered C may rapidly lose C and N following cultivation (Baisden and Amundson, 2003) but predictions remain contentious because data are not currently available to generalize for temperate ecosystems on a time scale of less than 10 years following cultivation (Davidson and Ackerman, 1993; West and Post, 2002; Miller et al., 2004).

Models predict that N_2O emissions in long-term no-till soils may be lower than those in tilled soils (Six et al., 2004). Over time, increased aggregation, decreased bulk density, and lower mineralization rates in no-till soils may limit nitrifier and denitrifier N_2O production. These changes, in addition to cessation of annual N fertilizer

amendments, reduce N_2O emissions when agricultural soils are converted to unfertilized successional communities (Robertson et al., 2000). Cultivation of no-till soils or other disturbances that increase soil N availability may accelerate N_2O emissions. Pinto et al. (2004) found that cultivation of a 17-year old, unfertilized perennial pasture increased N_2O emissions from 2.7 to 4.6 mg N_2O-N m^{-2} over 5 days of sampling. Keller et al. (1993) found that converting forest to pasture increased N_2O fluxes in the first ten years. In both of these studies authors attributed increases to accelerated SOM turnover producing excess inorganic N that was subsequently used by N_2O-producing nitrifiers and denitrifiers.

In temperate regions, the processes controlling C and N cycling responses to cultivation have been studied almost exclusively in soils with a recent history of disturbance (Martens, 2001). Studies have demonstrated that long-term tillage aerates soils, exposes C in protected microsites (Del Gado et al., 2003; DeGryze et al., 2004), increases soil temperature, modifies trace gas fluxes (Smith et al., 2001; Smith and Conen, 2004), and alters microbial community structure and function (Cavigelli and Robertson, 2000; Buckley and Schmidt, 2001). Over time, these changes lead to 30-60% declines in SOM (Davidson and Ackerman, 1993; Buyanovsky et al., 1997; West and Post, 2002; Lal, 2003).

The quantity and turnover rates of soil aggregates may be particularly important controls over SOM protection following changes in cultivation intensity (Paustian et al., 2000). Evidence for protection of SOM by aggregates includes low interior-aggregate oxygen concentrations that limit respiration rates, small intra-aggregate pore spaces that limit SOM access to decomposers, and increases in soil respiration rates when SOM is

released from within aggregates (Sexstone et al., 1985; Mikha and Rice, 2004). Field studies have demonstrated that C sequestration in afforested soils and restored grassland ecosystems principally occurs within soil aggregates (Jastrow et al., 1996; DeGryze et al., 2004).

We hypothesize here that if immediate and persistent reductions in soil aggregation occur after cultivation in conjunction with changes in soil temperature and other environmental controls over decomposition, rapid changes in SOM turnover, microbial activity and trace gas fluxes will occur after a single plowing. Our first objective is to measure changes in CO_2 and N_2O emissions for three years following cultivation of a previously uncultivated field at pre-cultivation levels of native soil C to evaluate the immediacy, magnitude, and persistence of change. Our second objective is to determine the persistence of soil aggregation and changes in the distribution of physically protected light-fraction SOM after initial cultivation. We additionally followed changes in soil inorganic N, nitrifier enzyme activity and soil moisture to better understand factors driving changes in gas fluxes.

MATERIALS AND METHODS

Site Description and Experimental Design

Our experimental site was a previously never-tilled field with little topography and no known soil gradients located at the W.K. Kellogg Biological Station (KBS) in southwest Michigan, USA (42° 24' latitude, 85° 24' longitude). In 1956 the site was cleared of trees and thereby converted from a northern hardwood forest to a midsuccessional grassland community that has since been mowed each fall. Soils at the

site are Kalamazoo (fine-loamy) and Oshtemo (coarse-loamy) mixed, mesic, Typic Hapludalfs developed on glacial outwash (Crum and Collins, 1995). The two series co-occur at KBS with variation within a series often as great as variation between series. Prior to initial cultivation, surface soils (0-7 cm) contained 31.8 (± 1.4) g C kg^{-1}, 2.59 (± 0.11) g N kg^{-1}, and soil pH averaged 5.93 (± 0.11); the same soil C levels had been measured 3 years and 12 years prior to this study and are statistically indistinguishable from C levels in nearby undisturbed forests on the same soil types (Robertson et al., 2000).

Dominant plant species at the site include *Bromus inermis, Rubus allegheniensis, Monarda fistulosa, Juncus* sp., *Solidago canadensis, Arrhenatherum elatius, Poa pratensis,* and *Elytrigia repens* (Robertson et al., 2000). In 2002 we established in this field eight 3 x 6 m plots to which we assigned 4 replicates of two tillage treatments (cultivated and uncultivated control) in a randomized complete block design. Plots were laid out in an east – west direction and arranged in two rows running north – south with 4 plots and two blocks in each row. There were 5 m between plots in north – south and east – west directions. Cultivated sites were mowed with biomass left in place prior to cultivation to 19 cm. We used a moldboard plow and a disc for primary and secondary cultivation, respectively, methods commonly used for preparing fallow land for agricultural production. Cultivation occurred in the same plots on 25 June 2002 (DOY 176), 15 June 2003 (DOY 166), and 20 June 2004 (DOY 172). To study soil disturbance effects separately from other factors associated with agricultural conversion (e.g. the use of annual plant monocultures and fertilizers), we left the site fallow following tillage to allow a diversity of annual and perennial plants to recolonize.

Soil and Plant Sampling

Samples for aggregate and total soil C and N analysis were collected prior to initial cultivation on 18 June 2002, and post-initial-cultivation on 24 August 2002, 27 May 2003, and 21 October 2003. An additional sample for total C analysis was taken 24 September 2004. Five 3.8 cm soil cores were taken from each plot to a depth of 20 cm, placed in plastic bags, and refrigerated (< 7 d) prior to sieving through an 8 mm sieve and air-drying at 20 °C.

At each of two locations within a plot two 2.5 cm soil cores were collected to a depth of 7 cm at each time of trace gas sampling. Cores were passed through an 8 mm sieve, homogenized, and used for gravimetric soil moisture determinations.

Plant and litter C estimates prior to initial cultivation were estimated by drying and analyzing litter and plant samples collected on 21 June 2002 (DOY 172) from two 625 cm^{-2} quadrats in each plot. Changes in the litter layer after cultivation were estimated on 15 September 2003 from two 625 cm^{-2} quadrats in each of four adjacent plots cultivated for the first time in 2003. Organic C and total N concentrations of plant and soil samples were determined by dry combustion and gas chromatography in a CHNS analyzer (Costech ECS 4010, Costech Analytical Technologies, Valencia CA.).

Trace Gas Fluxes

Gas fluxes were determined using a single 25 cm diameter static PVC chamber (5500 cm^{-3}) located within each plot (Livingston and Hutchinson, 1995). Prior to sampling, gas-tight lids with sampling ports were placed on chamber bases permanently

installed to a soil depth of 2.5 cm and accumulated headspace was then sampled four times over 90 min by removing 20 mL of headspace gas to 12 mL vials (Labco Unlimited, Buckinghamshire, UK). Gas sampling was generally performed between the hours of 0900 and 1400 and all plots were sampled on each sampling day. Within 48 h of collection CO_2 was analyzed using an infrared gas absorption analyzer (IRGA) and N_2O analyzed using a gas chromatograph outfitted with a ^{63}Ni electron capture detector (350 °C). Flux for each chamber was calculated as the linear portion of the gas accumulation curve for that chamber. Trace gas measurements were made 13 times in 2002 between 24 June and 22 August, 10 times in 2003 between 29 May and 13 October, and 19 times in 2004 between 15 April and 29 October. We interpolated daily gas fluxes from our periodic measurements to estimate total CO_2 and N_2O fluxes over the measurement period.

Particulate SOM Density Distribution

Cultivation effects on the distribution of particulate SOM in four density fractions was determined using a sequential fractionation technique in sodium polytungstate (NAPT). Whole soil samples (15 g) were dispersed in 0.5% sodium hexametaphosphate by shaking for 48 h on a rotary shaker set at 190 rpm. Dispersed samples were poured through a 53 μm sieve and rinsed thoroughly with deionized (DI) water. Sand and particulate organic matter (POM) remaining on the sieve were transferred to filter paper and then backwashed into a 100 mL beaker with 40mL NAPT (density = 1.9 g cm^{-3}). After equilibrating overnight we aspirated the floating material off the surface. POM remaining in the beaker was classified as having a density > 1.9 g cm^{-3}. POM with a

density < 1.9 g cm^{-3} was then sequentially suspended in NAPT with densities of 1.6 and 1.3 g cm^{-3}, resulting in POM in four density fractions (> 1.9, 1.6 – 1.9, 1.3 – 1.6, < 1.3 g cm^{-3}). Clay plus silt associated SOM was determined by difference.

Aggregate and Light Fraction Separation

Aggregate distribution was determined by hand on triplicate 35 g air-dried soil samples (0-20 cm soil depth) by wet-sieving in water through a series of 2000 μm, 250 μm, and 53 μm sieves. Soil was submerged for 5 min on the surface of the 2000 μm sieve which was then moved up and down for two minutes with a stroke length of 3 cm for 50 strokes. Sieving was repeated on the 250 μm (50 strokes) and 53 μm (30 strokes) sieves using the soil plus water that passed through the next larger sieve. Aggregates remaining on each sieve were dried at 60 °C. Sand content was determined on an aggregate subsample after dispersing soil in sodium hexametaphosphate (0.5%) for 48 h on a rotary shaker at 190 rpm.

Mean weight diameter (MWD) of sand-free aggregates was determined by calculating the sum of the products of the mean diameter of each size fraction and the proportion of the total sample weight in that fraction (Kemper and Rosenau, 1986).

The method we used to separate inter- and intra-aggregate light fraction (LF) is based on previously published protocols (Six et al., 1998; Gale et al., 2000). Aggregate subsamples were pre-wetted prior to LF analysis to minimize aggregate slaking during LF separation. An 8 g subsample of aggregates was divided in half and placed on two membrane filters (47 mm diameter; Pall Supor-450) overlaying two paper filters (70 mm diameter; Whatman 42) in a 10 cm petri dish. The paper filters conducted water to the

membrane filters, which, in turn, facilitated the smooth transfer of soil into beakers. Four mL of DI water were trickled onto the paper filters in order to slowly wet all of the aggregates by capillarity. Aggregates were transferred from the membrane filters to 100 mL beakers after 16 h with 5 mL aliquots of NAPT at a density of 1.62 g cm^{-3}. A total of 55 mL NAPT was used for each sample. A preliminary test showed that the final density of the sodium polytungstate was 1.60 g cm^{-3} following equilibration with the water contained in aggregates.

After 24 h, LF was aspirated from the surface of the sodium polytungstate and then rinsed on a hardened, ashless filter paper with at least 600 mL DI H_2O. We refer to this pool as inter-aggregate LF. After removal of this pool, we aspirated the remaining sodium polytungstate. Aggregates were then dispersed to release the intra-aggregate LF using sodium hexametaphosphate as described previously and resuspended in NAPT (d = 1.62 g cm^{-3}). The intra-aggregate LF was collected form the surface.

Soil Temperature and Inorganic Nitrogen Dynamics

Soil temperature was determined at the time of each trace gas sampling to a depth of 7 cm at a distance of 25-35 cm from the sampling chamber. NH_4^+ and NO_3^- were extracted with 1 M KCl from duplicate field-moist 10 g soil samples using a 1:5 soil/extractant ratio. Soil extracts were filtered with a syringe filter using a type A/E glass fiber filter (Pall Corporation, East Hills, NY). Filtrates were stored in 7 mL scintillation vials and frozen until analysis for NH_4^+ and NO_3^-. Both analyses were performed on an Alpkem 3550 Flow Injector Analyzer (OI Analytical, College Station, TX). Inorganic N was determined on samples collected to a depth of 7 cm in 2002 on

DOY 175, 178, 190, 200, 207, 228, and 234; in 2003 on DOY 149, 170, 178, 189, 216, 226, 245, 266, and 286; and in 2004 on DOY 131, 156, 180, 194, 208, 230, and 268. Additionally, inorganic N was also determined to a depth of 7-20 cm on three dates in 2003 and all dates in 2004. Trends at this depth were similar to those at 0-7 cm but generally smaller in magnitude and are not presented here.

We used a shaken slurry method (Hart et al., 1994) to test for changes in nitrifier enzyme activity to 0-7 and 7-20 cm soil depths following cultivation on 13 October 2003 (DOY 286) and 17 August 2004 (DOY 230). Briefly, 30 g field-moist soil was combined with 100 mL solution containing non-limiting quantities of NH_4^+ in a 160 mL jar capped with polyfilm. The polyfilm was perforated with a needle to allow rapid gas exchange while minimizing water loss. Jars were placed on a rotary shaker and rotated at 200 rpm. Each flask was sampled 4 times during a 28 h incubation period. Soil extracts were centrifuged, filtered, and frozen until analysis for NO_3^-.

Statistical Analysis

Soil aggregate size distributions were corrected for sand content of the same size as the aggregates since this is usually not part of the aggregate structure. Carbon content of soil organic matter fractions was calculated on an area basis by correcting for bulk density and soil sampling depth. Tillage effects on trace gas fluxes, soil moisture, temperature and inorganic N were analyzed by Proc Mixed (Version 8.2, SAS Institute, 1999) using a randomized complete block design analysis of variance (ANOVA) with repeated measures (SAS Version 8.2, SAS Institute, 1999). Treatment and sampling date were considered fixed effects and block a random effect. Where there were significant

day of year by treatment interactions, differences between treatments on separate days were determined using the slicing command in SAS. Cultivation effects on aggregation and SOM pools were similarly analyzed but without repeated measures. Analysis of covariance (Goldberg and Scheiner, 2001) was used to examine the relationship between soil temperature, moisture and trace gas fluxes in 2002, 2003, and 2004.

RESULTS

Trace Gas Fluxes

Within 2 days of initial cultivation in 2002 tillage had significantly ($P < 0.001$; n = 4) increased CO_2 fluxes > 100%, from 91 to 196 mg CO_2-C m^{-2} hr^{-1} (Figure 3.1). We estimate that within the first 33 d following cultivation 85 g C m^{-2} was lost due to tillage. During the next 30 d, tillage effects on CO_2 flux were somewhat lower, resulting in a daily average loss of 1.4 g C m^{-2} d^{-1} over the 60 d measurement period.

Prior to the second cultivation in 2003, CO_2 fluxes were again higher in the cultivated plots ($p<0.001$). In 2003 the tillage-induced CO_2 response was measurable for 80-120 days and average daily CO_2-C loss due to cultivation was 1.9 g m^{-2} d^{-1} for the 138 d sampling period.

Prior to cultivation in 2004 there were no significant differences ($p<0.05$) between treatments. Following cultivation (DOY 172), cultivated plots had significantly greater CO_2 fluxes on DOY 203 (104%), 204 (73.4%), and 211 (152%). Additional daily CO_2-C losses due to cultivation were 1.00 g C m^{-2} d^{-1} over the 198 sampling period in 2004.

Analysis of covariance showed that soil moisture and temperature (Fig. 1) were related to CO_2 flux differently in different years. In 2002 there was no temperature effect but a significant soil moisture by treatment interaction ($P < 0.05$), indicating that the relationship between CO_2 flux and moisture differed between treatments [control: $\log_{10}$ CO_2 flux = 0.28 + 0.77 ($\log_{10}$ moisture), $r^2 = 0.74$; cultivated: $\log_{10}$ CO_2 flux = 0.98 + 0.33 ($\log_{10}$ moisture), $r^2 = 0.21$; units in Figure 3.1]. Moisture effects were not significant in 2003 or 2004. In 2003 there was a significant ($p<0.05$) soil temperature effect [$\log_{10}$ CO_2 flux = -0.61 + 1.40 ($\log_{10}$ temperature), $r^2 = 0.42$]. In 2004 there was again a significant ($p<0.05$) soil temperature effect, although a smaller proportion of the flux was explained [$\log_{10}$ CO_2 flux = 0.05 + 0.89 ($\log_{10}$ temperature), $r^2 = 0.16$].

N_2O fluxes in 2002 increased in response to cultivation but not until 30 d after cultivation when fluxes in cultivated plots were as high as 87 μg N_2O-N m^{-2} h^{-1} (Figure 3.1). In 2003, mean fluxes were higher in cultivated plots on all days but significantly different only on DOY 170 and 202. Overall, N_2O fluxes were considerably higher in 2004 than in 2002 or 2003. In 2002, average N_2O fluxes were 2.87 in control plots and 22.17 μg N_2O-N m^{-2} h^{-1} in cultivated plots; in 2003 the average N_2O fluxes were 3.72 in control plots and 11.54 μg N_2O-N m^{-2} h^{-1} in cultivated plots; in 2004 control plots emitted 11.37 μg N_2O-N m^{-2} h^{-1} compared to 76.14 μg N_2O-N m^{-2} h^{-1} in cultivated sites. In 2004, there were extremely high fluxes due to cultivation on DOY 163 (217.4 ± 153.3 μg N_2O-N m^{-2} h^{-1}) and DOY 189 (1292 ± 573.2 μg N_2O-N m^{-2} h^{-1}).

Soil Aggregation

The mean weight diameter (MWD) of aggregates measured 63 d after tillage was 35% lower in cultivated than in control plots ($P < 0.01$, $n = 4$; Figure 3.2). These differences were still evident prior to tillage in 2003 ($P<0.01$, $n = 4$). In October 2003 (DOY 294) the reduction in MWD associated with tillage was 37% ($P < 0.05$; Figure 3.2), similar to the difference found in 2002. The size class of 2000 – 8000 μm aggregates declined to 19% from 34% in control plots by DOY 236, 2002 (Figure 3.3). In 2003, 2000 – 8000 μm aggregates remained lower in cultivated plots while aggregates in the 53 – 250 and <53 μm size classes increased.

Soil Organic Matter Distribution

There were no differences in total soil C to 20 cm depth between control and cultivated plots following cultivation in 2002 (4.36 ± 0.05 vs. 4.70 ± 0.15 kg m^{-2}), 2003 (4.38 ± 0.28 vs. 4.81 ± 0.26 kg m^{-2}), or 2004 (4.32 ± 0.09 vs. 4.74 ± 0.32 kg m^{-2}). Cultivation reduced litter C on the soil surface from 195 to 18 g C m^{-2} ($p<0.01$) after a single cultivation. In 2002 and 2003 POM with a density > 1.9 g C cm^{-3} was similar in control and cultivated plots (Table 3.1). In 2002, there was an increase of POM in the lower density fractions (< 1.3, 1.3 – 1.6, and 1.6 – 1.9). Cultivation did not significantly change POM distribution in 2003 ($p<0.05$), although there was a trend ($p < 0.1$) toward more POM with a density of 1.3 – 1.6 g C m^{-2} in cultivated plots (Table 3.1).

The proportion of intra-aggregate to total LF in 2000 – 8000 μm aggregates declined from 28 to 16% within 60 d of the first cultivation in 2002 ($p<0.05$; $n = 4$; Figure 3.4). In 2003 there was a trend towards a reduced proportion of intra-aggregate LF associated with 2000 – 8000 μm aggregates in cultivated (21%) compared to control

(27%) plots ($p<0.1$; $n = 4$) . The intra-aggregate LF in the 250 – 2000 μm size class increased 73% ($P<0.05$; $n = 4$) and there was a trend towards an increase in inter-aggregate LF from 56.7 to 118.0 g LF C m^{-2} ($p<0.1$). In the 53 – 250 μm size class there was an increase in the inter- and intra-aggregate LF and total LF.

Soil Inorganic Nitrogen

Cultivation in 2002 increased extractable soil NO_3^- concentrations to 3.18 μg NO_3^--N g^{-1} (compared to 0.41 μg NO_3^--N g^{-1} in control plots) after 24 d (Figure 3.5). NO_3^- concentrations in cultivated plots remained higher on DOY 207, 228 and 234 and peaked on DOY 228 (14.6 vs. 0.44 μg NO_3^--N g^{-1}). NH_4^+ concentrations were also increased by cultivation on DOY 190 (87%), 200 (312%), 207 (176%), 228 (193%), and 234 (82%).

In 2003 cultivation significantly increased NO_3^- concentrations on DOY 170 and 216 but there were no differences on the other sampling dates (Figure 3.5). NH_4^+ concentrations were significantly greater in cultivated plots on DOY 170 and 216 but lower in these plots relative to control plots on the following three sampling dates. In 2004 NO_3^- concentrations on DOY 180 were 11.8 μg NO_3^--N g^{-1} in cultivated plots and 0.55 μg NO_3^--N g^{-1} in control plots (Figure 3.5). NO_3^- concentrations remained higher in cultivated plots on DOY 194 and 208. NH_4^+ concentrations were reduced by cultivation on four of seven sampling dates in 2004.

Nitrifier enzyme activity (Figure 3.6) in 2003 was 5-fold higher in cultivated than uncultivated treatments (25.4 vs. 127 μg N kg^{-1} soil h^{-1}) to 7 cm soil depth and 3-fold higher at 7 – 20 cm (14.9 vs. 45.2 μg N kg^{-1} soil h^{-1}). Similar trends were observed in

2004 when control plots had lower nitrifier enzyme activity at 0 - 7 cm (35.5 vs. 109 μg N kg^{-1} soil h^{-1}) and 7 - 20 cm (10.5 vs. 45.7 μg N kg^{-1} soil h^{-1}).

DISCUSSION

We documented substantial, immediate losses of CO_2-C following cultivation. On average, cultivated plots lost an additional 1.4, 1.9 and 1.0 g C m^{-2} d^{-1} in 2002, 2003 and 2004, respectively, equivalent to 84 g C m^{-2} over the 60 d following cultivation in 2002, 260 g C over the 138 d sampling period in 2003, and 198 g C over 2004's 198 d sampling period. N_2O emissions also markedly increased during our measurement period following cultivation: 672% in 2002, 210% in 2003, and 570% in 2004. Based on IPCC calculations using a 100-y time horizon for N_2O these emission differences are equivalent to 22.5, 9.1, and 75.4 g CO_2-C equivalents m^{-2} for 2002, 2003, and 2004, respectively. The 3-year average of 35.7 g CO_2-C m^{-2} is similar to the average annual C gain (ca. 30 g C m^{-2} y^{-1}) under no-till systems in the U.S. Midwest (Robertson et al., 2000; Franzluebbers and Steiner, 2002). Below we discuss how changes in the soil environment and biological processes likely contributed to these fluxes and, more generally, to destabilizing soil C and N.

Soil Structure and Organic Matter

Many studies have demonstrated that long-term repeated cultivation reduces soil structural stability and changes the distribution of SOM (e.g. Six et al., 1998; Grandy et al., 2002; DeGryze et al., 2004). Our results indicate that a significant amount of the structural degradation and change in C distribution, particularly in large soil fractions,

occurs after plowing only once. There was no measurable additional decline in soil structure following cultivation in 2003, further demonstrating that cultivation effects occur immediately and are persistent. Mean weight diameter (MWD) differences measured 63 d after tillage were largely attributable to declines in 2000 - 8000 μm aggregates (Figure 3.4). In cultivated sites, 19% of the soil was in the 2000 - 8000 μm size class, compared to 34% in the control plots, identical to adjacent agricultural fields on the same soil type that have been tilled for > 50 y (data not shown). The persistence of these effects was evident prior to tillage in 2003 when aggregation in cultivated sites remained substantially lower, despite the potential for freeze-thaw and wetting-drying cycles to have affected aggregation during winter months.

Soil CO_2 emission responses to cultivation in previously cultivated soils may occur for only hours or days, suggesting that physical phenomenon such as diffusion rates are driving increases (Kessavalou et al., 1998; Calderón et al., 2001; Jackson et al., 2003). In our previously uncultivated soils, sustained CO_2 fluxes suggest that microbial respiration increased following cultivation due to increased substrate availability. These substrates included LF released from large aggregates in addition to aboveground C that entered low-density, unprotected, inter-aggregate POM pools (Table 3.1; Figure 3.4) that are rapidly oxidized following disturbance (Arrouays and Pelissier, 1994; Six et al., 1999; DeGryze et al., 2004).

In 2002 this aboveground C consisted of litter (142 ± 30 g C m^{-2}) and plant biomass (228 ± 11 g C m^{-2}). In agricultural systems, Lupwayi et al. (2004) found that incorporation of wheat litter with conventional tillage increased its decomposition rate by 48%; Burgess et al. (2002) found that litter decomposition rates at 20 cm were 52 - 105%

greater than those at the soil surface. Light fraction pools have been shown to be correlated with soil surface respiration rates (Janzen et al., 1992; Alvarez and Alvarez, 2000) and its depletion represents a major portion of C loss in cultivated soils (Cambardella and Elliott, 1992, 1994).

Slow recovery of the plant community following cultivation suggests that soil C turnover rather than increased root respiration accounts for the additional CO_2-C emissions. It generally took about four weeks for significant plant recovery to occur. During this time, heterotrophic respiration will have accounted for most of the CO_2 flux in the tilled plots while in the control plots autotrophic respiration may have produced 50% or more of the measured CO_2 (Hanson et al., 2000). Our inability to detect changes in total soil C is a common finding in short-term C loss studies due to the need to detect relatively small changes in soil C against large and spatially heterogeneous background pools (Sollins et al., 1999; Brye et al., 2002).

Soil Moisture and Temperature

Analysis of covariance showed that soil moisture and temperature were related to CO_2 flux differently in different years. In 2002, the significant soil moisture by treatment interaction resulted primarily from differential responses to low and high gravimetric soil water contents; specifically, at soil moisture contents less than 20 %, CO_2-C emissions were an average of 84% higher in cultivated sites (2.64 vs. 4.88 g C m^{-2} d^{-1}) whereas at higher soil moisture contents the CO_2-flux difference was only 21% (5.84 vs. 7.08 g C m^{-2} d^{-1}). These differences may be due to a greater influence of soil moisture on C availability in uncultivated sites. Soil moisture can stimulate C and N mineralization by

enhancing aggregate turnover (Denef et al., 2001), by accelerating diffusion of active C compounds (Borken et al., 2003), and by increasing lysis of microbial cells and the release of intra-cellular solutes (Fierer et al., 2002). These processes may have been important sources of C following wetting of undisturbed soils; in cultivated sites, however, increased C availability associated with aggregate destruction and changing SOM pool sizes following tillage may have elevated emissions in drier soils. Kessavalou et al. (1998) similarly found that wetting effects varied with disturbance intensity. They experimentally increased soil moisture content with 5.1 cm of water in a wheat-fallow cropping system and found that between 24 and 72 h after wetting increases in CO_2 emissions averaged 109% for subtill, 82% for no-till, and 24% for plowing treatments.

A significant temperature effect on CO_2 emissions suggests that measured increases in the average temperature of cultivated plots in 2003 (16.9 vs. 19.7 °C) and 2004 (16.2 vs. 18.4) contributed to increased CO_2 fluxes. Other studies have also shown that decomposition and CO_2 flux are related to soil temperature (e.g. Wagai et al., 1998; Inoue et al., 2004; Knorr et al., 2005). Davidson et al. (2000) argue that changes in soil temperature should not be viewed in isolation but rather in conjunction with changes in other controls over SOM. In our experiment, it is likely that SOM pools became more susceptible to the effects of warming after cultivation because of aggregate destruction and decreases in the physical protection of labile C pools. Although some studies addressing the effects of soil warming on CO_2 emissions indicate that soil respiration responses to increased temperature may decrease over time (Kirschbaum , 2000; Luo et al., 2001) or that only certain pools of C are susceptible to mineralization after soil warming (Davidson et al., 2000), the long-term effects of soil warming on resistant C

pools that represent the majority of SOM are difficult to infer from field experiments lasting only a few years (Knorr et al., 2005; Powlson, 2005).

N_2O fluxes and N Availability

N_2O fluxes did not increase in 2002 until 34 d after cultivation, likely due to the lack of adequate NO_3^- and moisture: not until then did cultivated soils exhibit both a high soil moisture content (>20%) and substantially elevated soil NO_3^- concentrations (Figures 3.1, 3.5). In 2003 and 2004 N_2O fluxes also were highest on sampling days with high soil NO_3^- concentrations. Pinto et al. (2004) similarly found that N_2O fluxes following cultivation of a perennial pasture increased synchronously with increases in soil NO_3^- following an initial lag period.

In these unfertilized soils, changes in nitrogen availability may be a particularly important control over N_2O emissions via denitrification. Low NO_3^- concentrations in our control soils suggest a tight coupling of plant N uptake and the microbial processes of N-mineralization and nitrification. High NO_3^- concentrations in the cultivated sites indicate that soil disturbance disrupted the synchrony between inorganic N production and consumption. Accelerated SOM mineralization and increased nitrifier enzyme activity likely enhanced NO_3^- production and in all three years soil NO_3^- concentrations and N_2O fluxes declined as vegetation re-established, likely due to plant N uptake. Additional changes in the synchrony between nitrogen availability and plant demand, including the use of supplemental N fertilizer or conversion to annual crops, will likely lead to additional N_2O emissions.

The effects of tillage on soil surface N_2O fluxes have been primarily studied following conversion of long-term, conventionally tilled cropping systems to no-till (Grant et al., 2004; Six et al., 2004; Mackenzie et al., 1997). These studies demonstrate the potential for changes in soil water content, pore space structure, nitrogen cycling, and plant productivity following adoption of no-till to modify denitrification rates and N_2O emissions (MacKenzie et al., 1997, 1998; Baggs et al., 2003). The results we present here demonstrate the potential for sizeable N_2O-N losses following cultivation of no-till ecosystems and corroborate measurements by Pinto et al. (2004) showing increases in N_2O emissions over 5 d of between 1.8 and 23 mg N_2O-N m^{-2} following the plowing of a perennial pasture.

Low N_2O emissions in the spring and fall of 2003 and 2004 suggest that we captured those seasonal periods with the highest flux, however, some studies have reported that winter and early spring N_2O emissions can be important components of the total annual budget and that spring thaw emissions, in particular, may be among the highest of the year (Flessa et al., 1995; Kammann et al., 1998). Previous winter and early spring N_2O sampling campaigns near our study site on the same soil type, however, have found low or undetectable emissions (Robertson, unpublished data). Laboratory experiments have demonstrated that unfrozen, super-cooled water films around clay particles can support denitrification at temperatures as low as -2 to -4°C (Dorland and Beauchamp, 1991; Kopenen et al., 2004). In our soils, with clay contents generally < 20% (Crum and Collins, 1995), the availability of unfrozen water to support denitrification in frozen soils may limit winter denitrification.

CONCLUSIONS

Overall our results illustrate the rapid and destabilizing effect of cultivation on C and N cycling in a soil at pre-cultivation levels of native C. Following a single tillage event, 19% of the soil was present as aggregates in the 2000-8000 μm size class, identical to adjacent agricultural fields on the same soil type tilled for > 50 y, compared to 34% in control plots. Aggregate destruction directly released light-fraction organic matter from within intra-aggregate microsites and limited the incorporation of particulate organic matter originating from aboveground C pools into aggregates. These processes increased substrate availability that, along with changes in temperature, contributed to higher mineralization rates. As a result, cultivated plots lost an additional 1.4, 1.9 and 1.0 g C m^{-2} d^{-1} in 2002, 2003 and 2004, respectively, equivalent to 84 g C m^{-2} over the 60 d following cultivation in 2002, 260 g C m^{-2} over the 138 d sampling in 2003, and 198 g C m^{-2} over 2004's 198 d sampling period. Increased mineralization rates also increased nitrifier enzyme activity and soil NO_3^--N, which approached 15 μg g^{-1} in cultivated plots. Cultivation increased N_2O emissions by 672% in 2002, 210% in 2003, and 570% in 2004. Based on IPCC calculations using a 100-y time horizon for N_2O, these emission differences are equivalent to a 3-year average of 35.7 g CO_2-C m^{-2}, which is similar to the average annual C gain under no-till systems in the U.S. Midwest. Our results demonstrate that successional communities at pre-cultivation levels of native C experience rapid increases in ecosystem C and N cycling immediately following cultivation. This acceleration translates into substantive destabilization of soil C and N stocks and dramatic increases in trace gas fluxes, suggesting that policies designed to promote soil C sequestration need to protect soils from even periodic plowing.

Table 3.1. Cultivation effects on the distribution of soil organic matter in 2002 (DOY 236) and 2003 (DOY 294) to 20 cm. Values are means (n = 4) with standard errors in parentheses.[†]

			Particulate organic matter density (g cm^{-3})			
		Mineral-associated C	> 1.9	1.6 - 1.9	1.3 - 1.6	< 1.3
		g C m^{-2}				
2002	Control	3160 (89.59)	856.1 (42.07)	107.8 (9.49)*	148.5 (17.41)*	83.52 (7.46)*
	Cultivated	3316 (92.19)	849.3 (56.20)	163.4 (6.44)	219.9 (19.12)	146.8 (11.76)
2003	Control	2858 (528.0)	1039 (239.1)	138.0 (14.69)	222.8 (18.24)	127.3 (17.56)
	Cultivated	3366 (331.6)	834.9 (95.60)	153.5 (5.86)	290.6 (25.59)	167.0 (15.01)

* Indicates significant differences between control and cultivated plots within a year and SOM fraction.

[†] There were no differences in total soil C to 20 cm between control and cultivated plots in 2002 (4.36 ± 0.05 vs. 4.70 ± 0.15 kg m^{-2}), 2003 (4.38 ± 0.28 vs. 4.81 ± 0.26 kg m^{-2}), or 2004 (4.32 ± 0.09 vs. 4.74 ± 0.32 kg m^{-2}).

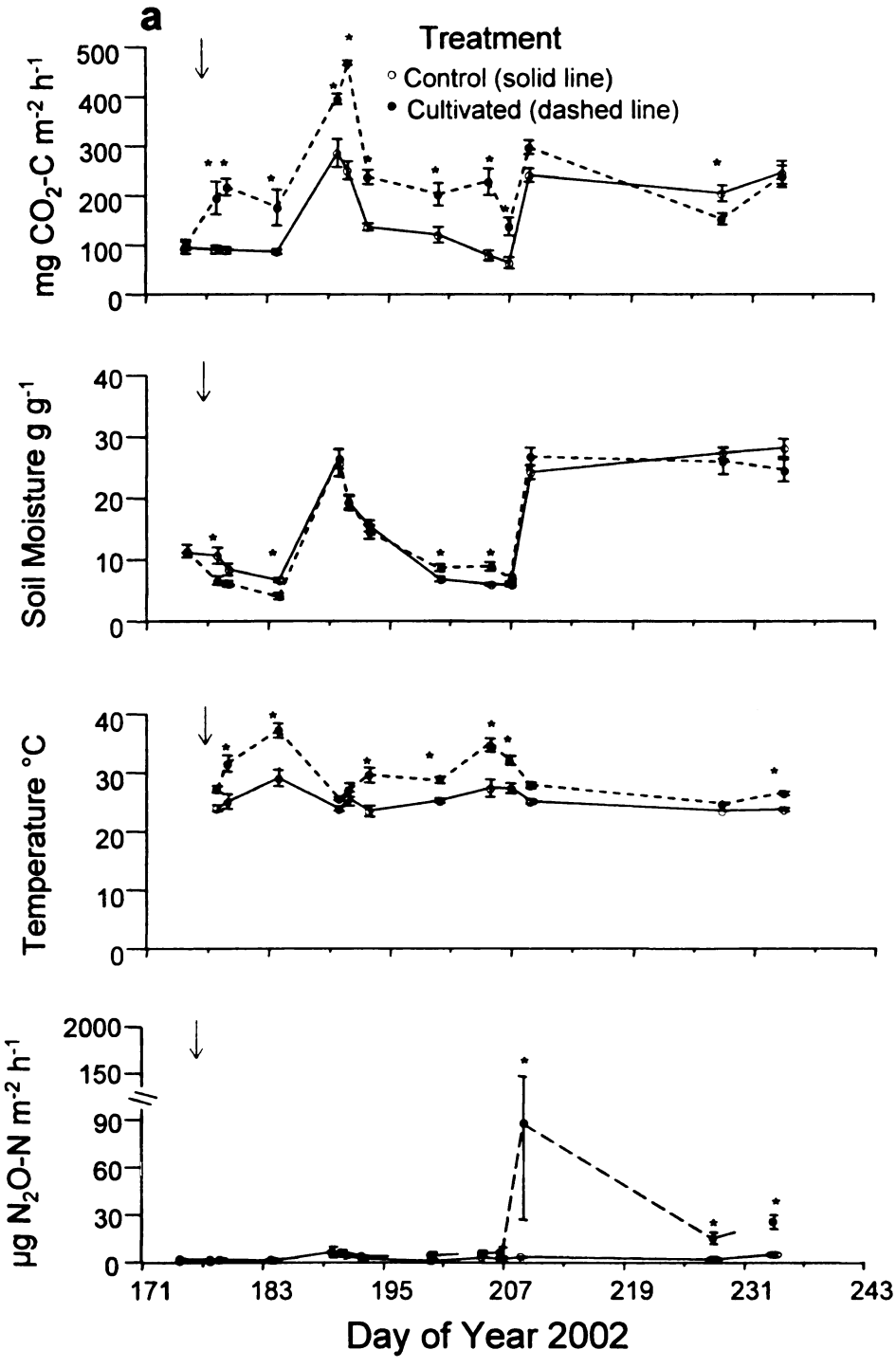

Figure 3.1. Changes in soil CO_2 flux, moisture, temperature, and N2O flux following cultivation of a previously never tilled soil in 2002. Measurements were made in 2002 (a; this page), 2003 (b; following page), and 2004 (c; two pages ahead). Arrows indicate cultivation dates. Note different x-axis ranges in each year, reflecting different durations of sampling. Soil temperature and moisture samples were determined for the 0-7 cm depth. Treatment means are shown ± standard error (n = 4); * indicates statistically significant ($P < 0.05$) differences between control and cultivated treatments within a single day of year (DOY) where there was a significant treatment by DOY interaction.

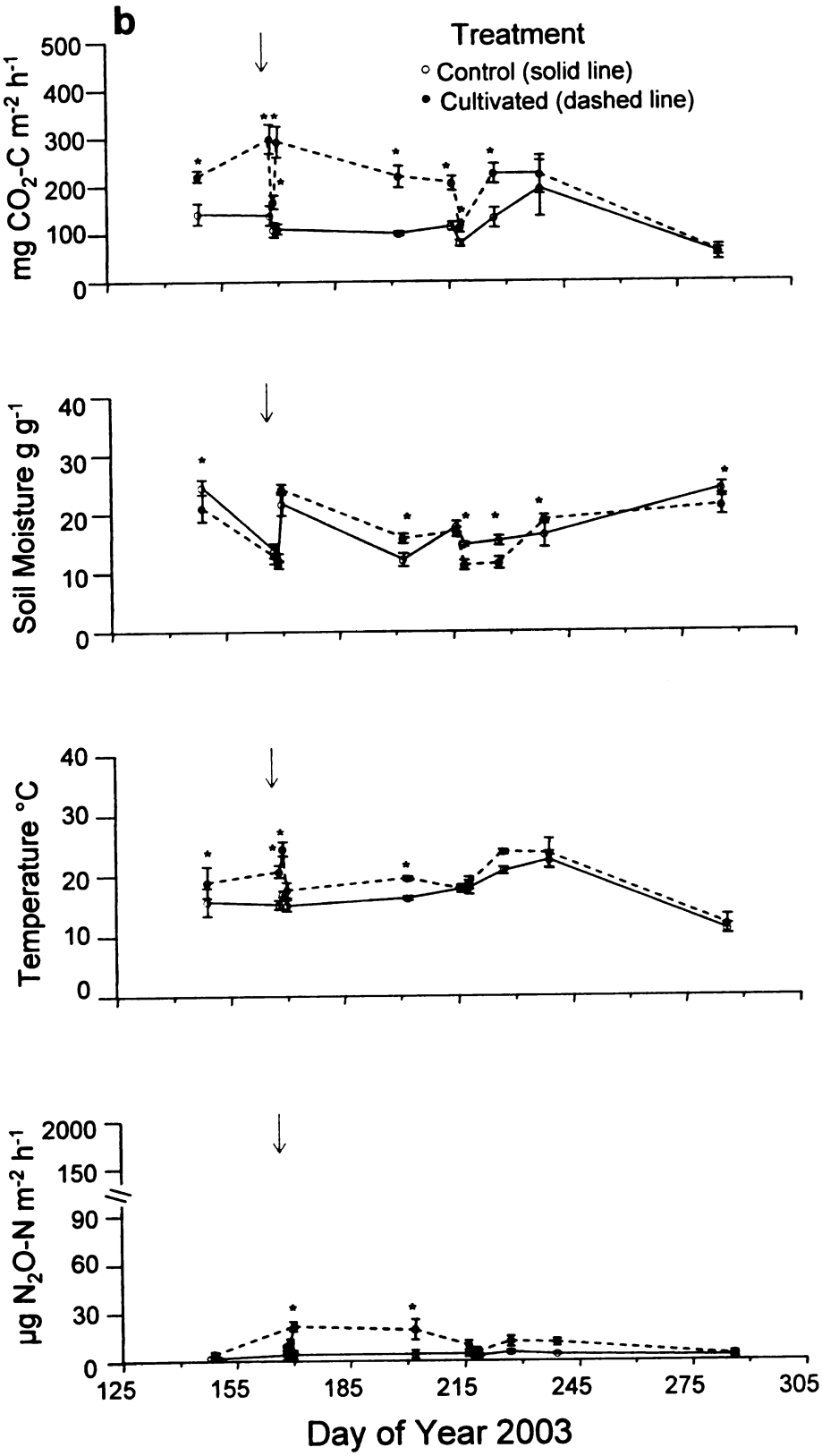

Figure 3.1. Cnt'd

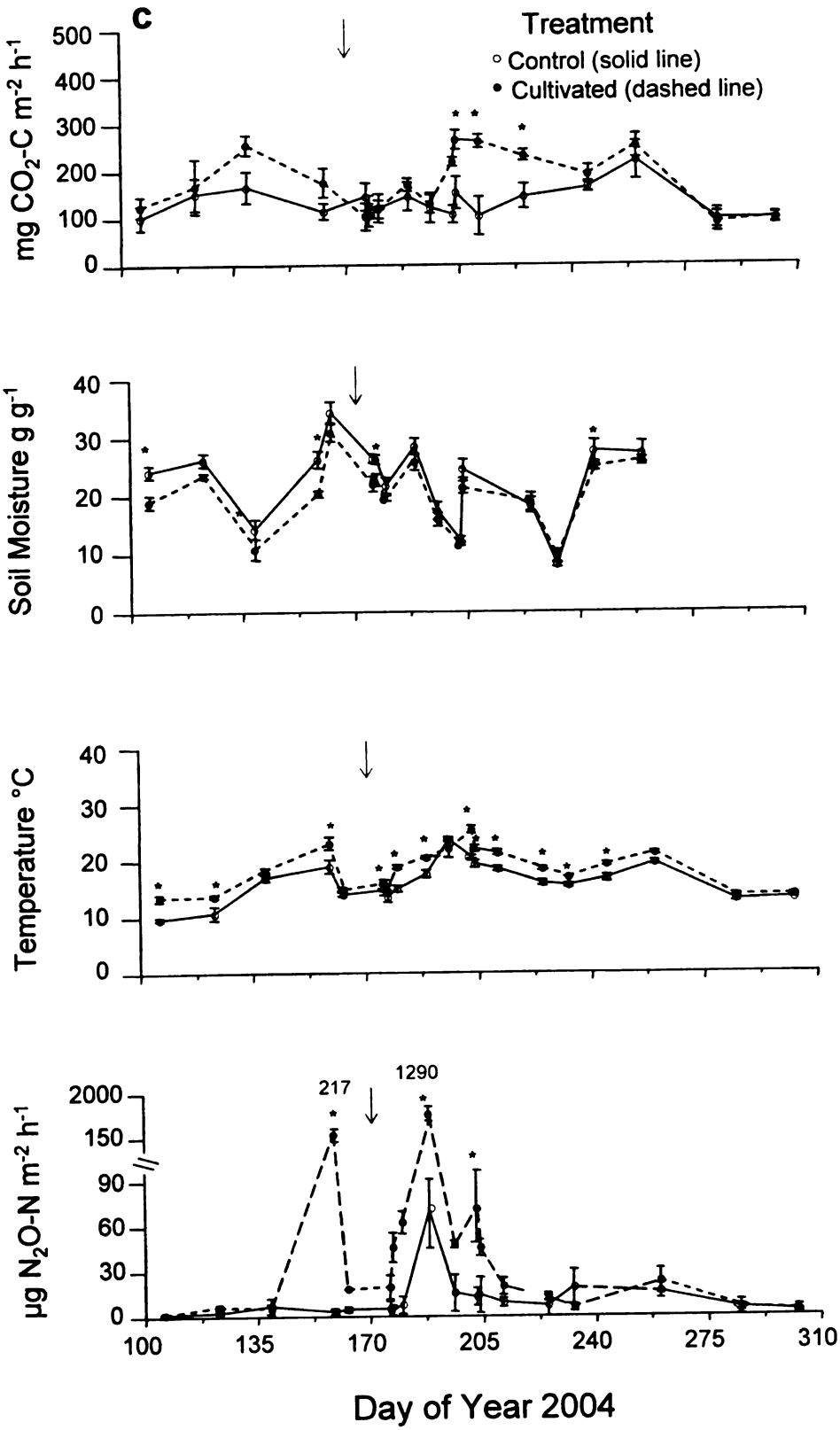

Figure 3.1. Cnt'd

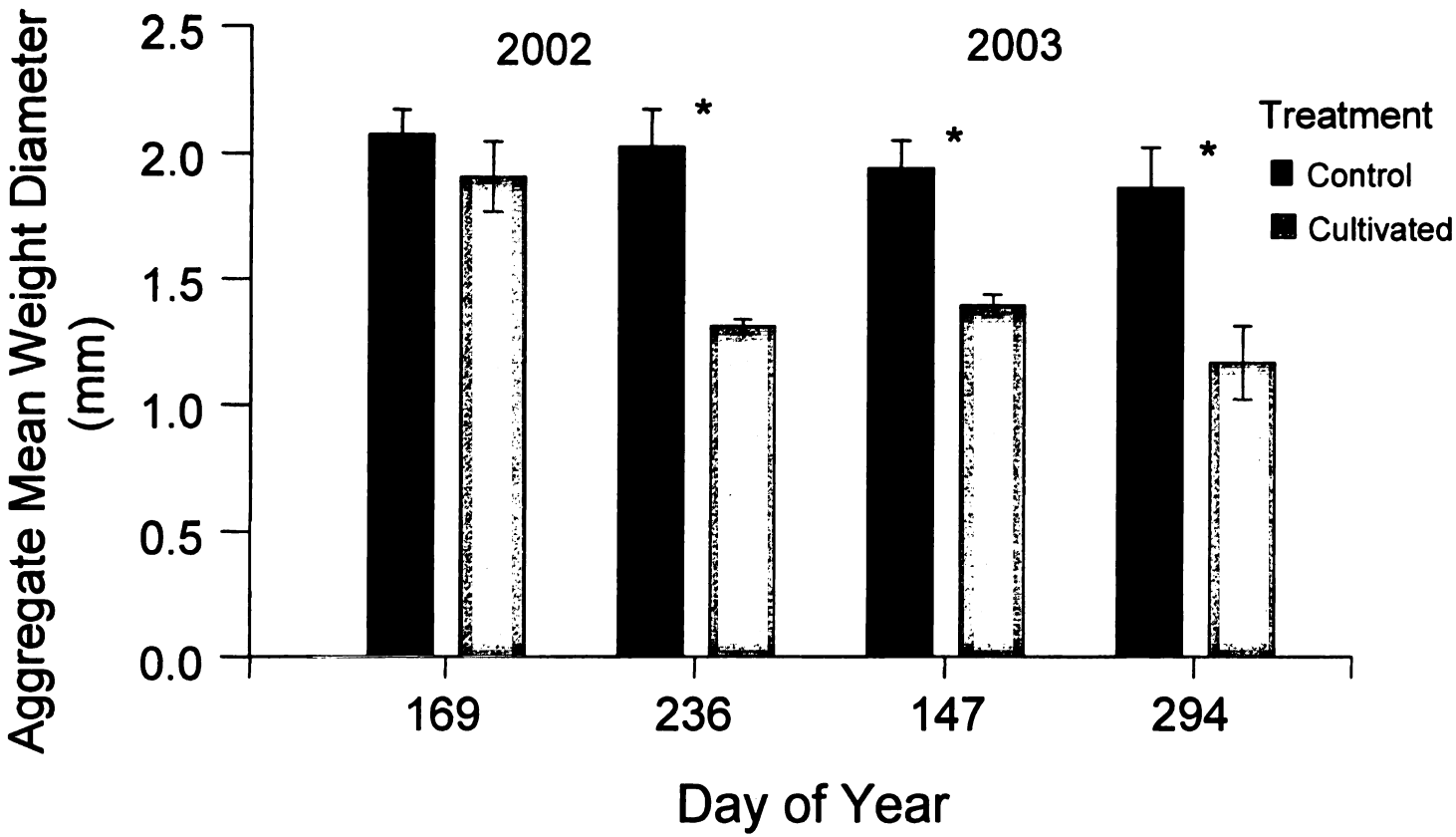

Figure 3.2. Mean weight diameter (WMD) of soil aggregates following cultivation of a previously uncultivated soil. Tillage occurred on day of year (DOY) 176 in 2002 and DOY 166 in 2003. Aggregation was determined for the 0-20 cm depth. Treatment means are shown ± standard error (n = 4); * indicates statistically significant ($P < 0.05$) differences between control and cultivated treatments within a single DOY.

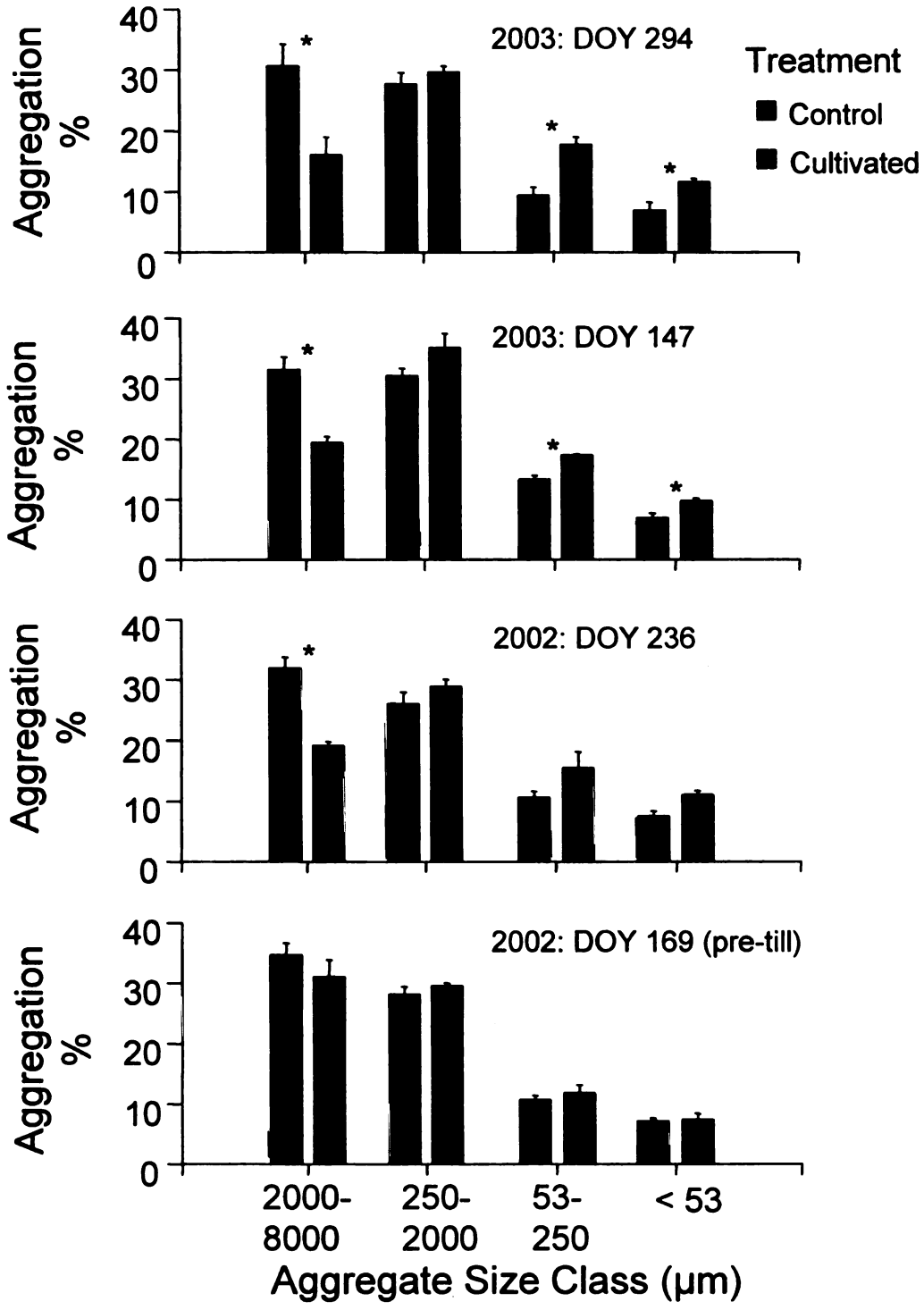

Figure 3.3. Soil aggregate distribution in four size classes. Black bars represent control plots; patterned bars, cultivated plots. Tillage occurred on day of year (DOY) 176 in 2002 and DOY 166 in 2003. Aggregation was determined for the 0-20 cm depth. Treatment means are shown ± standard error (n = 4); * indicates statistically significant ($P < 0.05$) differences between control and cultivated treatments within a size class and DOY

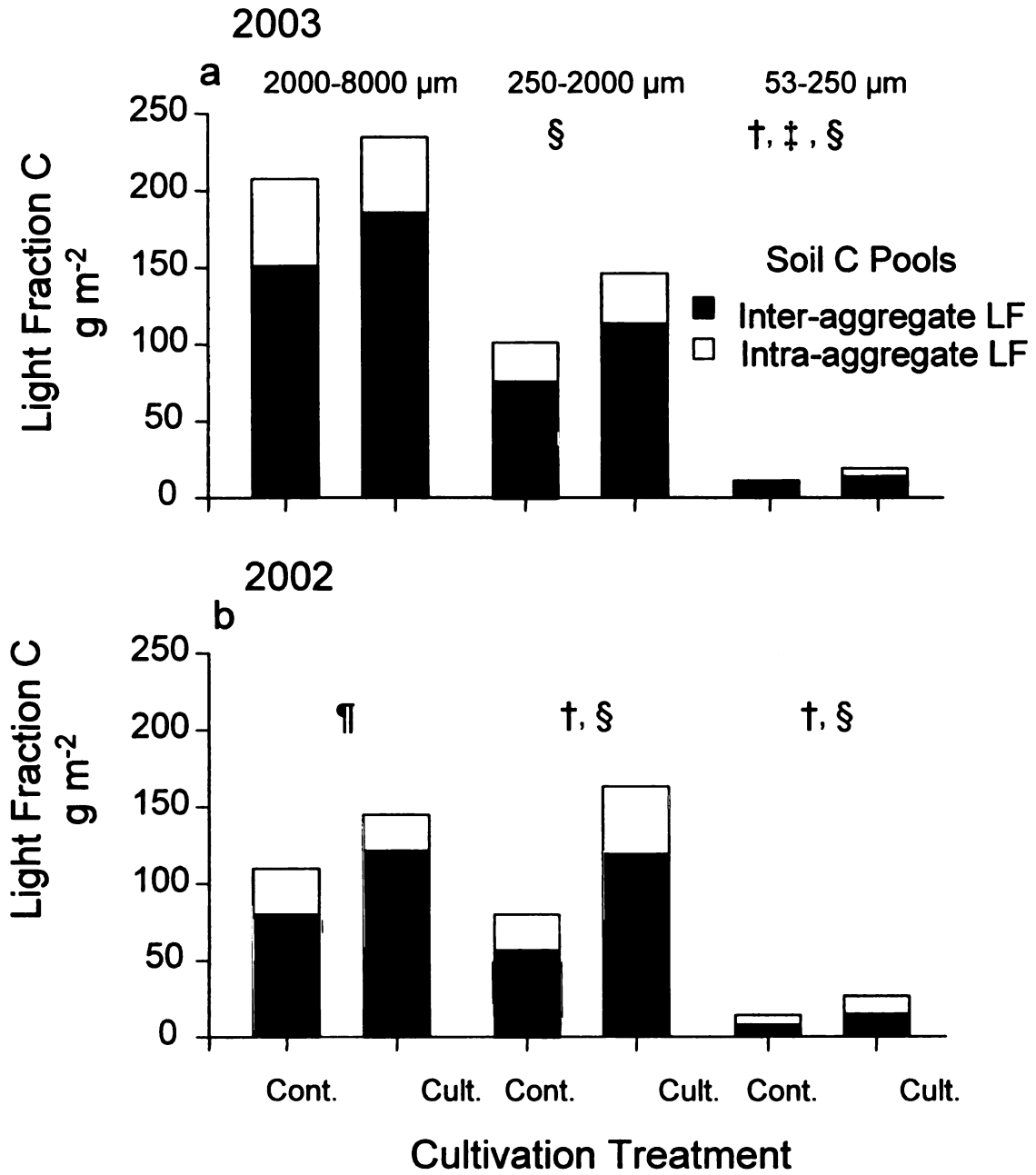

Figure 3.4. Distribution of inter- and intra-aggregate light fraction organic matter (LF) in control and cultivated plots in 2002 and 2003. Samples were collected to a depth of 20 cm on 24 August 2002, day of year (DOY) 236 and 15 June 2003 (DOY 166) and wet-sieved into aggregate size classes. †, ‡, § and ¶ indicate significant differences ($P<0.05$) within an aggregate size class between cultivation treatments for inter-aggregate LF, intra-aggregate LF, total LF and the proportion of total LF within aggregates, respectively. In 2003 there was trend towards a decreased proportion of intra-aggregate LF ($p<0.054$) in 2000 – 8000 µm aggregates.

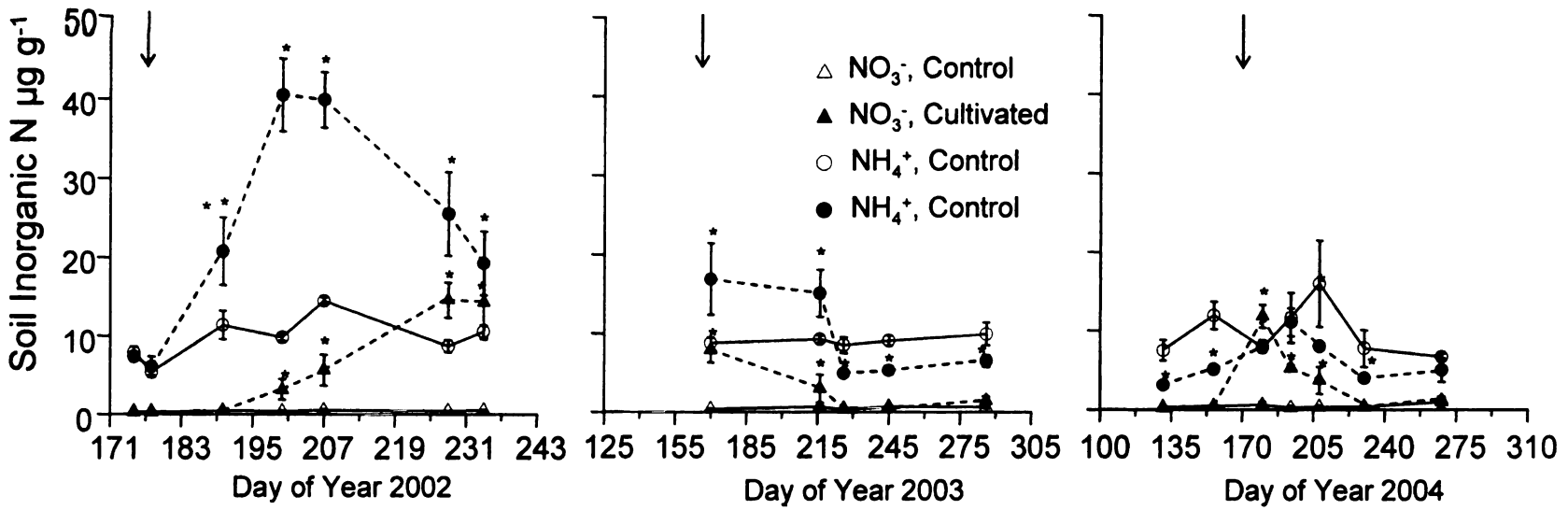

Figure 3.5. Changes in inorganic N following initial cultivation. Arrows indicate cultivation dates for 2002 (left panel), 2003 (middle panel), and 2004 (right panel). Inorganic N was determined for the 0-7 cm depth. Treatment means are shown ± standard error (n = 4); * indicates statistically significant ($P < 0.05$) differences between control and cultivated treatments within a day of year (DOY) where there was a significant treatment by DOY interaction.

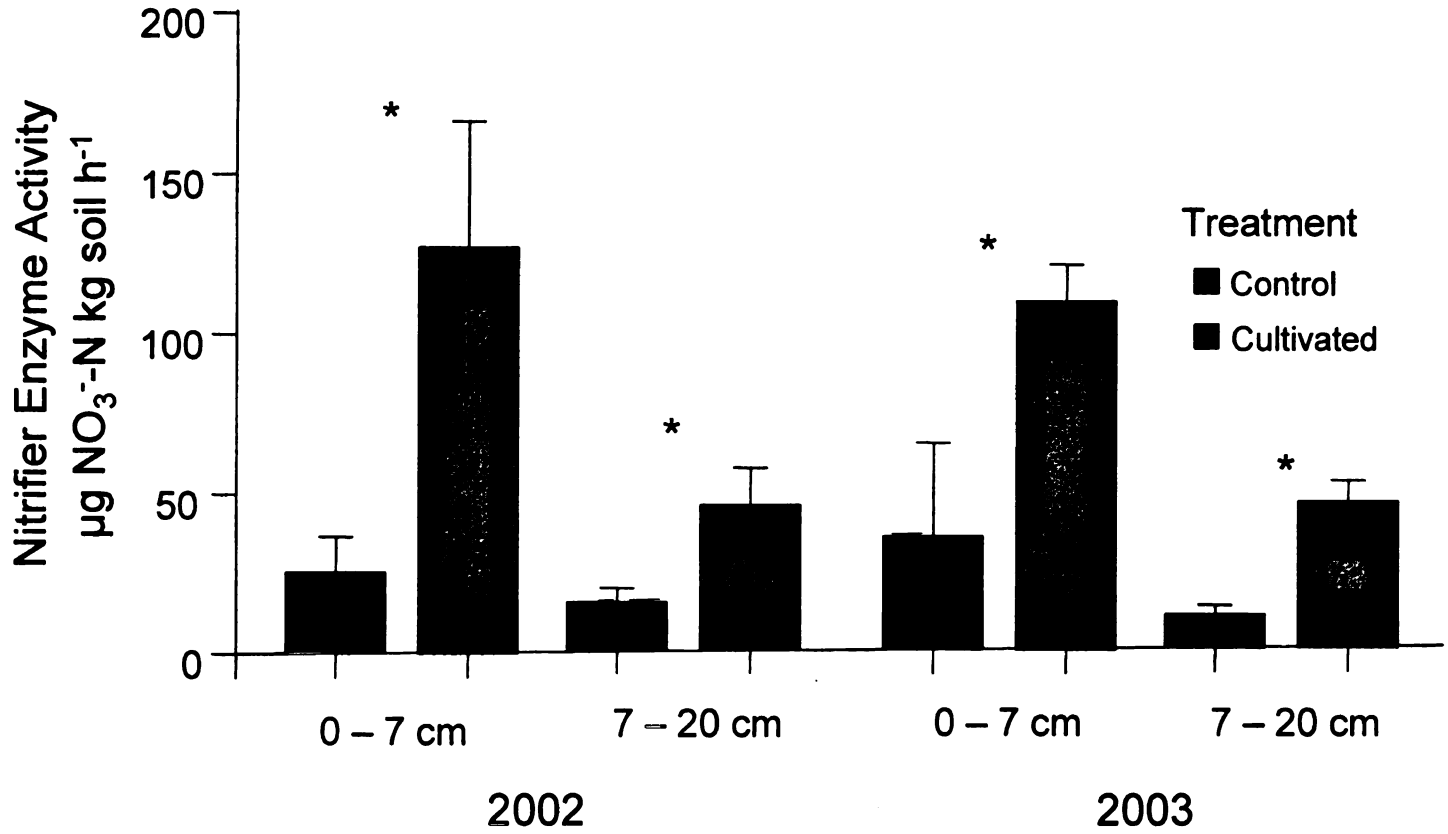

Figure 3.6. Changes in nitrifier enzyme activity following cultivation on 13 October 2003 (DOY 286) and 17 August 2004 (DOY 230). Treatment means are shown ± standard error (n = 4); * indicates statistically significant ($P < 0.05$) differences between control and cultivated treatments within a day of year (DOY) where there was a significant treatment by DOY interactions.

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CHAPTER 4

Changes in Aggregate-Protected C, Inorganic Nitrogen and Enzymes Following Initial Tillage of an Undisturbed Soil Profile

ABSTRACT

Understanding the short-term effects of tillage on the persistence of soil organic matter following years or decades of no-till is critical to developing C conservation strategies. We annually plowed replicated plots in a previously uncultivated midsuccessional soil between 2002 and 2004 and investigated changes in aggregation, soil organic matter dynamics, inorganic N, and N enzyme activities. Within 60 d of initial cultivation, soil aggregates in the 2000-8000 μm size class at 0-7 cm depth declined to levels indistinguishable from those in an adjacent agricultural soil cultivated for >50 y. Inter-aggregate, unprotected light fraction (LF) increased following cultivation, as did particulate C in soil fractions with densities < 1.9 g cm^{-3}. Nitrifier enzyme activity in 2003 was 5-fold higher in cultivated than in uncultivated treatments (127 vs. 25.4 μg N kg^{-1} soil h^{-1}) to 7 cm soil depth and 3-fold higher at 7 – 20 cm depth (45.2 vs. 14.9 vs. μg N kg^{-1} soil h^{-1}). Both nitrification and denitrification were stimulated by tillage in 2004. Changes in the mass of total soil C were not detectable over the 3 years of this study but there was significant vertical homogenization of C within the profile across all soil C pools. Our study demonstrates that plowing once immediately and substantially alters aggregation, the vertical distribution of C in the soil profile, light-fraction and particulate C dynamics, and N-cycle enzyme activities. The effects of even

occasional cultivation in no-till soils may thus be detrimental to soil structure and C stocks. Results highlight the value of permanent no-till.

INTRODUCTION

Changes in soil organic matter (SOM) and other soil properties following the conversion of native ecosystems to agriculture are well known. SOM declines, C is redistributed between surface and subsurface horizons and is released from protected microsites (e.g. Del Gado et al., 2003; DeGryze et al., 2004). Accompanying changes include increased soil temperature, modified trace gas fluxes (e.g. Smith et al., 2001; Smith and Conen, 2004), and altered microbial community structure and function (e.g. Cavigelli and Robertson, 2000; Buckley and Schmidt, 2001), among others. Typically SOM declines over time to 30-60% of original values (e.g. Davidson and Ackerman, 1993; Buyanovsky et al., 1997; West and Post, 2002; Lal, 2003). In tropical ecosystems change occurs rapidly, within months of initial cultivation (Houghton et al., 1985; Detwiler, 1986; Brown and Lugo, 1990). In temperate ecosystems it is more difficult to generalize because data for the first 10 y of cultivation are not currently available (Davidson and Ackerman, 1993; West and Post, 2002; Miller et al., 2004).

Ultimately, net greenhouse gas mitigation by soil C storage depends on the persistence of stored SOM. But an important challenge to sequester C in soils is the vulnerability of stored carbon to oxidation following intermittent tillage, a common practice in U.S. soils under no-till management (Paustian et al., 2000; Lal, 2004; Pacala and Socolow, 2004). Theory and a handful of relevant studies suggest that immediate C losses from temperate region soils may be less dramatic than from tropical ecosystems

(c.f. Bowman et al. 1990). Tiessen and Stewart (1983) found no difference four years after cultivating a silt loam. Other authors (e.g. Pierce et al., 1994; VandenBygaart and Kay, 2004) have also failed to find an effect within the first few years of initial cultivation, perhaps because of detection difficulties: short-term total soil C changes are notoriously difficult to detect because of high background C concentrations in most arable soils, spatial variability, and the redistribution of above-ground pools upon cultivation (Sollins et al., 1999). Consequently, soil C permanence may be better predicted by changes in more readily detected factors known to affect C storage such as soil aggregation, the distribution of C in the soil profile, the dynamics of specific SOM fractions in soil, and enzyme activities that reflect soil microbial activity.

Macroaggregates (>250 μm soil particles) may be especially good predictors because of their importance for protecting recently deposited, labile organic matter (Angers and Giroux 1996; Jastrow 1996). Isotope studies and ^{13}C CP/MAS NMR spectroscopy have demonstrated that newly incorporated crop residues, root derived carbon, and young SOM are found in macroaggregates (Golchin et al. 1994; Six et al. 1998; Gale et al. 2000a; 2000b). In ecosystems with frequent soil disturbance, accelerated turnover rates of macroaggregates limit the physical stabilization of labile SOM compounds such as particulate C (SOM > 53 μm). In a recent study Grandy and Robertson (2005, in review) demonstrated C protection within 2000-8000 μm aggregates reduces its turnover rate by as much as 50%.

In this paper we investigate the short-term effects of tillage on soil physical and microbial processes that control C and N turnover and persistence. We observed aggregation, inter and intra-aggregate LF pools, particulate organic matter, and nitrifier

and denitrifier enzyme activities in a previously uncultivated mid-successional community subjected to three years of cultivation. By studying a previously undisturbed soil profile and leaving the site fallow following cultivation we are able to isolate and measure cultivation effects independent of changes in plant community composition and prior cultivation.

MATERIALS AND METHODS

Site Description and Experimental Design

Our experimental site was a previously never-tilled field at the W.K. Kellogg Biological Station (KBS) Long-Term Ecological Research site in southwest Michigan, USA (42° 24' latitude, 85° 24' longitude). In 1956 the site was cleared of trees and thereby converted from a northern hardwood forest to a midsuccessional grassland community that has since been mowed each fall, with mown biomass left in place. Soils at the site are Kalamazoo (fine-loamy) and Oshtemo (coarse-loamy) mixed, mesic, Typic Hapludalfs developed on glacial outwash (Crum and Collins, 1995). The two series co-occur at KBS with variation within a series often as great as variation between series. Prior to initial cultivation, soils contained 31.8 (± 1.4) g C kg^{-1} and 2.59 (± 0.11) g N kg^{-1}, and soil pH averaged 5.93 (± 0.11); the same soil C levels had been measured 3 years and 12 years prior to this study and are statistically indistinguishable from C levels in nearby undisturbed forests on the same soil types (Robertson et al., 2000).

In 2002 we established in this field eight 3 x 6 m plots to which we assigned 4 replicates of two tillage treatments (cultivated and uncultivated control) in a randomized complete block design. Cultivated sites were mowed with biomass left in place prior to

cultivation to 19 cm. We used a moldboard plow and a disc for primary and secondary cultivation, respectively. Cultivation occurred on 25 June 2002 (DOY 176), 15 June 2003 (DOY 166), and 20 June 2004 (DOY 172).

Soil and Plant Sampling

Samples for aggregate and total C and N analysis were collected prior to initial cultivation on 18 June 2002, and post-initial-cultivation on 24 August 2002, 21 October 2003, and 24 September 2004. An additional sample was taken on 27 May 2003 for aggregate analysis. On each sample date, five 3.8 cm diameter soil cores were taken from each plot to a depth of 20 cm, placed in plastic bags, and refrigerated (< 7 d) prior to sieving through an 8 mm sieve and air-drying at 20 °C.

Plant and litter C estimates prior to initial cultivation were estimated by drying and analyzing litter and plant samples collected on 21 June 2002 from two 625 cm^{-2} quadrats in each plot. Changes in the litter layer after cultivation were estimated on 15 September 2003 from two 625 cm^{-2} quadrats in each of four adjacent plots cultivated for the first time in 2003. Organic C and total N concentrations of plant and soil samples were determined by dry combustion and gas chromatography in a CHNS analyzer (Costech ECS 4010, Costech Analytical Technologies, Valencia CA.).

Particulate SOM Density Distribution

Cultivation effects on the distribution of particulate SOM in four density fractions were determined using a sequential fractionation technique in sodium polytungstate (NAPT). Whole soil samples (15 g) were dispersed in 0.5% sodium hexametaphosphate

and poured through a 53 μm sieve. Particulate materials were sequentially fractionated in sodium polytungstate at four densities to separate POM into four density fractions: (> 1.9, 1.6 – 1.9, 1.3 – 1.6, < 1.3 g cm^{-3}). Clay plus silt associated SOM (< 53 μm) was determined by the difference between whole-soil C and total particulate C.

Aggregate and Light Fraction Separation

Aggregate distribution was determined by hand on triplicate 35 g air-dried soil samples by wet-sieving in water through a series of 2000 μm, 250 μm, and 53 μm sieves. Mean weight diameters (MWD) of sand-free aggregates were determined by calculating the sum of the products of the mean diameter of each size fraction and the proportion of the total sample weight in that fraction (Kemper and Rosenau, 1986).

The method we used to separate inter- and intra-aggregate light fraction (LF) is based on previously published protocols (Six et al., 1998; Gale et al., 2000). Aggregate subsamples (8 g) were pre-wetted with 4 mL H_2O prior to LF analysis to minimize aggregate slaking during LF separation. Aggregates were transferred from the membrane filters to 100 mL beakers after 16 h with 5 mL aliquots of NAPT at a density of 1.62 g cm^{-3}. A total of 55 mL NAPT was used for each sample. A preliminary test showed that the final density of the sodium polytungstate was 1.60 g cm^{-3} following equilibration with the water contained in aggregates.

After 24 h, inter-aggregate LF was aspirated from the surface of the sodium polytungstate and then rinsed with at least 600 mL DI H_2O. Aggregates were then dispersed to release the intra-aggregate LF using sodium hexametaphosphate and

resuspended in NAPT (d = 1.62 g cm^{-3}). The intra-aggregate LF was collected form the surface.

Inorganic Nitrogen Dynamics

NH_4^+ and NO_3^- were extracted with 1 M KCl from duplicate field-moist 10 g soil samples using a 1:5 soil/extractant ratio. Soil extracts were filtered with a syringe filter using a type A/E glass fiber filter (Pall Corporation, East Hills, NY). Filtrates were stored in 7 mL scintillation vials and frozen until analysis for NH_4^+ and NO_3^-. Both analyses were performed on an Alpkem 3550 Flow Injector Analyzer (OI Analytical, College Station, TX). Additionally, inorganic N was determined to a depth of 7-20 cm on three dates in 2003 and on all dates in 2004.

Nitrifier and Denitrifier Enzymes

We used a shaken slurry method (Hart et al., 1994) to test for changes in nitrifier enzyme activity to 0-7 and 7-20 cm soil depths following cultivation on 13 October 2003 (DOY 286) and 17 August 2004 (DOY 230). Briefly, 30 g field-moist soil was combined with 100 mL solution containing non-limiting quantities of NH_4^+ in a 160 mL jar capped with parafilm. The parafilm was perforated with a needle to allow rapid gas exchange while minimizing water loss. Jars were placed on a rotary shaker and rotated at 200 rpm. Each flask was sampled 4 times during a 28 h incubation period. Soil extracts were centrifuged, filtered, and frozen until analysis for NO_3^-.

Denitrifier enzyme activity was measured to 0-7 and 7-20 cm soil depths on soil samples collected on 17 August 2004 using methods described in Groffman et al. (1999).

We combined 10 g field-moist soil with a 10 mL solution consisting of $NaC_4H_4O_4$ (2 mM) and KNO_3^- (1mM) in 160 mL serum vials. We capped these vials with rubber septa and flushed them with N_2 five times over 10 min to create anaerobic conditions prior to adding acetylene (15%). Acetylene blocks the conversion of N_2O to N_2 so that N_2O measurements alone can be used to estimate total denitrification potential. High microsite substrate availability was maintained in the jars by keeping them on a rotary shaker (190 rpm) during the incubation. N_2O concentrations were determined on four 0.5 mL headspace samples collected every 12 min. We completed the entire analysis within 60 min of the substrate additions in order to reduce the potential for cell growth or reproduction to alter N_2O production during incubation (Tiedje, 1987; Luo et al., 1996). N_2O was analyzed using a gas chromatograph outfitted with a ^{63}Ni electron capture detector (350 °C).

Statistical Analysis

Soil aggregate size distributions and aggregate-associated C pools were corrected for sand content > 53 μm. Carbon content of soil organic matter fractions was calculated on an area basis by correcting for bulk density. Tillage effects on SOM pools and enzyme activities were analyzed by Proc Mixed (Version 8.2, SAS Institute, 1999) using a randomized complete block design analysis of variance (ANOVA). Treatment, soil depth and, in the case of C pools, SOM fraction were considered fixed effects and block a random effect.

Single degree of freedom comparisons were made to compare differences among soil fractions and treatments at different depths using the LSD statistic to calculate a 95%

confidence interval around the differences between means generated using the diff option in Proc Mixed. The LSD was carried out using the PDMIX800 algorithm (Saxton, 1998). Soil inorganic N concentrations were analyzed by Proc Mixed using a randomized complete block design analysis of variance (ANOVA) with repeated measures (SAS Version 8.2, SAS Institute, 1999). Treatment and sampling date were considered fixed effects and block a random effect. Where there were significant day of year by treatment interactions, differences between treatments on separate days were determined using the slicing command in SAS.

RESULTS

Total Soil C and N

On 24 August 2002, 60 d after initial tillage, there were significant changes in the depth distribution of soil C and N (Table 4.1). C and N concentrations in tilled plots were significantly lower than those in conventional systems at 0-7 cm but greater at 7-20 cm. Expressed on an areal basis, soil C and N showed the same trend, despite tilled plots having a slightly higher bulk density at 0-7 cm and lower bulk density at 7-20 cm. These changes in soil C and N generally persisted in 2003 and 2004 although differences between C concentrations at 7-20 cm were not significantly different (Table 4.1). Expressed on an areal basis, total soil C at 0-20 cm was not different between treatments in any year.

Aggregation

Prior to tillage in June 2002 there were no differences in soil aggregation between treatments (Figures 4.1, 4.2). Within 60 d, tillage decreased 2000-8000 μm aggregates at both soil depths and increased the amount of soil in the 53-250 μm and <53 μm size classes at 0-7 cm. 2000-8000 μm aggregates declined from 0.47 to 0.15 g g^{-1}. These changes persisted over the winter and into the following spring when control plots had 0.40 g g^{-1} in the 2000-8000 μm size class and tilled plots had 0.24 g g^{-1}. Differences in 2000-8000 μm aggregates at both depths were also evident in October 2003 but not in September 2004. Tillage increased soil in size classes < 250 μm at 0-7 cm throughout the experiment although there were generally no differences in these classes at 7-20 cm. Shifts in soil from 2000-8000 μm size classes into smaller sizes following cultivation are also evident from statistical comparisons among size classes within treatments. Control plots generally contained greater soil in macroaggregate than microaggregate classes, particularly at 0-7 cm, whereas in tilled plots the 53-250 μm class contained amounts of soil frequently equal to that in the 250-2000 μm class and higher than that in the 2000-8000 μm class.

Particulate and Aggregate-associated C

Initial cultivation had significant effects on the distribution of SOM in different particulate organic matter POM density fractions and soil depths (Table 4.2). On 24 August 2002 cultivation reduced SOM in clay fractions and POM > 1.9 g at 0-7 cm and increased all POM classes and clay-associated SOM at 7-20 cm. Cultivation effects on POM distribution were similar in 2003 and 2004 although they were not as consistent across density fractions. Treatment differences were related to a homogenization of C

availability at 0-20 cm due to cultivation. In control plots, there were significant differences in all POM fractions and clay associated C at all sampling dates after tillage (except the 1.3 – 1.6 g cm^{-3} in 2004); in contrast, the tilled plots had few differences in C concentrations between depths. At 0-20 cm POM increased in densities < 1.9 g cm^{-3} while there were no increases in the clay fractions or POM pools > 1.9 g cm^{-3} (Table 4.2).

Tillage changed the depth distribution of C associated with aggregate size fractions (Figure 4.3) and increased inter-aggregate light fraction C in the 2000-8000 μm class at 0-7 and 7-20 cm in 2002 and also in the 250-2000 μm class at 7-20 cm in 2002 (Figure 4.4). In 2003, tillage increased inter-aggregate LF C in the 250-2000 μm class at 0-7 cm and LF C in the 2000-8000 μm class at 7-20 cm. Similar to whole soil C, there was a redistribution of inter-aggregate and intra-aggregate LF in the soil profile following cultivation. Intra-aggregate LF concentrations were generally indistinguishable among treatments; however, tillage increased intra-aggregate LF in the 250-2000 μm class in 2002 and the 2000-8000 μm class in 2003 (Figure 4.5). Tillage, soil fraction and depth effects on C/N ratios are presented in Table 4.3.

Inorganic Soil N and Enzymes

Cultivation in 2002 increased extractable soil NO_3^- concentrations at 0-7 cm to 3.18 μg NO_3^--N g^{-1} from 0.41 μg NO_3^--N g^{-1} in control plots after 24 d (Figure 4.6). NO_3^- concentrations in cultivated plots remained higher on DOY 207, 228 and 234 and peaked on DOY 228 (14.6 vs. 0.44 μg NO_3^--N g^{-1}). NH_4^+ concentrations were also increased by cultivation on DOY 190 (87%), 200 (312%), 207 (176%), 228 (193%), and 234 (82%). No data are available for 7-20 cm in 2002.

In 2003 cultivation significantly increased NO_3^- concentrations on DOY 170 and 216 but there were no differences on the other sampling dates (Figure 4.6). NH_4^+ concentrations were significantly greater in cultivated plots on DOY 170 and 216 but lower in these plots relative to control plots on the following three sampling dates. At 7-20 cm there were no differences in NO_3^- or NH_4^+ between treatments on the three dates for which data are available. In 2004 NO_3^- concentrations on DOY 180 were 11.8 μg NO_3^--N g^{-1} in cultivated plots and 0.55 μg NO_3^--N g^{-1} in control plots. NO_3^- concentrations remained higher in cultivated plots on DOY 194 and 208. NH_4^+ concentrations were reduced by cultivation on four of seven sampling dates in 2004. At 7-20 cm, results were similar but treatment differences were smaller in magnitude. Tillage increased NO_3^- concentrations on DOY 180, 194, 208, 230, and 268. The greatest differences occurred on DOY 180 when tilled plots had 6.09 and control plots 1.40 μg NO_3^--N g^{-1}. Tillage increased NH_4^+ concentrations on DOY 194 (Figure 4.6).

N Enzyme Activity

Nitrifier enzyme activity in 2003 (Figure 4.7) was 5-fold higher in cultivated than in uncultivated treatments (127 vs. 25.4 μg N kg^{-1} soil h^{-1}) to 7 cm soil depth and 3-fold higher at 7 – 20 cm depth (45.2 vs. 14.9 μg N kg^{-1} soil h^{-1}). Similar trends were observed in 2004 when cultivated plots had higher nitrifier enzyme activity at 0 - 7 cm (109 vs. 35.5 μg N kg^{-1} soil h^{-1}) and 7 - 20 cm depths (45.7 vs. 10.5 μg N kg^{-1} soil h^{-1}). Denitrifier enzyme activity (Figure 4.8) was similar at 0-7 cm depth; at 7-20 cm depth tillage increased DEA from 15.6 to 153 μg N_2O-N kg^{-1} h^{-1}.

DISCUSSION

Soil Aggregates

Changes in soil aggregates were immediate, significant, and persistent following initial cultivation (Figure 4.1). Within 60 d of cultivation, soil in the 2000-8000 μm size class at 0-7 cm soil (Figure 4.2) declined to levels indistinguishable from those in an adjacent agricultural soil cultivated for >50 y (Grandy and Robertson, 2005 in review). Cultivation also led to fewer aggregates in this size class at 7-20 cm depth, demonstrating that declines at the soil surface were not solely due to a redistribution of aggregates to increased soil depths.

Many prior studies have demonstrated that long-term cultivation reduces soil aggregation (e.g. Gupta and Germida, 1988; Jastrow et al., 1996; Mikha and Rice, 2004). DeGryze et al. (2004) reported that at 0-7 cm soil depth, soil aggregate mean weight diameter (MWD) in conventionally managed agricultural soils was ca. 3-fold lower than in early successional, afforested, and native forest ecosystems; at 7-25 cm depth reductions in MWD were also significant and due largely to declines in aggregates > 2000 μm. Six et al. (2000) showed that cultivation reduced aggregation in four soils ranging in texture from sandy loam to silty clay loam. Our results show that tillage immediately destroys macroaggregates and that these declines in soil structure persist throughout the growing season and following winter.

Increased turnover rates of macroaggregates following cultivation are due to the direct effects of tillage and also to changes in annual plant cover and long-term changes in soil C and microbial activity. Fungal hyphae and fine-root networks contribute to macroaggregate stability (Bethlenfalvay et al., 1994). These networks are particularly

susceptible to disruption following disturbance (Jansa et al., 2003). Bare soil surfaces following tillage expose aggregates to the direct impacts of rainfall and make them more susceptible to slaking and dispersion following rapid wetting (Kooistra and van Noordwijk, 1996). A combination of direct and indirect effects of tillage likely contributed to the rapid declines in soil structure at our site.

The persistence of cultivation effects on soil structure was evident prior to tillage in 2003 when aggregation in cultivated sites remained substantially lower (Figures 4.1, 4.2), despite the potential for root growth and freeze-thaw and wet-dry cycles to have affected aggregation in both tillage systems. Plant roots can increase aggregation by enmeshing small particles into stable macroaggregates; by supplying organic substrates such as root hairs, sloughed cells and mucilage; and by influencing soil moisture content (Reid and Gross, 1982; Perfect et al., 1990). Freeze-thaw and wetting drying cycles can directly break down aggregates but also influence structure by changing the proximity and alignment of mineral and organic particles to facilitate aggregation (Kemper and Rosenau, 1986; Rasiah et al., 1992; Denef et al., 2001). The direct and indirect destructive effects of tillage were apparently greater than these other forces that serve to equalize aggregation in the two treatments.

Soil Organic Matter

The vertical homogenization of soil C and N in the profile following cultivation is consistent with results from other studies (Tables 4.1, 4.2). VandenBygaart and Kay (2004) studied the effects of moldboard plowing soils in three different textural classes (sandy clay loam, silty clay loam, and sandy loam) after 22 yr of no-till management. In

each of these textural classes they found that soil C declined at 0-5 cm and increased at lower depths with the net result being no change in total soil C. Kettler et al. (2000) studied the effects of moldboard plowing a soil that had been in a no-till wheat-fallow system. Five yr after plowing there remained differences in SOM stratification to 30 cm but no differences in total soil C. Pierce et al. (1994) found in Michigan that plowing fields in no-till for 6 to 7 yr redistributed C to 150 mm depth and that this effect persisted for 4 to 5 yr after plowing although there were no changes in total soil C. We similarly found changes in SOM stratification and that this homogenization occurred across SOM pools of different densities.

After three years of annual tillage we were still unable to detect differences in total soil C. Rates of SOM loss from temperate ecosystems immediately following cultivation have rarely been studied and the available data are inconclusive. Bowman et al. (1990), for example, found 40% organic C losses within three years of cultivation (15.7 vs. 9.4 kg m^{-3}) and that between three and twenty years after cultivation there was little additional C loss. These rapid rates of soil C change are unusual and may have been due to their sandy soil texture (Davidson and Ackerman, 1993; West and Post, 2002) as well as changes in vegetation that accompanied tillage. Substantial decreases in litter inputs associated with agricultural conversion may also have contributed to the C losses as vegetation changed from short-grass steppe to an unfertilized wheat-fallow rotation. Tiessen and Stewart (1983) found no change in soil C concentration after four years of cultivating a previously no-till silt loam soil. The high silt (49%) and clay (20 %) contents of these soils may have increased their protective capacity and made them more resistant to the destructive effects of cultivation (Wander and Bidart, 2000).

Despite the lack of change in total soil C we measured significant changes in POM pools to 20 cm and also in inter-aggregate SOM (Table 4.2; Figure 4.2). Collectively, these changes suggest increases in organic matter availability. POM concentrations and quantity to 20 cm in 2002 increased in fractions < 1.9 g cm^{-3} while in 2004 changes occurred in fractions <1.6 g cm^{-3}. Light fraction pools have been shown to be correlated with soil surface respiration rates (Janzen et al., 1992; Alvarez et al., 2000) and its depletion represents a major portion of C loss in cultivated soils (Cambardella and Elliott, 1992, 1994). We measured no changes in clay fractions or in POM fractions with a density > 1.9 g cm^{-3}. Both these fractions tend to be more stable than LF POM (Golchin et al., 1994). Heavy fraction SOM is considered both more chemically resistant to decomposition (recalcitrant) and more physically protected than LF (Swanston et al., 2000). Decreased physical protection of SOM is also suggested by the increases in inter-aggregate LF at both 0-7 at 7-20 cm. The biomass incorporated with tillage thus moved primarily into POM pools with low densities and inter-aggregate light fraction pools. In 2002 this aboveground C consisted of litter (142 ± 30 g C m^{-2}) and plant biomass (228 ± 11 g C m^{-2}).

Soil N Pools and Enzymatic Activity

Long-term cultivation changes microbial community structure and function (Cavigelli and Robertson, 2000; Steenwerth et al., 2002). Buckley and Schmidt (2001) quantified the effects of plant community composition, fertilization, tillage and historical cultivation on microbial community structure at the KBS LTER. They found that changes in crop management and recent variations (7 yr) in tillage intensity had no

influence on microbial community structure although agricultural systems differed from the never-tilled midsuccessional community examined in our study. Further, a 50-yr-old historically-tilled midsuccessional community had similar microbial community structure to annual and perennial cropping systems, demonstrating the persistent effects of agricultural management on microbial communities. The persistent effects of agricultural management on microbes was also demonstrated by Compton and Boone (2000) who found that nitrification rates remained higher in agricultural than in undisturbed soils more than a century after agricultural abandonment. Our study demonstrates that tillage effects on microbial processes begin to occur immediately after cultivation (Figures 4.7, 4.8). We found changes in nitrifier enzyme activity the year following initial tillage (2003) and continued to detect differences due to tillage in 2004, when we were also able to detect differences in denitrifier enzyme activity. Increased nitrifier enzyme activities likely contributed to the greatly increased soil NO_3^- concentrations as did the slow recovery of the plant community following tillage. It is also likely that increased denitrifier enzyme activities accelerated N_2O fluxes, which increased 6-fold in 2002, 2-fold in 2003 and 6-fold in 2004 (Grandy and Robertson, 2005, in review).

Other studies have also demonstrated immediate changes in microbial processes following soil disturbance. Calderón and Jackson (2000) demonstrated that sieving can alter microbial biomass C and phospholipid fatty acid abundance and distribution within 2 d of cultivation. In a follow-up study, Calderón et al. (2001) showed in the field that tillage of a soil used for vegetable production changed denitrification rates and phospholipid fatty acid profiles, indicating that microbial communities may have changed.

Tillage thus has both immediate and persistent effects on microorganisms. One explanation for this is that soil properties influencing microbes respond rapidly to cultivation and also do not recover quickly. Changes in the spatial distribution and protection of resources may be critical. Our results show that vertical homogenization of soil C occurs after one plowing. Other studies have demonstrated that the spatial distribution of resources responds rapidly to cultivation and may take decades to recover (Robertson et al., 1988; 1997). Further the relocation of resources to deeper in the soil profile may influence long-term decomposition dynamics and microbial processes. In agricultural systems, Lupwayi et al. (2004) found that incorporation of wheat litter with conventional tillage increased its decomposition rate by 48%; Burgess et al. (2002) found that litter decomposition rates at 20 cm were 52 - 105% greater than those at the soil surface.

Implications for Soil management

Marland et al. (2001) identified soil C permanence as the fundamental challenge to C sequestration. Although tillage is the major threat to stored soil C, most farmers for agronomic reasons periodically cultivate otherwise no-till fields. Rather than focussing on total soil C responses, which can be extremely hard to detect in short-term studies, we measured changes in the mechanisms underlying C and N cycling to infer potential long-term changes. We show immediate reductions in the physical protection of C, homogenization of C in the soil profile and changes in microbial processes following cultivation of a previously uncultivated soil. These changes drastically increased CO_2 fluxes (1.0-1.9 g CO_2-C m^{-2} d^{-1} over three growing seasons; Grandy and Robertson, 2005, in review) and over a longer time this enhanced CO2 efflux would likely lead to

detectable C losses. These results and recent research demonstrating that long-term no-till cropping can reduce soil N_2O emissions (Six et al., 2004) with no significant ecological or yield tradeoffs (Grandy and Robertson, 2005, in review) highlight the need to maintain no-till management. Efforts should be made in specific regions to identify and overcome the agronomic limitations to no-till associated with different soil types.

CONCLUSIONS

We tilled a previously uncultivated field to investigate the immediate effects of tillage on aggregation, SOM dynamics, and nitrifer and denitrifier enzyme activities. By choosing a previously undisturbed soil profile and leaving the site fallow following cultivation we were able to isolate and measure the effects of cultivation without the confounding effects of changes in plant communities or prior cultivation. Results include:

1) Within 60 d of cultivation, soil in the 2000-8000 μm size class at 0-7 cm declined to levels indistinguishable from those in an adjacent agricultural soil cultivated for >50 y (Grandy and Robertson, 2005, in review). These changes were persistent.
2) Inter-aggregate, unprotected LF increased as a result of cultivation.
3) Nitrifier enzyme activity one year after initial cultivation was 5-fold higher in cultivated than uncultivated treatments (25.4 vs. 127 μg N kg^{-1} soil h^{-1}) to 7 cm soil depth and 3-fold higher at 7 – 20 cm depth (14.9 vs. 45.2 μg N kg^{-1} soil h^{-1}). In the following year there were significant tillage effects on both nitrifier and denitrifier activity.

4) Total soil C changes were not detectable by the end of year 3 but there was significant vertical homogenization of C within the profile.

Our results demonstrate that soil C and N are immediately influenced by cultivation and that these changes are persistent through the growing season and subsequent winter.

Table 4.1. Tillage effects on soil C and N and bulk density at 0-7 and 7-20 cm depths. Initial cultivation occurred on 25 June 2002 and again in the spring of 2003 and 2004 on the same plots.[†‡]

		24 August 2002					21 October 2003					24 September 2004				
		C	N	Bulk Density	C	N	C	N	Bulk Density	C	N	C	N	Bulk Density	C	N
		%	%	g cm^{-3}	kg m^{-2}	kg m^{-2}	%	%	g cm^{-3}	kg m^{-2}	kg m^{-2}	%	%	g cm^{-3}	kg m^{-2}	kg m^{-2}
0-7 cm	control	3.21a	0.27a	1.06d	2.38b	0.20b	3.17a	0.23	1.06c	2.33b	0.17b	3.13a	0.26a	0.92b	2.01b	0.17bc
	tilled	2.34b	0.21b	1.15c	1.89c	0.17c	2.42b	0.19	1.13c	1.90b	0.15b	1.84b	0.16b	1.33a	1.71b	0.15c
7-20 cm	control	1.09d	0.10c	1.40a	1.98c	0.19bc	1.09c	0.09	1.45a	2.05b	0.18b	1.26c	0.12c	1.42a	2.30b	0.21b
	tilled	1.66c	0.15d	1.30b	2.80a	0.25a	1.71bc	0.14	1.31b	2.91a	0.24a	1.67b	0.15bc	1.41a	3.03a	0.27a

ANOVA F-Tests

Source	P values														
Tillage	0.103	0.199	0.989	0.114	0.182	0.788	0.978	0.242	0.247	0.254	0.063	0.015	0.002	0.323	0.202
Depth	0.001	0.001	0.001	0.026	0.005	0.002	0.001	0.001	0.064	0.011	0.001	0.001	0.001	0.003	0.001
Tillage* depth	0.001	0.001	0.001	0.001	0.004	0.017	0.050	0.005	0.005	0.022	0.001	0.002	0.001	0.032	0.046

[†]Means within a column followed by different letters are statistically different (P,).05) using an lsd test.
‡There were no differences in total soil C to 20 cm between control and cultivated plots in any year.

Table 4.2. Tillage and depth effects on the concentration of particulate organic matter (POM) in four density fractions and clay-associated C (<53 μm) on three sampling dates. Initial cultivation occurred on 25 June 2002 and again in the spring of 2003 and 2004 on the same plots.[‡]

		24 August 2002					21 October 2003					24 September 2004				
		Clay	>1.9	1.6-1.9	1.3-1.6	<1.3	Clay	>1.9	1.6-1.9	1.3-1.6	<1.3	Clay	>1.9	1.6-1.9	1.3-1.6	<1.3
		mg C g^{-1} soil														
0-7	Control	21.43*†a	8.06b*†	0.91†cd	1.08†c	0.64†d	22.10†a	5.66*†b	1.47†cd	1.59†c	0.85†d	25.41*†a	2.49†b	1.33*†b	1.32b	0.75†c
	Tilled	16.77†a	4.43†b	0.65c	0.80c	0.74c	16.67†a	3.21b	0.87d	1.61c	0.84d	13.77a	1.83†b	0.71d	1.29c	0.84cd
7-20	Control	8.67*a	1.42*b	0.23*c	0.38*c	0.20*c	6.54*a	3.31b	0.16c	0.56c	0.35c	11.12a	0.56b	0.25d	0.35*c	0.28cd
	Tilled	11.60a	2.93b	0.66cd	0.92c	0.52d	11.63a	3.41b	0.50c	0.96c	0.59c	13.83a	0.88bc	0.46d	0.98b	0.51cd
0-20	Control	12.35a	3.34b	0.42*d	0.58*c	0.33*d	11.02a	3.98b	0.53c	0.85c	0.49c	14.83a	1.05b	0.53cd	0.61*c	0.40*d
	Tilled	13.01a	3.41b	0.65d	0.88c	0.59d	13.38a	3.34b	0.62c	1.16c	0.68c	13.37a	1.19b	0.54c	1.08b	0.62c
		kg m^{-2}														
0-20	Control	3.16a	0.86b	0.11*cd	0.15*c	0.84*e	2.86a	1.04b	0.14d	0.22c	0.13b	3.67a	0.26b	0.13c	0.15*c	0.10*d
	Tilled	3.32a	0.85b	0.16d	0.22c	0.15d	3.37a	0.84b	0.15d	0.29c	0.17d	3.79a	0.33b	0.15c	0.30b	0.17c

*Indicates significant differences between tillage treatments within a depth, POM fraction, and sampling date.
†Indicates differences between depths within a treatment, POM fraction, and sampling date.
‡Means followed by different letters indicate significant differences ($p<0.05$) between POM fractions within a treatment, depth, and sampling date.

Table 4.3. Tillage, depth and organic matter fraction effects on C/N ratios of different soil fractions in 2002, 2003 and 2004. Initial cultivation occurred on 25 June 2002 and again in the spring of 2003 and 2004 on the same plots.[‡]

		24 August 2002				21 October 2003				24 September 2004			
		0-7		7-20		0-7		7-20		0-7		7-20	
Soil Fraction		Cont	Till	Cont	Till	Cont	Till	Cont	Till	Cont	Till	Cont	Till
Whole soil		11.79e	11.34f	10.60ef	11.39d	13.84de	12.75ef	11.53f	12.32de	12.10de	11.34g	10.80de	11.18gh
Aggregate	2000-8000	11.74e	12.94f	11.18ef	11.61d	12.90de	13.32ef	11.66f	11.63de	12.46de	12.32fg	11.65de	11.38gh
size class	250-2000	11.55e	11.87f	11.07ef	11.48d	12.16de	12.20ef	11.48f	11.67de	12.13de	11.65fg	11.19de	12.27gh
	53-250	11.50e	11.78f	9.97ef	10.91d	12.18de	11.71ef	10.06f	10.93e	11.24de	10.60g	9.74e	9.88h
	<53	8.68e	9.77f	7.57f	7.94d	10.27e	10.11f	7.86f	9.04e	10.31e	9.58g	8.12e	8.34h
Inter-POM	2000-8000	23.58*[†]d	30.12cd	30.91bcd	29.61bc	23.57[†]ab	25.32ab	29.54*cd	22.12c	22.82[†]ab	23.63[†]bc	37.23*a	29.18b
	250-2000	21.75d	26.62de	30.06[†]bcd	28.34bc	22.38[†]ab	21.17bc	31.94*bc	24.98c	23.43a	23.06bc	27.29b	26.20bc
	53-250	23.95d	22.42e	29.02bcd	25.61c	20.62[†]bc	19.32cd	25.07d	21.90c	15.44[†]cd	18.17de	20.62c	18.33ef
Intra-POM	2000-8000	30.62abc	32.02cd	28.39cd	32.32b	23.61[†]ab	23.98abc	29.69*cd	22.58c	25.65a	24.51b	27.84b	24.24bcd
	250-2000	24.93*[†]cd	41.30a	33.58abc	38.91a	25.67[†]ab	27.13[†]a	35.69b	32.72b	24.69a	22.55bcd	28.88*b	23.73cd
	53-250	35.37a	38.60ab	35.14ab	38.54a	21.79[†]ab	25.52[†]ab	34.47bc	31.86b	25.92[†]a	30.51[†]a	37.16a	35.41a
WS POM	Clay	10.67e	10.49f	9.49ef	10.21d	11.45de	11.28ef	7.81f	9.91e	11.24de	9.94g	10.21de	10.32h
	>1.9	13.18e	11.50f	15.50e	12.35d	21.62[†]b	22.15abc	34.83*bc	22.84c	15.59cd	16.12ef	14.68d	15.91fg
	1.6-1.9	20.79[†]d	22.01e	27.41d	26.09c	16.14cd	15.76de	17.77e	16.37d	18.42bc	19.56cde	20.21c	18.07f
	1.3-1.6	31.54*[†]ab	35.19abc	39.13*a	30.96bc	23.10[†]ab	21.00bc	32.20*bc	25.66c	23.24a	22.72bcd	27.29b	22.85cde
	<1.3	25.72[†]bcd	31.79cd	33.13abcd	27.80bc	26.83[†]a	26.41[†]a	45.76*a	39.62a	26.22a	23.47bc	29.35b	25.64bc

*Indicates significant differences between tillage treatments within a depth, soil fraction, and sampling date.

[†]Indicates differences between depths within a treatment, soil fraction, and sampling date.

[‡]Means followed by different letters within a column indicate significant differences ($p<0.05$) between soil fractions within a treatment, depth, and sampling date.

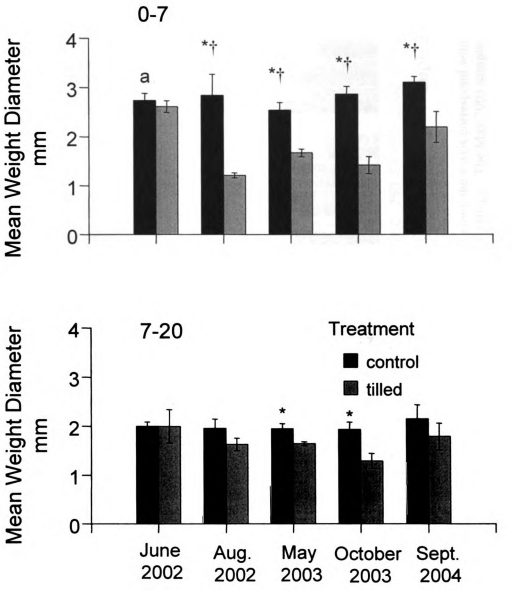

Figure 4.1. Mean weight diameter (MWD) of soil aggregates. The June 2002 sampling was done prior to cultivation. The May 2003 samples were taken prior to the second cultivation. Treatment means are shown ± standard error (n = 4); * indicates statistically significant ($P < 0.05$) differences between control and tilled treatments within a single sampling date. †Indicates significant differences between depths within a treatment and sampling date.

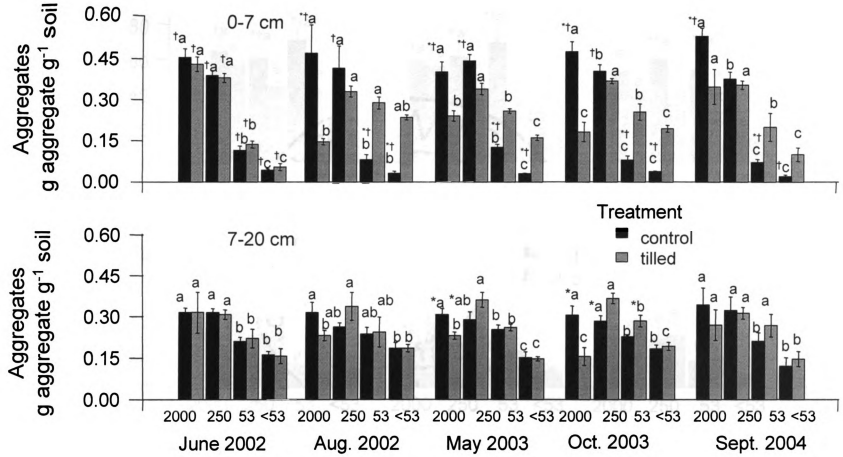

Figure 4.2. Tillage effects on the distribution of soil in four aggregate size classes. Numbers along the x-axis correspond with aggregate size: 2000 = 2000-8000 μm; 250 = 250 – 2000 μm; 53 = 53-250 μm; and <53 = 53-250 μm. The May 2003 samples were taken prior to the second cultivation. Treatment means are shown ± standard error (n = 4); * indicates statistically significant ($P < 0.05$) differences between control and tilled treatments within a single sampling date. †Indicates significant differences between depths within a treatment and sampling date. Size classes within a sampling date and treatment with different letters are significantly different.

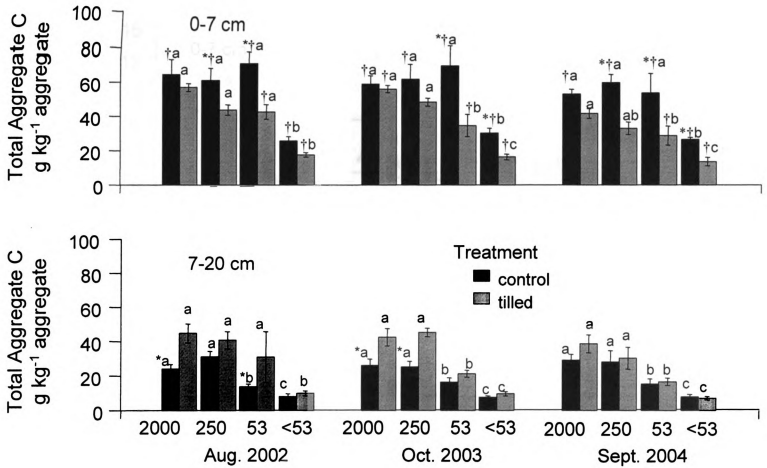

Figure 4.3. Tillage effects on total sand-free aggregate C in 2002, 2003 and 2004. Initial cultivation was in June 2002. Treatment means are shown ± standard error (n = 4); * indicates statistically significant ($P < 0.05$) differences between control and tilled treatments within a single sampling date. †Indicates significant differences between depths within a treatment and sampling date. Size classes within a sampling date and treatment with different letters are significantly different.

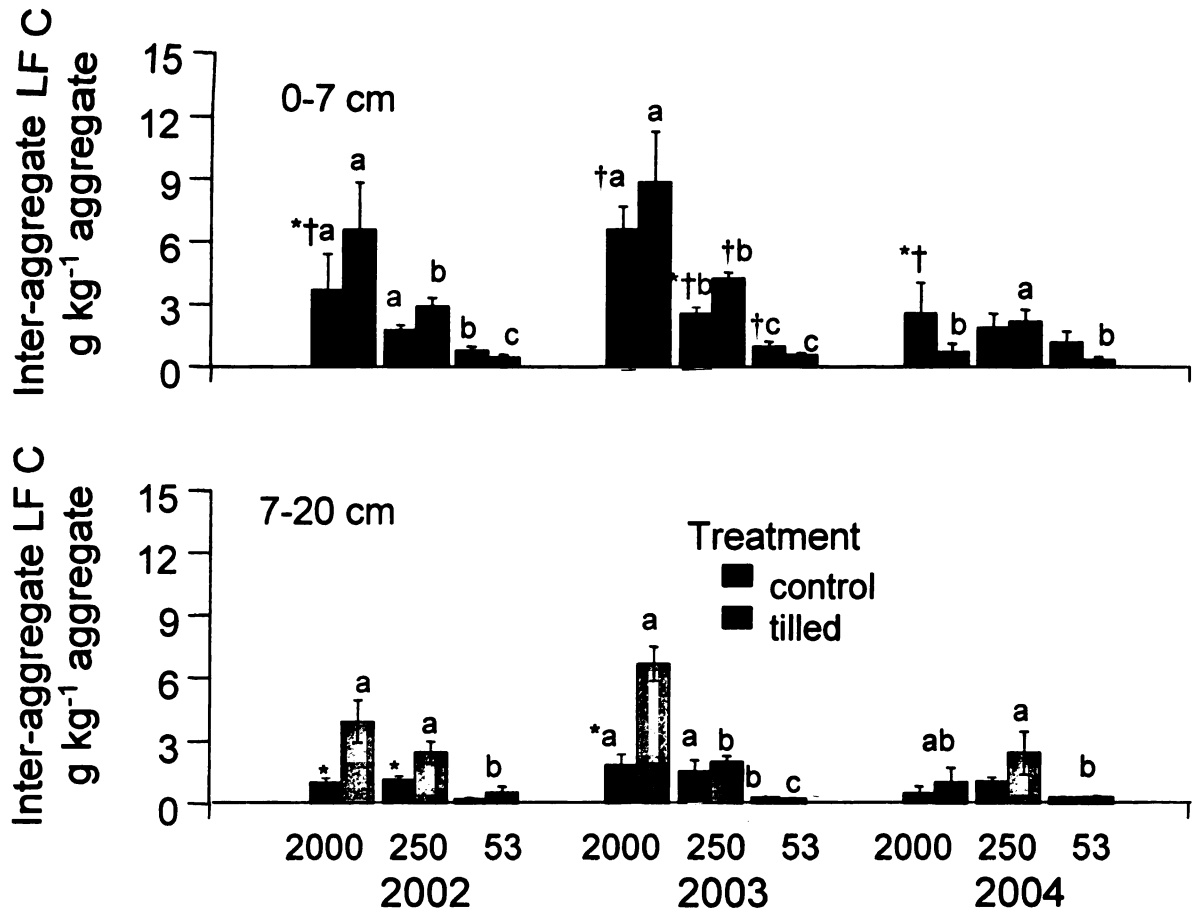

Figure 4.4. Tillage effects on the distribution of sand-free inter-aggregate light fraction organic matter in 2002, 2003 and 2004. Initial cultivation was in June 2002. Treatment means are shown ± standard error (n = 4); * indicates statistically significant ($P < 0.05$) differences between control and tilled treatments within a single sampling date. †Indicates significant differences between depths within a treatment and sampling date. Size classes within a sampling date and treatment with different letters are significantly different.

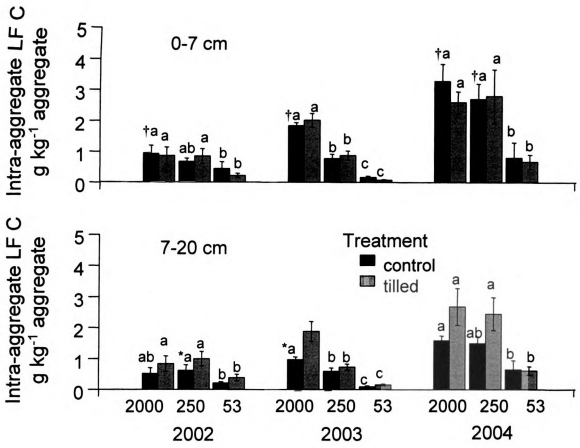

Figure 4.5. Tillage effects on the distribution of sand-free intra-aggregate light fraction organic matter in 2002, 2003 and 2004. Initial cultivation was in June 2002. Treatment means are shown ± standard error (n = 4); * indicates statistically significant ($P < 0.05$) differences between control and tilled treatments within a single sampling date. †Indicates significant differences between depths within a treatment and sampling date. Size classes within a sampling date and treatment with different letters are significantly different.

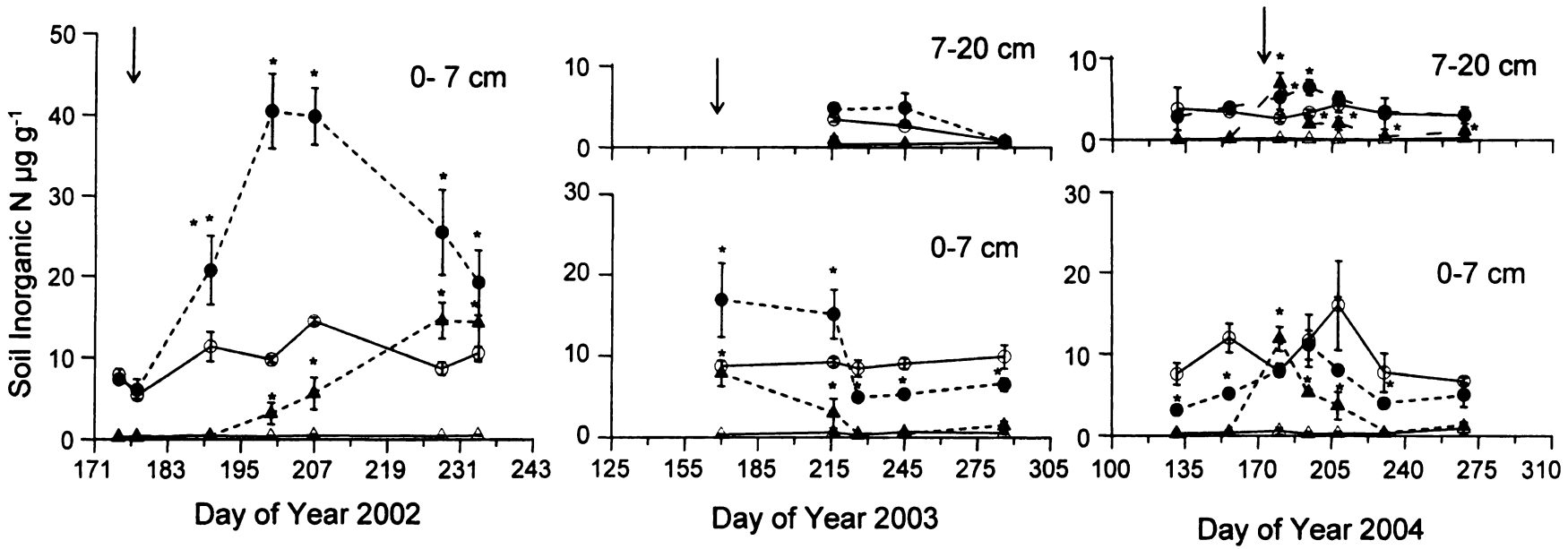

Figure 4.6. Changes in inorganic N following initial cultivation. NH_4^+ (circles) and NO_3^- (triangles) in control plots (open symbol, solid line) and cultivated plots (closed symbol, dashed line). Arrows indicate cultivation dates for 2002 (left panel), 2003 (middle panel), and 2004 (right panel). Inorganic N was determined for the 0-7 cm depth. Treatment means are shown ± standard error (n = 4); * indicates statistically significant ($P < 0.05$) differences between control and cultivated treatments within a day of year (DOY) and sampling depth.

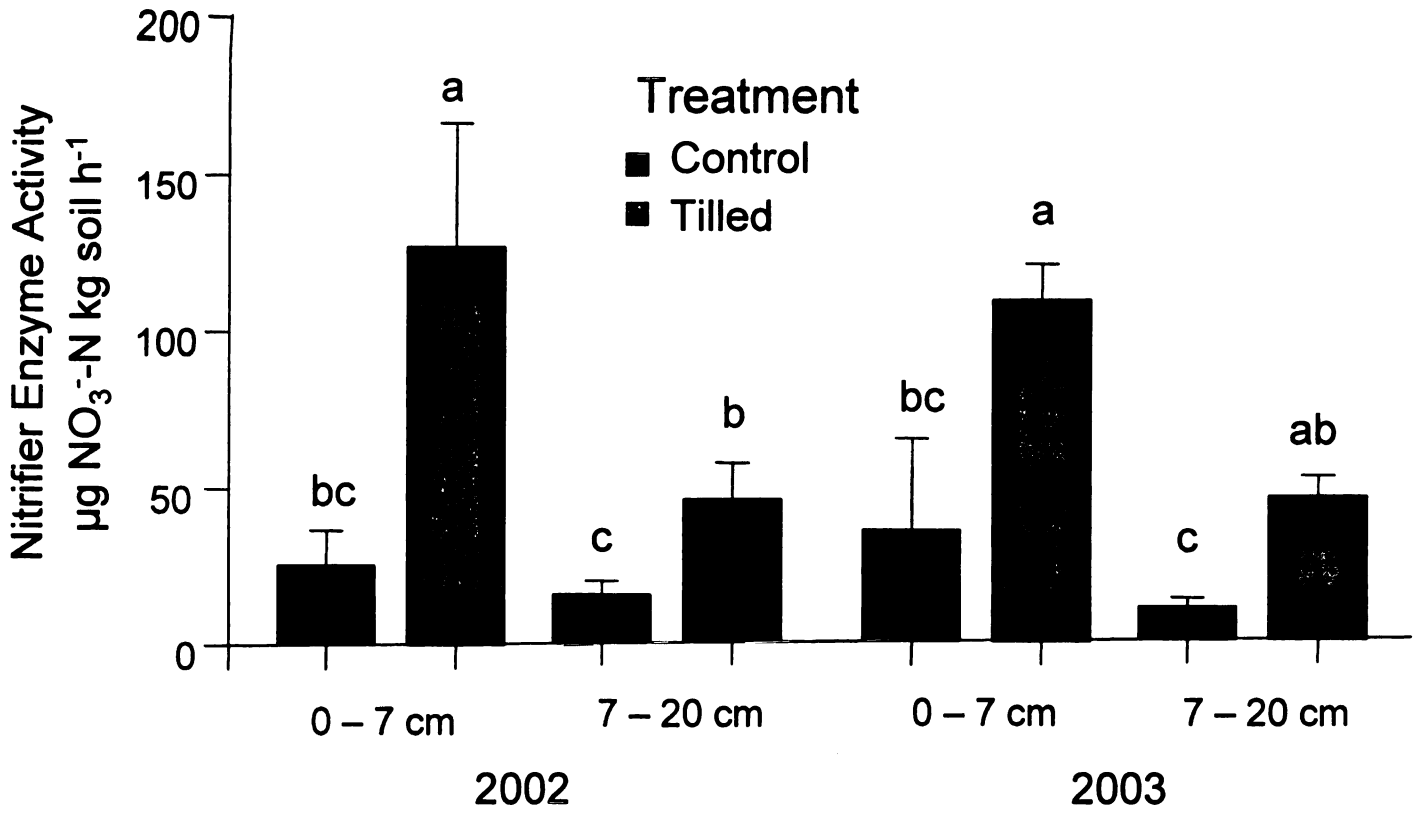

Figure 4.7. Changes in nitrifier enzyme activity following cultivation on 13 October 2003 (DOY 286) and 17 August 2004 (DOY 230). Black bars represent control plots; patterned bars, cultivated plots. Treatment means are shown ± standard error (n = 4); Bars with different letters are statistically different ($P < 0.05$).

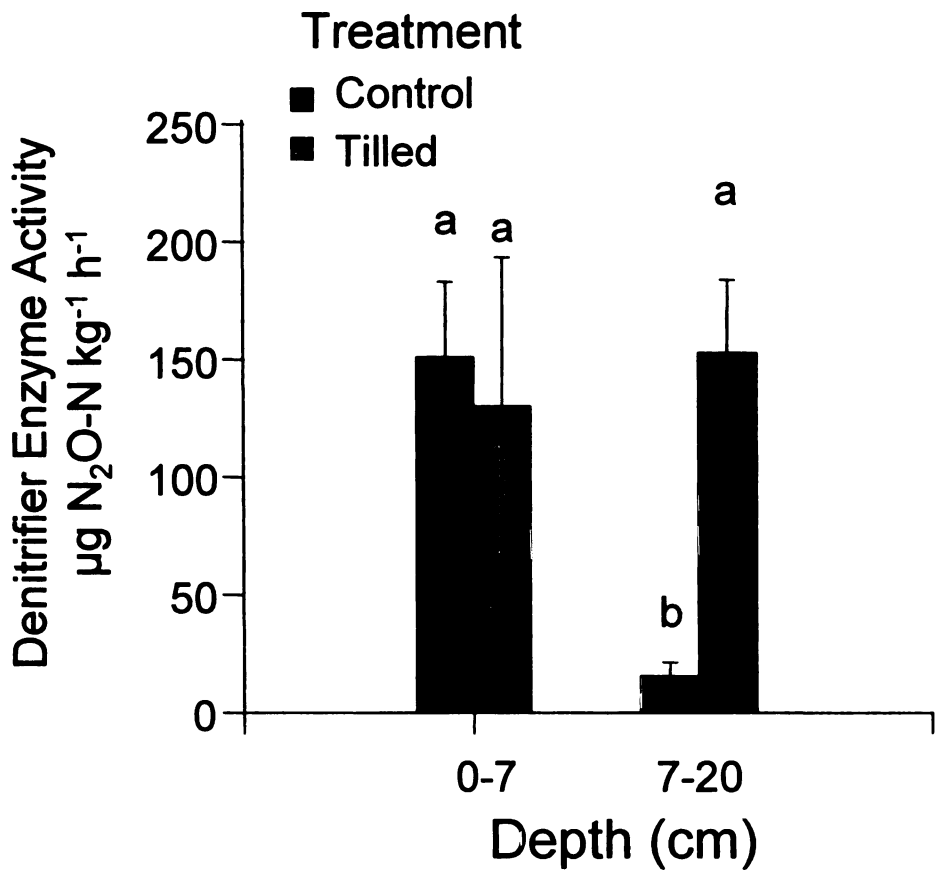

Figure 4.8. Changes in denitrifier enzyme activity in August, 2004 after three tillage events. Treatment means are shown ± standard error (n = 4); Bars with different letters are statistically different ($P < 0.05$).

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CHAPTER 5

Plant-Mediated Effects of Initial Cultivation on Carbon Stabilization, Carbon Dioxide, Nitrous Oxide, and Methane

ABSTRACT

Tillage can significantly alter trace gas fluxes and the stability of soil C and N. The effects of soil disturbance, however, are usually confounded by fertilizer inputs, annual cropping or prior tillage. We cultivated a previously uncultivated midsuccessional community and left the site fallow and unfertilized to study cultivation effects separate from other agricultural disturbances. We hypothesized that rapid changes in aggregation, microbial activity and trace gas fluxes would occur after a single plowing and that both plant litter and growing plants will modify responses to tillage. On one set of cultivated plots we removed aboveground biomass prior to cultivation and in another set of cultivated plots we removed vegetation throughout the growing season to examine plant litter and growing plant effects on trace gas fluxes, aggregation and microbial processes following tillage. Tillage with litter plowed under increased CO_2 fluxes by 72% in year 2 and 61% in year 3 and 40 and 54% of these increases were due to moving aboveground C belowground. N_2O fluxes doubled the first year and increased seven-fold the second year due to cultivation in plots with litter. These changes were related to increased nitrate concentrations in the soil which reached levels commonly found in fertilized fields (10-20 $\mu g\ N\ g^{-1}$) and increased nitrifier and denitrifier enzyme activities. Plant litter had little effect on N cycling but growing plants strongly modified soil NO_3^- concentrations and N_2O fluxes. Global warming potential increases in cultivated plots over six month

periods in 2003 and 2004 represent 12 and 13 times more C, respectively, than is annually gained (30 g C m^{-2} y^{-1}) under no-till cropping in the Midwest U.S.. Our results demonstrate that tillage alone can immediately change aggregation, trace gas fluxes and microbial processes and thereby destabilize soil C and N stocks.

INTRODUCTION

Radiative forcing of the Earth's atmosphere is increasing at unprecedented rates due to human activities. Since 1750, atmospheric concentrations of CO_2, N_2O, and CH_4 have increased by more than 30, 150, and 17%, respectively (IPCC, 2001). Agriculture has contributed substantially to global fluxes of these gases and now represents an important mitigation option as carbon sequestration and greenhouse gas abatement in soils are two of a limited number of rapidly-deployable, high impact CO_2 stabilization options now available to policy makers (Kauppi et al., 2001; Caldeira et al., 2004). Although management strategies such as cover-cropping and improved N fertilizer management potentially reduce the global warming potential (GWP) of agricultural ecosystems, eliminating tillage may have the broadest impact because of its potential to alter soil-atmosphere exchanges of all three biogenic greenhouse gases.

Eliminating tillage by converting agricultural land to perennial crops or to successional communities has the potential to sequester ca. 60 g soil C m^{-2} y^{-1} (CAST, 2004) and conversion to no-till annual crops has the potential to sequester C at about half this rate (West and Post, 2002; Lal, 2003). Ultimately, however, net greenhouse gas mitigation by soil C storage depends on the persistence of stored soil organic matter (SOM) and an important challenge to persistent C and N is the potential reversibility of C

sequestration (Paustian et al., 2000; Lal, 2004; Pacala and Socolow, 2004). No-till soil management in the U.S, although increasing, continues to be rotated with tillage because of perceptions that no-till limits N availability and decreases yields (Martens, 2001). Further, there is currently little incentive to maintain the C stocks in long-term no-till soils or successional communities. Some models predict that soils with sequestered C may rapidly lose C and N following cultivation (Baisden and Amundson, 2003) but predictions remain contentious because data are not currently available to generalize for temperate ecosystems on a time scale of less than 10 years following cultivation (Davidson and Ackerman, 1993; West and Post, 2002; Miller et al., 2004).

The effects of tillage on N_2O emissions are related to changes in soil structure, moisture, porosity and other factors that influence C, N and O_2 availability. Higher N_2O emissions in no-till than in conventionally tilled cropping systems have been frequently reported (MacKenzie et al., 1997; Ball et al., 1999; Baggs et al., 2003) although some studies have found lower emissions in no-till soils or no difference between tillage systems (Robertson et al., 2000; Elmi et al., 2003), demonstrating the potential for responses to vary across cropping systems, soil types, and the time since no-till implementation. In an assessment of potential European land-use changes on non-CO_2 greenhouse gases, Smith et al. (2001) calculated that converting all possible arable land to no-till could result in an increase in N_2O emissions equivalent to 20.5 Tg CO_2-C y^{-1}. In a recent review Six et al. (2004) concluded that differences in N_2O fluxes between till and no-till change over time. Grandy et al (2005, in review) reported that no-till had no effects on total N_2O emissions from corn-soybean-wheat cropping systems over 12 yr.

Tillage has been reported to decrease methane oxidation in some agricultural and natural soils (Hütch et al., 1994; Cochran et al., 1997; Mosier et al., 1997) but in others increase it (Kruse and Iversen, 1995) or have no effect (Mosier et al., 1998; Burke et al., 1999). Suwanwaree and Robertson (2005) found that tillage per se – separate from fertilization effects, a confounding factor in earlier studies – had no effect on CH_4 oxidation in agricultural, forest, or successional ecosystems.

These studies indicate that despite recent efforts to quantify tillage effects on trace gases, significant uncertainties remain. Tillage may influence CO_2, CH_4 and N_2O fluxes but its net effect on greenhouse gas exchange is largely unknown. Further, tillage in temperate ecosystems has been examined almost exclusively in soils with long histories of agricultural management. The legacies of prior tillage, fertilization and annual cropping effects on soil structure, microbial communities, aggregation, and other factors that control trace gas fluxes may last for more than a century, confounding the influence of recent tillage. Because of this, our basic understanding of tillage effects on soils and, in particular, our ability to predict future effects of tillage in long-term no-till soils is limited.

In this study our overall objective was to determine the effects of tillage on trace gas fluxes and their underlying physical and microbial controls, and the extent to which plants modify tillage effects. Our specific objectives were to: 1) determine tillage effects on CO_2, N_2O and CH_4 emissions in a previously uncultivated mid-successional soil to determine the effects of cultivation on GWP independently of other factors; 2) quantify the persistence of aggregation and aggregate-associated C immediately following tillage; and 3) examine disturbance effects on soil N cycling and microbial processes that control

C and N turnover. By cultivating a never-previously cultivated field and minimizing plant community changes we were able to look at soil disturbance free from the influence of other agricultural management practices, something that, to best of our knowledge, has never been done before. We hypothesized that rapid changes in aggregation, microbial activity and trace gas fluxes will occur after a single plowing and that both plant litter and growing plants will modify responses to tillage.

MATERIALS AND METHODS

Site Description and Experimental Design

Our experimental site was a previously never-tilled field at the W.K. Kellogg Biological Station (KBS) in southwest Michigan, USA (42° 24' latitude, 85° 24' longitude). The soils, plant communities, and management at this site have been described previously (Grandy, 2005) and the six dominant plant species are listed in Table 5.1. Briefly, soils at the site are Kalamazoo (fine-loamy) and Oshtemo (coarse-loamy) mixed, mesic, Typic Hapludalfs developed on glacial outwash (Crum and Collins, 1995). Prior to initial cultivation, soils contained 31.8 (± 1.4) g C kg^{-1} and 2.59 (± 0.11) g N kg^{-1}, and soil pH averaged 5.93 (± 0.11); the same soil C levels had been measured 3 years and 12 years prior to this study and are statistically indistinguishable from C levels in nearby undisturbed forests on the same soil types (Robertson et al., 2000).

In 2002, four uncultivated 3 x 6 m control sites were established in this field as part of a related study (Grandy, in review). In 2003 we established in this field eight 3 x 6 m plots to which we assigned 4 replicates of two tillage treatments with differing aboveground biomass inputs. In one treatment we removed all aboveground plant and

litter biomass prior to cultivation and in the other treatment all aboveground biomass was mowed and left in place prior to cultivation. Vegetation was clipped at ground-level. After raking as much biomass from the site as possible, remaining plant material was blown off of the soil surface with a push-blower. Within each of the four plots in which aboveground biomass was tilled into the soil, a single 1m x 1m bare-ground microplot was established. These microplots were separated from the surrounding plots by root-exclusion barriers inserted to 30 cm. Bare-ground microplots were established on 29 June 2003 and maintained by removing aboveground plant biomass weekly. Microplots were removed prior to cultivation in 2004 and then re-established in the same location.

Cultivation occurred in the same plots on 15 June 2003 and 20 June 2004. To study soil disturbance effects separately from other factors associated with agricultural conversion (e.g. the use of annual plant monocultures and fertilizers), we left the site fallow following tillage to allow a diversity of annual and perennial plants to recolonize. We anticipated that cultivation would alter dominant plant species to a degree but that this change would be relatively small compared to the establishment of annual or perennial monocultures.

Trace Gas Fluxes

Field protocols for determining trace gas emissions and laboratory analysis of CO_2 and N_2O fluxes have been described previously (Grandy, in review). Gas fluxes were determined using a single 25 cm diameter static PVC chamber (5500 cm^{-3}) located within each plot (Livingston and Hutchinson, 1995). Prior to sampling, gas-tight lids with

sampling ports were placed on chamber bases permanently installed to a soil depth of 2.5 cm and accumulated headspace was then sampled four times over 90 min by removing 20 mL of headspace gas to 12 mL vials (Labco Unlimited, Buckinghamshire, UK). Gas sampling was generally performed between the hours of 0900 and 1400 and all plots were sampled on each sampling day. Within 48 h of collection, CO_2 was analyzed using an infrared gas absorption analyzer (IRGA); N_2O analyzed using a gas chromatograph outfitted with a ^{63}Ni electron capture detector (350 °C); and CH_4 analyzed using a gas chromatograph equipped with a flame ionization detector (FID). Flux for each chamber was calculated as the linear portion of the gas accumulation curve for that chamber. Trace gas measurements were made 1 times in 2002 between 24 June and 22 August, 1times in 2003 between 29 May and 13 October, and 19 times in 2004 between 15 April and 29 October. Gas fluxes were measured 19 times in 2003 between 25 May 2003 and 13 October 2003 and 19 times in 2004 between 15 April 2004 and 29 October 2004. CH_4 measurements are not available for 2004.

Soil Inorganic N

NH_4^+ and NO_3^- were extracted with 1 M KCl from duplicate field-moist 10 g soil samples using a 1:5 soil/extractant ratio. Soil extracts were filtered with a syringe filter using a type A/E glass fiber filter (Pall Corporation, East Hills, NY). Filtrates were stored in 7 mL scintillation vials and frozen until analysis for NH_4^+ and NO_3^-. Both analyses were performed on an Alpkem 3550 Flow Injection Analyzer (OI Analytical, College Station, TX).

Enzyme Dynamics

We used a shaken slurry method (Hart et al., 1994) to test for nitrifier enzyme activity to 0-7 and 7-20 cm soil depths on 27 May 2003 (pre-cultivation), 02 September 2003, 13 October 2003, 10 May 2004 and 17 August 2004. Briefly, 30 g field-moist soil was combined with 100 mL solution containing non-limiting quantities of NH_4^+ in a 160 mL jar capped with parafilm. The parafilm was perforated with a needle to allow rapid gas exchange while minimizing water loss. Jars were placed on a rotary shaker and rotated at 200 rpm. Each flask was sampled 4 times during a 28 h incubation period. Soil extracts were centrifuged, filtered, and frozen until analysis for NO_3^-.

Denitrifier enzyme activity was measured at 0-7 and 7-20 cm soil depths on soil samples collected on 16 July 2003, 18 August 2003, 21 October 2003 17 and 17 August 2004 using methods described in Groffman et al. (1999). We combined 10 g field-moist soil with 10 mL solution consisting of $NaC_4H_4O_4$ (2 mM) and KNO_3^- (1 mM) in 160 mL serum vials. We capped these vials with rubber septa and flushed them with N_2 five times over 10 min to create anaerobic conditions prior to adding acetylene (15%). Acetylene blocks the conversion of N_2O to N_2 so that N_2O measurements alone can be used to estimate total denitrification potential. High microsite substrate availability was maintained in the jars by keeping them on a rotary shaker (190 rpm) during the incubation. N_2O concentrations were determined on four 0.5 mL headspace samples collected every 12 min. We completed the entire analysis within 60 min of the substrate additions in order to reduce the potential for cell growth or reproduction during the incubation to alter N_2O production (Luo et al., 1996). N_2O was analyzed using a gas chromatograph outfitted with a ^{63}Ni electron capture detector (350 °C).

Readily mineralizable C was measured in 2003 on May 27 (pre-till), July 16, September 2 and October 13. A subsample of soil (15-20 g) was transferred into 60 mL glass serum vials with a 13 mm diameter opening. We estimated the bulk density of each of our samples after tamping down the serum vials 10 times on a laboratory bench and from this determined the amount of water needed to bring them to 55% WFPS. After wetting the samples, the serum vials were placed in a ½ pint jar with ca. 60 mL of water in the bottom. These jars were covered with polyfilm that permits relatively free O_2 and CO_2 exchange but retains water. Jars were put into boxes and then into dark incubation chambers maintained at 25 °C for 7d. After 7 d, serum vials were flushed for 45 s with a humidified air stream. After flushing, bottles were sequentially capped with a rubber septum. A 0.5 mL sample of headspace was immediately drawn with a syringe and then two additional samples were taken over a 90 min sampling interval. CO_2 content of each gas sample was analyzed using an infrared gas absorption (IRGA) analyzer, followed by calculation of the respiration potential for the time interval (Robertson et al. 1999).

Aggregation

Soil aggregation was determined on samples collected 27 May 2003, 16 July 2003, and 21 October 2003. Aggregate distribution was determined by hand on triplicate 35 g air-dried soil samples by wet-sieving in water through a series of 2000 μm, 250 μm, and 53 μm sieves (Six et al. 1998). The method we used to separate inter- and intra-aggregate light fraction (LF) is based on previously published protocols (Six et al., 1998; Gale et al., 2000). Aggregate subsamples (8 g) were pre-wetted with 4 mL H_2O prior to LF analysis to minimize aggregate slaking during LF separation. Aggregates were

transferred from the membrane filters to 100 mL beakers after 16 h with 5 mL aliquots of NAPT at a density of 1.62 g cm^{-3}. A total of 55 mL NAPT was used for each sample. A preliminary test showed that the final density of the sodium polytungstate was 1.60 g cm^{-3} following equilibration with the water contained in aggregates.

After 24 h, inter-aggregate LF was aspirated from the surface of the sodium polytungstate and then rinsed with at least 600 mL DI H_2O. Aggregates were then dispersed to release the intra-aggregate LF using sodium hexametaphosphate and resuspended in NAPT (d = 1.62 g cm^{-3}). The intra-aggregate LF was collected form the surface.

Data Analysis

Soil aggregate size distributions and aggregate-associated C pools were corrected for sand content > 53 μm. Carbon content of soil organic matter fractions was calculated on an area basis by correcting for bulk density. Tillage effects on aggregation and aggregate-associated SOM pools were analyzed by Proc Mixed (Version 8.2, SAS Institute, 1999) using a randomized complete block design analysis of variance (ANOVA). Treatment, soil depth and aggregate size fraction were considered fixed effects and block a random effect. Treatment effects on bulk density, total soil C, denitrifier and nitrifier enzyme activities and mineralizeable C were analyzed similarly but without the size class effect. Soil inorganic N concentrations were analyzed by Proc Mixed using a randomized complete block design analysis of variance (ANOVA) with repeated measures (SAS Version 8.2, SAS Institute, 1999). Treatment and sampling date were considered fixed effects and block a random effect.

We interpolated daily gas fluxes from our periodic measurements to estimate total CO_2 and N_2O fluxes over the measurement period. Individual trace gas contributions to global warming potential were made using 100-yr time horizon factors of 310 for N_2O and 21 for CH_4 and calculated, conservatively, for a six month period, which corresponds approximately with the length of our growing season and sampling campaign. The difference in global warming potential was calculated as the sum of the difference in N_2O, CO_2 and CH_4 between tilled and control plots with CH_4 oxidation considered a negative offset of CO_2 and N_2O emissions. Gas fluxes and differences in GWP were analyzed similarly as other variables except treatment was the only fixed effect. Analysis of covariance (Goldberg and Scheiner, 2001) was used to examine the relationship between soil temperature, moisture and CO_2 fluxes in 2003 and 2004.

To determine gas fluxes in bare-ground plots in 2003, prior to the regeneration of vegetation and establishment of the microplots, we used emission rates corresponding to the same replicate of the tilled plots with litter to create a data set that covered the entire sampling period. These emissions should have been identical to those from the bare-ground microplots. This data set was used to determine mean fluxes for the sampling period. In 2004, measurements from the bare-ground microplots spanned the entire sampling period and these were used for interpolation.

Single degree of freedom comparisons were made to using the LSD statistic to calculate a 95% confidence interval around the differences between means generated using the diff option in Proc Mixed. The LSD was carried out using the PDMIX800 algorithm (Saxton, 1998). Data were log transformed where necessary to improve homogeneity of variance

Plant and Soil Analysis

Cultivation effects on plant litter were determined on 15 September 2003 from two 625 cm^{-2} quadrats in each plot. Cultivation effects on plant species, biomass and litter were determined on 26 May 2004 prior to the second cultivation in two 1250 cm^{-2} microplots. Organic C and total N concentrations of plant and soil samples were determined by combustion and gas chromatography in a CHNS analyzer (Costech ECS 4010, Costech Analytical Technologies, Valencia CA.).

RESULTS

Plant and Litter Dynamics

Changes in the plant communities following cultivation were mainly due to the reduction in *Rubus accidentalis* (Table 5.1). Although some regenerated following tillage, it was no longer one of the top six species. Even in the control plots, however, *Rubus accidentalis* was patchily distributed as indicated by the high standard error. There was none in one of the replicates and only 8 g m^{-2} in another while there was 830 g m^{-2} in another replicate. *Bromus inermis*, *Agropyron repens*, and *Poa Pratensis L.* were among the top six biomass producers in all of the treatments.

Prior to the second cultivation in Spring 2004, we estimated that there was 192 g C m^{-2} in aboveground plant pools and 58 g C m^{-2} in litter pools of plots where vegetation was removed; in plots where aboveground biomass is incorporated with tillage, we estimated that C in the plant and litter pools was 260 and 43 g C m^{-2}, respectively (Table 5.2). These estimates are similar to estimates made in 2002 prior to the initiation of this

experiment when there was 228 ± 11 g C m^{-2} in plant pools and 142 ± 30 g C m^{-2} in litter pools.

Total Soil C and N

Cultivation redistributed C and N from the soil surface to 7-20 cm (Table 5.3). C concentrations in cultivated plots were reduced by 23-34% at 0-7 cm and increased by 52-54% at 7-20 cm. Control plots had 23-30% higher N concentration at the 0-7 cm depth than any of the cultivated treatments; at 7-20 cm cultivated plots had a 33-40% higher N concentrations. There were no differences in overall soil C and N concentration at 0-20 cm depth. Expressed on an areal basis, the biomass removal treatment and bare-ground microplots had more C than control plots to 20 cm but this was at least partially due to higher bulk densities in these plots than control plots.

Aggregation and Aggregate-Associated C

Cultivation immediately changed soil aggregate distributions (Figure 5.1). On 16 May 2003, 31 days after tillage, the proportion of soil in the 2000-8000 μm aggregate size class at 0-7 cm soil depth was reduced from 0.35 g g^{-1} in control plots to 0.20 in no litter plots and 0.16 g g^{-1} in litter plots. These trends were still evident in September 2003 when cultivation reduced soil in the 2000-8000 μm aggregate size from 0.48 in control to 0.21 in no litter and 0.18 g g^{-1} in litter plots. Cultivation increased soil in the microaggregate size classes (<250 μm). There were also reductions at the 7-20 cm soil depth, although they were proportionally less than those near the soil surface (Figure 5.1). At the 0-20 cm soil depth of cultivated plots there was a lower proportion of soil in the

2000-8000 μm size class on 16 July 2003 and 21 October 2003. On 24 September 2004 cultivated plots with litter and bare-ground microplots had lower soil in the 2000-8000 μm size class than control plots. There were generally no differences among no litter, litter and bare-ground plots except at 0-7 cm where bare-ground plots had a lower proportion of soil in the 250-2000 μm size class than vegetated plots with litter incorporated at tillage.

Cultivation generally homogenized the distribution of aggregate-associated C at 0-7 and 7-20 cm soil depth (Figure 5.2). There were no differences among depths for any of the cultivated treatments while control sites generally had more inter, intra and heavy fraction C at 0-7 cm. Cultivation reduced inter-aggregate C associated with 2000-8000 μm aggregates at 0-7 cm in no litter and bare-ground plots. Bare-ground plots also reduced inter-aggregate C associated with the 53-250 μm size class relative to control plots. At 7-20 cm, cultivated plots with litter increased C in the inter- and intra-aggregate pools of the 250-2000 μm size class (Figure 5.2).

Trace Gases and GWP

No litter and litter plots had higher CO_2 fluxes after mowing but before cultivation on 20 May 2003 (Figure 5.3). Cultivation immediately increased CO_2 emissions in litter and no litter plots and these emissions remained higher throughout 2003 (Table 5.4; Figure 5.3). Bare-ground microplots established after the recovery of vegetation generally had similar CO_2 fluxes to the plots with biomass incorporated at tillage. Tillage effects were still evident prior to cultivation in 2004 when cultivation again accelerated CO_2 fluxes from all plots. Mean CO_2 fluxes in 2003 were 33% greater in plots with no

litter, 72% greater in plots with litter and 61% greater in bare-ground microplots (Table 5.4). In 2004, no litter increased CO_2 emissions by 36% and litter by 61% relative to control plots (Table 5.4). N_2O fluxes were also accelerated by tillage and removal of plants throughout the growing season (Figure 5.4). In 2003, no-litter and litter doubled N_2O emissions while bare-ground increased N_2O emissions four-fold (Table 5.4). In 2004 cultivation of litter and no-litter treatments increased N_2O emissions seven-fold and in bare-ground plots 12-fold. CH_4 oxidation was reduced by tillage and plant manipulations (Figure 5.5; Table 5.4), although reductions in methane oxidation in bare-ground and no-litter plots were relatively small compared to cultivation effects on CO_2 and N_2O emissions. CO_2 contributions to GWP were far greater than those from CH_4 or N_2O.

Soil Temperature and Moisture

Soil moisture and temperature (Figure 5.6) were influenced by tillage. In 2003, mean soil temperature in cultivated treatments ranged from 18.2 in bare-ground plots to 19.3 °C in litter plots. All cultivated plots had a higher ($p<0.05$) temperature than control plots (16.6 °C). In 2004, bare-ground plots had the highest temperature (19.5) but all of the tilled treatments had a higher temperature than control (16.2). Analysis of covariance results showed a significant temperature effect in 2003 ($p<0.003$): CO_2 flux = 14.9 + 0.026 (temperature), $r^2 = 0.22$ ($p<0.000$) (Figure 5.7). In 2004 (Figure 5.7) there was a temperature by treatment interaction ($p<0.001$) indicating the slope of the line describing the relationship between temperature and CO_2 emissions differed between treatments. Control plots: CO_2 flux = 13.3 + 0.020 (temperature), $r^2 = 0.08$ ($p<0.000$); no litter plots:

CO_2 flux = 12.1 + 0.033 (temperature), r^2 = 0.40 ($p<0.000$); litter plots: CO_2 flux = 13.8 + 0.018 (temperature), r^2 = 0.22 ($p<0.000$); bare-ground plots: CO_2 flux = 15.5 + 0.026 (temperature), r^2 = 0.20 ($p<0.000$). In 2004, there was additionally a moisture effect ($p<0.02$), although the relationship with CO_2 was weak (r^2=0.02; data not shown).

Soil Inorganic N

Cultivation increased soil NO_3^- concentrations at 0-7 and 7-20 cm soil depth (Figure 5.8). In litter plots, NO_3^- peaked four days after tillage (DOY 170), when it exceeded 20 $\mu g\ g^{-1}$ and exceeded 10 $\mu g\ g^{-1}$ on DOY 215. On three sampling dates in 2003, NO_3^- concentrations were greater in litter plots than no-litter plots. The highest NO_3^- concentrations were in bare-ground microplots where concentrations exceeded 30 $\mu g\ g^{-1}$ at 0-7 cm and 20 $\mu g\ g^{-1}$ at 0-20 cm in 2003. In 2004 bare-ground microplots had the highest NO_3^- at both soil depths on DOY 208 and 230. Ammonium concentrations (Figure 5.9) responded strongly to tillage in 2003 at 0-7 cm but tillage effects were smaller at 7-20 cm and at both depth strata in 2004.

Microbial Processes

Potentially mineralizeable C was generally greater at 0-7 than 7-20 cm depth across treatments, except for litter plots on 13 October 2003 (Figure 5.10). Control plots had greater mineralizeable C at 0-7 cm on 16 July 2003 and again on 13 October 2003. Litter plots increased mineralizeable C at 7-20 cm on 02 September 2003 and again on 13 October 2003 and increased mineralizeable C at 7-20 cm on 02 September 2003. Cultivation decreased denitrifier enzyme activity at 0-7 cm and increased it at 7-20 cm.

Overall, the only significant cultivation effect at 0-20 cm was between no litter and control plots on 20 August 2004 (Figure 5.11). In contrast to denitrifier enzyme activity, cultivation increased nitrifier enzyme activity at 0-7, 7-20 and overall at 0-20 cm depths (Figure 5.12).

DISCUSSION

Soil Aggregation

Within 60 d of cultivation, soil in the 2000-8000 μm size class at 0-7 cm depth (Figure 5.1) declined to levels indistinguishable from those in an adjacent agricultural soil cultivated for >50 y (Grandy and Robertson, 2000, in review). There were also declines at 7-20 cm in the 2000-8000 μm size class demonstrating that declines at the soil surface were not solely due to a redistribution of aggregates to increased soil depths. Multiple studies have demonstrated that long-term cultivation reduces soil aggregation (e.g. Jastrow et al., 1996; Six et al., 2000; Mikha and Rice, 2004); our results demonstrate that tillage immediately destroys macroaggregates and that these declines in soil structure persist. Plant residues and growing plants are known to modify soil aggregation in multiple ways but we found few differences between litter, no litter and bare-ground plots. The effects of vegetation on aggregation were small compared to tillage effects.

Global Warming Potential (GWP) Impact

We documented (Table 5.4) immediate changes in trace gas fluxes after cultivation that dramatically altered global warming potentials (GWP). Global warming potential increases in cultivated plots with litter over a 6 month period in 2003 and 2004 represent

12 and 13 times more C, respectively, than is annually gained (30 g C m^{-2} y^{-1}) under no-till cropping in the midwest (Robertson et al. 2000; Franzluebbers and Steiner, 2002). These differences were primarily attributable to variation in CO_2 flux but in 2004 differences in N_2O flux between tilled plots with litter and control plots were equivalent to 35 g C m^{-2} over 6 months (Table 5.4). Methane oxidation offset little of the GWP contributions from CO_2 and N_2O. Similar differences in GWP were found in bare-ground microplots, although no litter plots had reduced GWP (though not significant) relative to litter plots. This difference was primarily due to reduced CO_2 flux in control plots.

Carbon Dioxide

Based on differences between no litter and control plots relative to differences between litter and control plots, between 40 and 54% of the soil surface CO_2 emissions in litter plots were from the incorporation of aboveground biomass (Table 5.4). We estimate that cultivation incorporated 302 g C m^{-2} of aboveground C in 2004 (Table 5.2). Although we do not have aboveground C estimates from pre-tillage in 2003, sampling of the control sites in spring 2002 provided an estimate of 370 g C m^{-2}, which is our best estimate of the 2003 C contribution with tillage. In 2003, the difference in CO_2-C loss between litter and no litter treatments was 188 g C m^{-2}, or 51% of the aboveground residue C. In 2004, the difference in CO_2-C loss between litter and no litter treatments was 147 g C m^{-2}, or 49% of the aboveground residue C (Table 5.4). Rochette et al (1999) found that 40% of maize residue C was oxidized in a single season. Burgess et al. (2002) found somewhat higher residue decomposition rates so that after two years 89-98% of buried corn residue was lost. Lupwayi et al. (2004) estimated that within 12 months

decomposition losses from canola residue were 50% in conventional tillage. Our estimates of aboveground C contributions to CO_2 flux are thus in line with prior estimates.

Striking declines in soil aggregation (Figure 5.1), increases in inter-aggregate LF (Figure 5.2), and greater mineralizeable C at 7-20 cm (Figure 5.10) indicate aboveground biomass C principally entered low-density, inter-aggregate POM pools that are rapidly oxidized following disturbance (Arrouays and Pelissier, 1994; Six et al., 1999; DeGryze et al., 2004). Lupwayi et al. (2004) found that incorporation of wheat litter with conventional tillage increased its decomposition rate by 48%; Burgess et al. (2002) found that litter decomposition rates at 20 cm were 52 - 105% greater than those at the soil surface. Increases in soil temperature may have also contributed to increased CO_2 emissions.

Bare-ground microplots in 2003 give us an estimate of the plant contributions to CO_2 flux after tillage. Similar fluxes in these plots and the tilled plots with litter indicate that plant contributions to CO_2 flux were very low during the season (Table 5.4). This was expected since recovery of the plant community generally took 3-4 weeks after cultivation, during which time heterotrophic respiration (mainly bacteria and fungi) would have accounted for most of the difference in CO_2 flux. In contrast, autotrophic (vascular plant roots) respiration may have accounted for 50% or more of the measured CO_2 in control plots (Hanson et al., 2000). Low mean CO_2 fluxes from bare-ground plots in 2004 may have been partially due to the absence of autotrophic respiration but may have also been due to decreases in C inputs. By establishing the plots in the same location in 2004 as in 2003 we minimized the above and below-ground C inputs.

Nitrous Oxide

The effects of tillage on soil surface N_2O fluxes have been primarily studied following conversion of long-term, conventionally tilled cropping systems to no-till (Grant et al., 2004; Six et al., 2004; Mackenzie et al., 1997). We found that tillage without fertilizer inputs or annual cropping doubled N_2O fluxes in 2003 and increased emissions seven-fold in 2004 (Table 5.4). Sizeable N_2O-N losses following cultivation of no-till ecosystems were also demonstrated by Pinto et al. (2004) who reported increases over 5 d of between 1.8 and 23 mg N_2O-N m^{-2} following the plowing of a perennial pasture. Keller et al. (1993) found that converting forest to pasture increased N_2O fluxes in the first ten years. In both of these studies, authors attributed increased N_2O emissions to accelerated SOM turnover producing excess inorganic N that was subsequently used by N_2O -producing nitrifiers and denitrifiers.

Our results also point to the importance of N availability because the highest N_2O emissions (Figure 5.4) generally coincided with elevated NO_3^- concentrations (Figure 5.8). Low NO_3^- concentrations in the control soils suggest a tight coupling of plant N uptake and the microbial processes of N-mineralization and nitrification. Accelerated SOM turnover, increased nitrifier enzyme activity (Figure 5.12) and reduced plant uptake following cultivation disrupted the synchrony between N availability and plant uptake. As a result, NO_3^- concentrations reached levels equal to or exceeding those typically found in fertilized agricultural soils (10-20 μg N g^{-1} soil).

If this site were converted to an annual cropping system, N_2O would contribute proportionally more to GWP over time due to soil C loss and increases in N_2O flux. For

example, Robertson et al. (2000) demonstrated that once an agricultural soil has reached equilibrium N_2O dominates contributions to GWP from soil surface gas emissions. Even in no-till cropping systems that are accruing C, annual C gains can be nearly offset by N_2O emissions (Grandy et al. 2005). Also, additional changes in the synchrony between nitrogen availability and plant demand, including the use of supplemental N fertilizer or conversion to annual crops, would lead to additional N_2O emissions (Mosier et al., 1998; Mosier, 2001).

Removing aboveground biomass prior to cultivation had little effect on N_2O emissions or on soil NO_3^- or NH_4^+ concentrations. Bare-ground microplots, however, greatly increased N_2O fluxes and soil NO_3^- concentrations in both years. Both of these results demonstrate the importance of growing plants in buffering nitrogen pulses after tillage; i.e., greater C turnover in litter than no litter plots, as evidenced by increased CO_2 fluxes, and its potential influence on N mineralization-immobilization dynamics had little effect on N cycling compared to the presence or absence of plants.

Methane

We detected small decreases in CH_4 oxidation with cultivation that were statistically significant in the no litter plots (Table 5.4). Other studies have also reported decreases in methane oxidation following tillage. In a grassland soil, Mosier et al. (1997) found that tillage reduced immediately CH_4 consumption by 34% and that this effect persisted for three years. Kessavalou (1998b) found that over three years CH_4 uptake in no-till systems averaged 7.4-8.1 g C ha^{-1} d^{-1} and in plowed soils averaged 5.9-8.0 g C ha^{-1} d^{-1}. In contrast, other studies have found tillage had no effects on CH_4 uptake (Robertson

et al., 2000; Suwanwaree and Robertson, 2005) or even increased oxidation (Kessavalou et al., 1998a). Differences in climate and soil texture may modify the direct effects of tillage but the indirect effects on pH and N turnover and availability may also be important (Bédard and Knowles, 1989; Hütsch et al. 1994; Suwanwaree and Robertson, 2005). In our study, tillage, per se, may not be decreasing CH_4 oxidation – despite declines in soil aggregation -- but having an indirect effect by increasing soil N cycling. Increases in N availability and turnover frequently decrease CH_4 oxidation rates (Hütsch et al. 1993; Hilger et al. 2000).

Tillage Management

Cultivating this previously uncultivated soil immediately changed physical and microbial processes regulating trace gas fluxes. Release of C from physical protection within macroaggregates > 250 μm, coupled with movement of aboveground C pools into unprotected belowground pools, greatly increased C substrate availability. Associated with this was a series of changes in microbial activity, soil inorganic N concentration, and trace gas fluxes. Although we detected no difference in soil C concentrations after two years and other studies have suggested that periodic cultivation may have little effect on otherwise no-till soils (Pierce et al., 1994; VandenBygaart and Kay, 2004), our results suggest that plowing once destabilizes C and N stocks. These same processes are likely occurring in agricultural soils rotated between till and no-till but are undetectable because SOM pools and aggregation are relatively low. Detecting C losses due to periodic cultivating may thus require observing these systems over many years.

The effects of cultivation on N_2O fluxes and soil NO_3^- concentrations can be strongly mediated by plants. Reducing the period of time following cultivation that soils are bare by using catch crops, cover crops or other strategies will reduce N losses.

CONCLUSIONS

We found that cultivation reduced the proportion of soil in the 2000-8000 μm aggregate size classes from 36% to 16% at 0-7 cm within 30 days. Declines in aggregate stability were also detected at 7-20 cm. These declines persisted throughout the study. Plant litter and growing plants had little effect on aggregation. Tillage with litter plowed under increased CO_2 fluxes by 72% in 2003 and 61% in 2004 and between 40 and 54% of these increases were due to moving aboveground C belowground. N_2O fluxes doubled in 2002 and increased seven-fold in 2003 due to cultivation in plots with litter. These changes were related to increased nitrate concentrations in the soil which achieved levels commonly found in fertilized fields (10-20 μg N g^{-1}) and increased nitrifier and denitrifier enzyme activities. Plant litter had little effect on N cycling but growing plants strongly modified soil NO_3^- concentrations and N_2O fluxes. Global warming potential increases in cultivated plots with litter over a 6 month period in 2003 and 2004 represent 12 and 13 times more C, respectively, than is annually gained (30 g C m^{-2} y^{-1}) under no-till cropping in the U.S. Midwest. These differences were primarily attributable to variation in CO_2 flux but in 2004 differences in N_2O flux between tilled plots with litter and control plots were equivalent to 35 g C m^{-2} over 6 months. Methane oxidation offset little of the GWP contributions from CO_2 and N_2O. Our results demonstrate the

importance of maintaining long-term no-till soils and protecting them from future disturbance.

Table 5.1. Tillage effects on top six dominant plant species by biomass and proportion of total biomass. Samples were collected on 26 May 2004, approximately one year after initial cultivation and before the second cultivation. Means with standard errors in parentheses.

Treatment	Plant Species	Plant Biomass $g\ m^{-2}$	Proportion Total Biomass
Control	*Rubus accidentalis*	246 (198)	0.19 (0.12)
	Dactylis glomerata L.	83.3 (38.9)	0.10 (0.05)
	Bromus inermis	71.4 (31.5)	0.11 (0.05)
	Poa pratensis L.	63.2 (23.9)	0.09 (0.04)
	Arrhenatherum elatius L.	48.8 (21.5)	0.06 (0.04)
	Agropyron repens	25.8 (15.0)	0.02 (0.01)
No Litter†	*Agropyron repens*	125 (58.3)	0.25 (0.09)
	Dactylis glomerata L.	92.1 (57.3)	0.20 (0.14)
	Bromus inermis	26.4 (8.30)	0.06 (0.02)
	Aster sp?	18.8 (8.05)	0.05 (0.03)
	Daucus carrota L.	11.4 (6.66)	0.03 (0.02)
	Poa pratensis L.	9.6 (6.47)	0.02 (0.01)
Litter	*Agropyron repens*	136 (53.7)	0.22 (0.07)
	Bromus inermis	90.7 (44.2)	0.15 (0.07)
	Dactylis glomerata L.	85.4 (34.4)	0.15 (0.06)
	Poa pratensis L.	37.0 (21.6)	0.06 (0.04)
	Barbarea vulgaria	23.6 (18.6)	0.04 (0.03)
	Daucus carrota L.	21.1 (7.22)	0.04 (0.01)

†No litter plots had all aboveground biomass removed prior to cultivation; litter plots had all aboveground biomass left in place.

Table 5.2. Aboveground C and N in plant and litter pools after initial cultivation in 2003 and prior to the second cultivation in spring 2004. Means with standard errors in parentheses.[†]

Date	C Source		Carbon	Nitrogen
			g m^{-2}	g m^{-2}
15 Sept. 2003	Litter	Control	195 (40.1)A	5.79 (1.32)A
		No Litter	20.84 (14.5)B	0.33 (0.23)B
		Litter	17.8 (4.42)B	0.39 (0.10)B
26 May 2004	Plant	Control	423 (110)A	13.7 (3.57)A
		No Litter	192 (25.0)B	6.23 (0.81)B
		Litter	259 (24.1)AB	8.39 (0.78)AB
	Litter	Control	192 (27.6)A	5.09 (0.73)A
		No Litter	58.3 (7.14)B	1.55 (0.19)B
		Litter	43.3 (2.63)B	1.15 (0.07)B

[†]Means within a column, date and C source followed by different letters are significantly different ($p<0.05$).

Table 5.3. Initial cultivation effects on soil C and N concentrations and bulk density at 0-7 and 7-20 cm sampling depths . Initial cultivation was on 15 June 2003 and again on the same plots in the spring of 2004.[†‡]

		N	C	Bulk Density	N	C
		%	%	g cm^{-3}	kg m^{-2}	kg m^{-2}
0-7 cm	Control	0.26 (0.01)a*	3.13 (0.15)a*	0.92 (0.06)b*	0.17 (0.01)	2.01 (0.15)
	No Litter	0.19 (0.01)b	2.23 (0.15)b	1.32 (0.06)a	0.18 (0.01)*	2.05 (0.12)*
	Litter	0.20 (0.01)b	2.40 (0.16)b	1.15 (0.07)a	0.16 (0.01)*	1.92 (0.15)*
	Bare-ground	0.18 (0.01)b	2.05 (0.11)b	1.36 (0.02)a	0.17 (0.01)*	1.94 (0.09)*
7-20 cm	Control	0.12 (0.01)b	1.26 (0.11)b	1.42 (0.02)	0.21 (0.01)b	2.30 (0.18)a
	No Litter	0.17 (0.02)a	1.91 (0.25)a	1.41 (0.02)	0.31 (0.03)a	3.48 (0.44)b
	Litter	0.17 (0.03)a	1.94 (0.34)a	1.28 (0.13)	0.28 (0.05)a	3.16 (0.54)b
	Bare-ground	0.16 (0.01)a	1.91 (0.15)a	1.46 (0.04)	0.31 (0.01)a	3.61 (0.22)b
0-20 cm	Control	0.15 (0.01)	1.74 (0.07)	1.29 (0.02)AB	0.38 (0.01)B	4.32 (0.09)B
	No Litter	0.18 (0.01)	2.02 (0.19)	1.38 (0.03)AB	0.49 (0.03)AB	5.53 (0.45)A
	Litter	0.18 (0.02)	2.09 (0.25)	1.25 (0.08)B	0.44 (0.04)AB	5.08 (0.53)AB
	Bare-ground	0.17 (0.01)	1.95 (0.13)	1.43 (0.03)A	0.47 (0.02)A	5.55 (0.31)A

[†]Means followed by different lowercase letters within a column and soil depth are statistically different at $p<0.05$.
[‡] Means followed by different uppercase letters within a column are statistically different for the 0-20 cm depth at $p<0.05$.
*Indicates differences between sampling depths for means within a column and treatment.

Table 5.4 Mean (interpolated) CO_2, N_2O and CH_4 fluxes following initial cultivation of sites with different vegetation management in 2003 and 2004. Individual trace gas contributions to global warming potential (GWP) were calculated for a period of 6 mo (corresponding approximately with the lengths of our sampling campaign and growing season) using 100-yr time horizon factors of 310 for N_2O and 21 for CH_4.[†§,¶]

		Mean Gas Fluxes			Trace Gas Contributions to GWP (CO_2 equivalents)			
		CO_2-C	N_2O-N	CH_4-C	CO_2	N_2O	CH_4	$\Delta GWP^{‡}$
		mg m^{-2} h^{-1}	μg m^{-2} h^{-1}	μg m^{-2} h^{-1}	g CO^2 m^{-2} y^{-1}	g CO^2 m^{-2} y^{-1}	g CO^2 m^{-2} y^{-1}	
2003	Control	112 (12.8)B	5.37 (2.16)C	-33.8 (2.44)A	1770 (203)	11.30 (4.54)	-6.23 (0.45)	-
	No Litter	149 (11.6)A	11.9 (1.36)B	-29.0 (2.76)AB	2360 (184)	24.9 (2.90)	-5.34 (0.51)	607 (302)
	Litter	193 (15.0)A	13.4 (1.75)AB	-26.0 (1.20)B	3050 (238)	28.1 (3.68)	-4.80 (0.22)	1300 (265)
	Bare-ground	180 (15.5)A	25.6 (4.30)A	-25.2 (1.04)B	2860 (245)	53.9 (9.04)	-4.65 (0.49)	1130 (312)
2004	Control	138 (17.4)C	10.8 (3.67)B	-	2190 (275)	22.8 (7.72)	-	-
	No Litter	188 (6.07)AB	77.9 (32.2)A	-	2980 (96.2)	164 (67.9)	-	929 (258)AB
	Litter	222 (16.3)A	71.7 (9.84)A	-	3520 (258)	151 (20.7)	-	1459 (374)A
	Bare-ground	151 (19.2)BC	132 (37.3)A	-	2390 (304)	278 (78.5)	-	461 (565)B

[†]Means are shown with standard errors in parentheses.

[‡]Difference in total GWP (sum of CO_2, N_2O and CH_4) from control plots.

[§]In 2004 there were fewer sampling points in bare-ground microplots so the high points are weighted more heavily in the interpolation.

[¶]Means within a column and year followed by a different letter are statistically different ($p<0.05$).

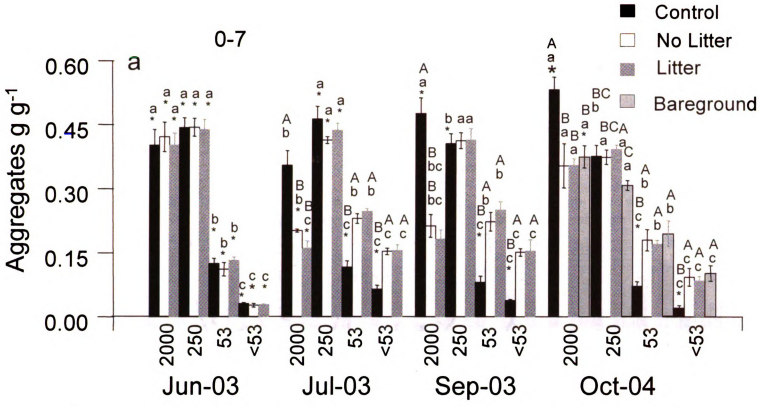

Figure 5.1 Cultivation effects in a previously uncultivated field on soil aggregation in 2003 and 2004 at 0-7 (a, this page), 7-20 (b, next page), and 0-20 (c, two pages ahead) cm depth. Bars are arranged within sampling date by size class going left to right: 2000-8000 μm, 250-2000 μm, 53-250 μm and <53 μm. No litter plots had all biomass removed prior to cultivation. Bare-ground plots were weeded and contained root exclusion barriers. Bargeround plots were measured in October 2004 only. Treatment means within a sampling date, depth, and size class followed by different uppercase letters are statistically different ($p<0.05$). Within a sampling date, depth and treatment, size classes followed by different lowercase letters are statistically different ($p<0.05$). Within a sampling date, treatment and size class, means at 0-7 cm followed by an asterisk are different from those at 7-20 cm.

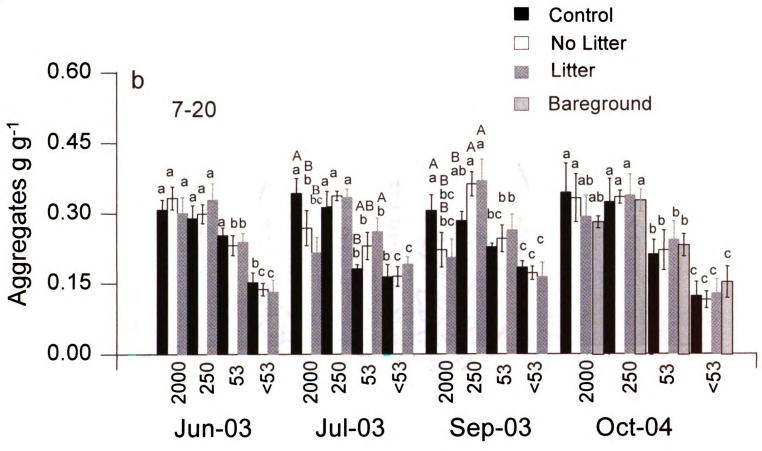

Figure 5.1. cont'd (7-20 cm soil depth)

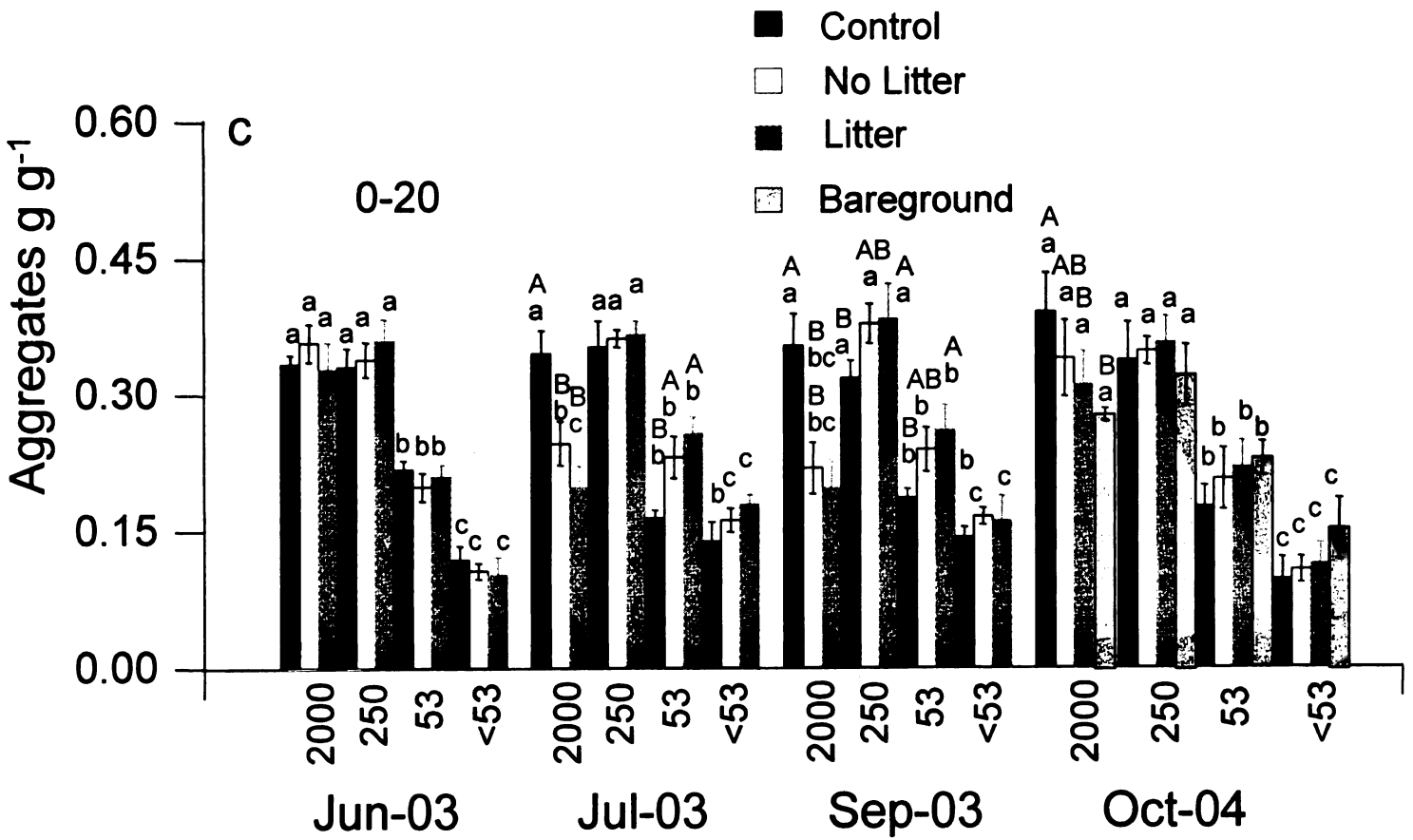

Figure 5.1. cont'd (0-20 cm).

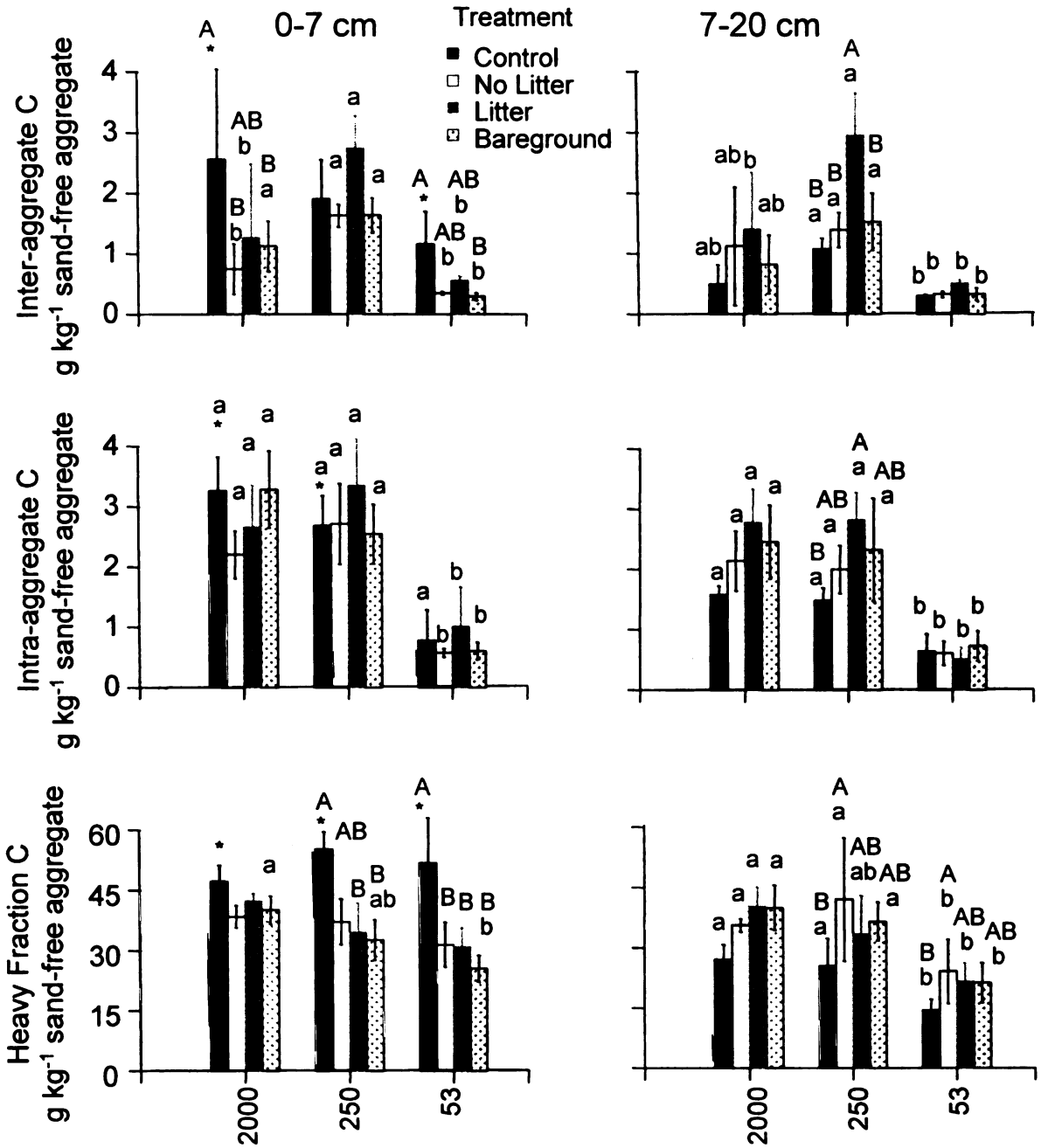

Figure 5.2. Cultivation effects in a previously uncultivated field on soil organic matter distribution in 2004 at 0-7 and 7-20 cm depth. Values going left to right on the x-axis represent aggregate size classes: 250-2000 μm, 53-250 μm and <53 μm, respectively. No-litter plots had all biomass removed prior to cultivation. Bare-ground plots were weeded and contained root exclusion barriers. Treatment means within a depth and size class followed by different uppercase letters are statistically different ($p<0.05$). Within a depth and treatment, size classes followed by different lowercase letters are statistically different ($p<0.05$). Within a treatment and size class, means at 0-7 cm followed by an asterisk are different from those at 7-20 cm.

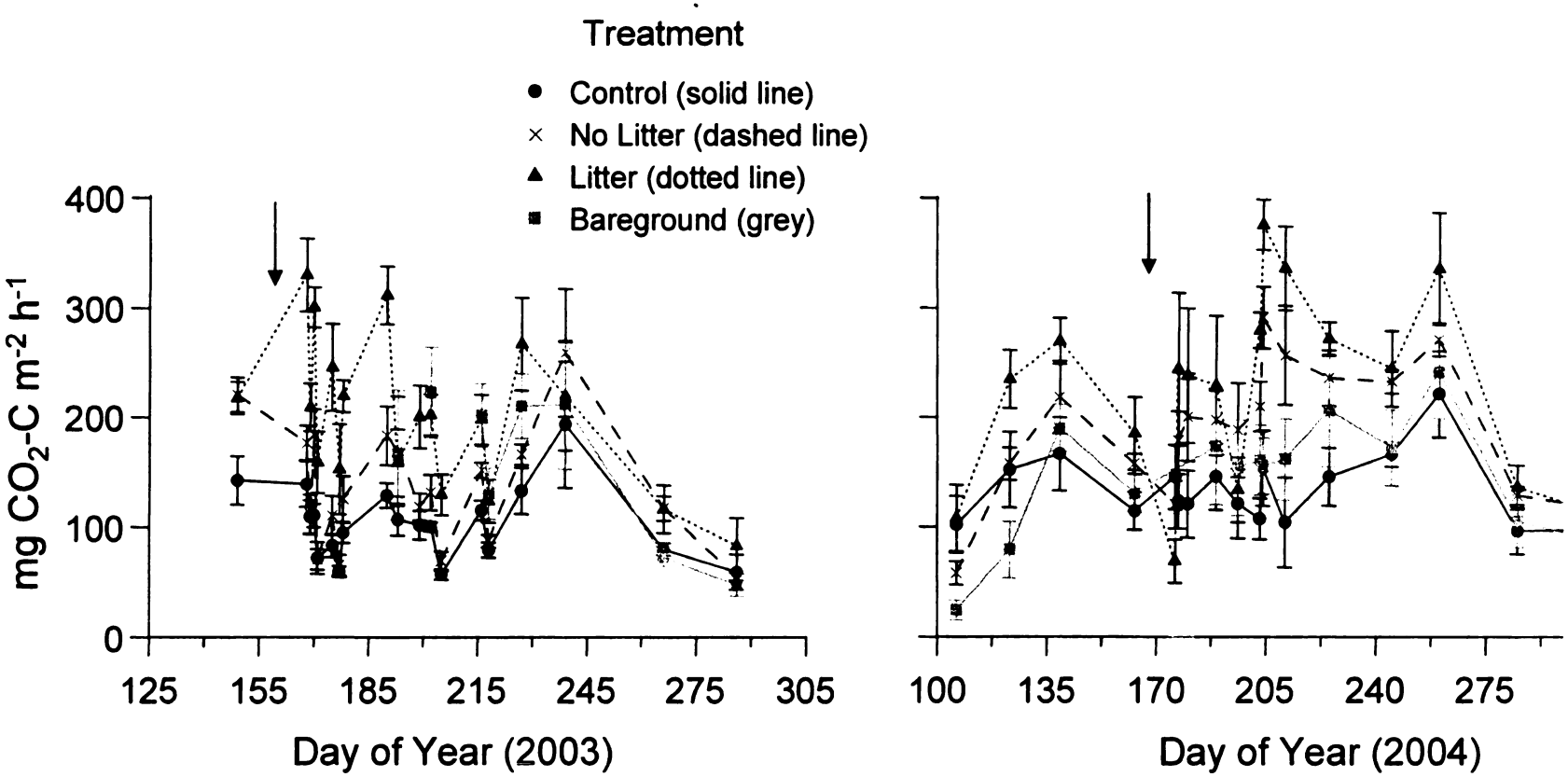

Figure 5.3. Cultivation effects in a previously uncultivated field on soil surface CO_2 fluxes in 2003 and 2004. No litter plots had all biomass removed prior to cultivation. Litter plots had all aboveground biomass incorporated with tillage. Bare-ground plots were weeded and contained root exclusion barriers. Arrows indicate cultivation dates. Bars are standard errors (n=4).

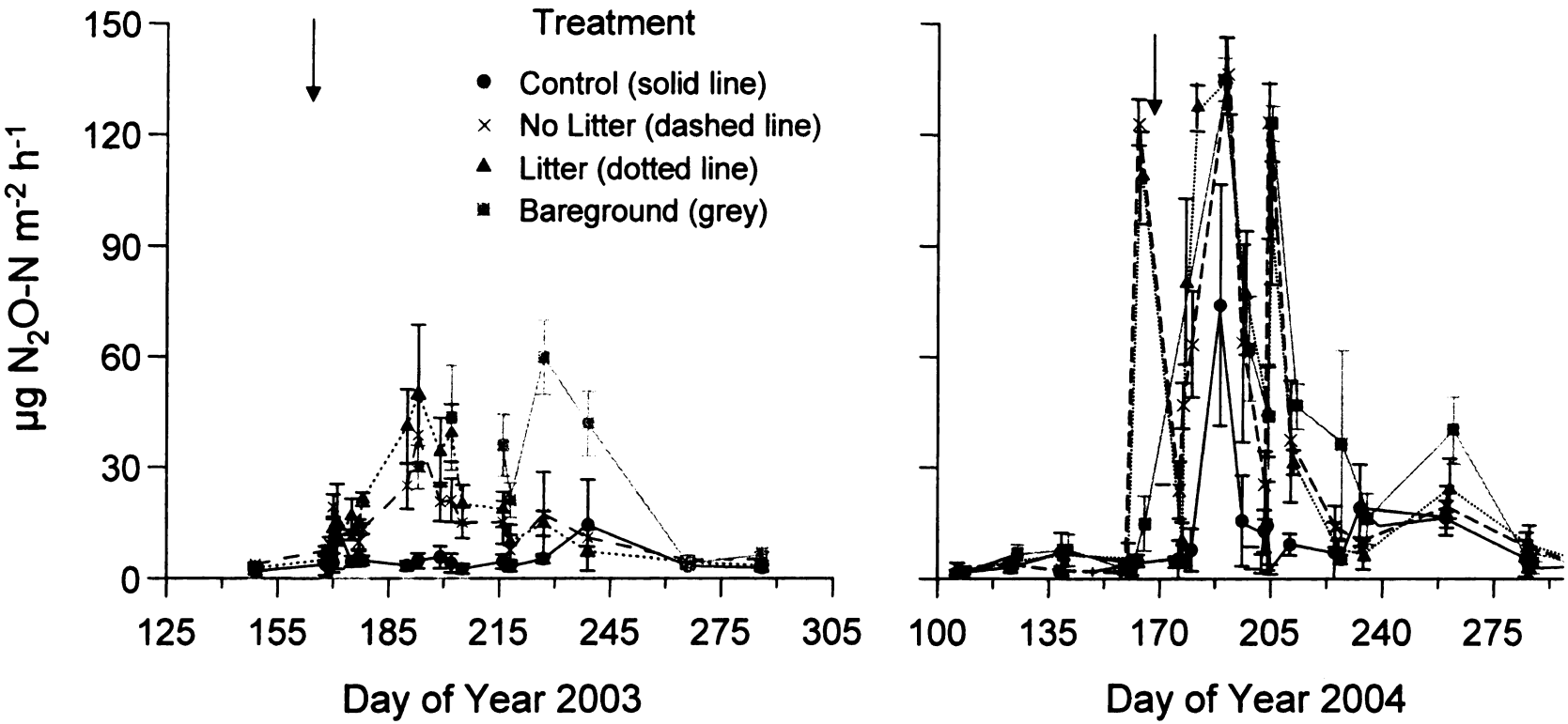

Figure 5.4. Cultivation effects in a previously uncultivated field on soil surface N_2O fluxes in 2003 and 2004. No litter plots had all biomass removed prior to cultivation. Litter plots had all aboveground biomass incorporated with tillage. Bare-ground plots were weeded and contained root exclusion barriers. Arrows indicate cultivation dates. Bars are standard errors (n=4).

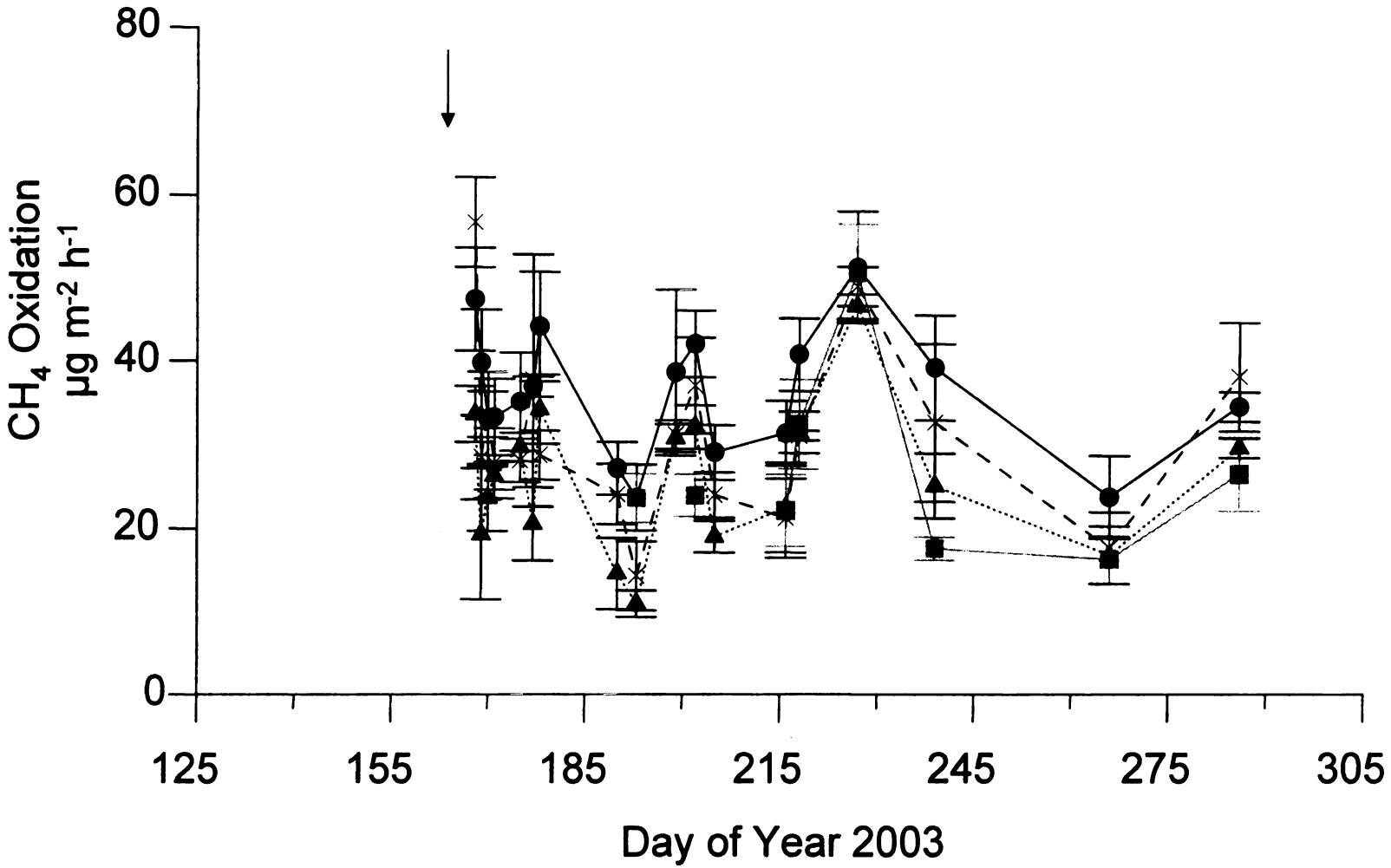

Figure 5.5. Cultivation effects in a previously uncultivated field on CH_4 oxidation in 2003. Measurements are not available for 2004. No litter plots had all biomass removed prior to cultivation. Litter plots had all aboveground biomass incorporated with tillage. Bare-ground plots were weeded and contained root exclusion barriers. The arrow indicates the cultivation date. Bars are standard errors (n=4).

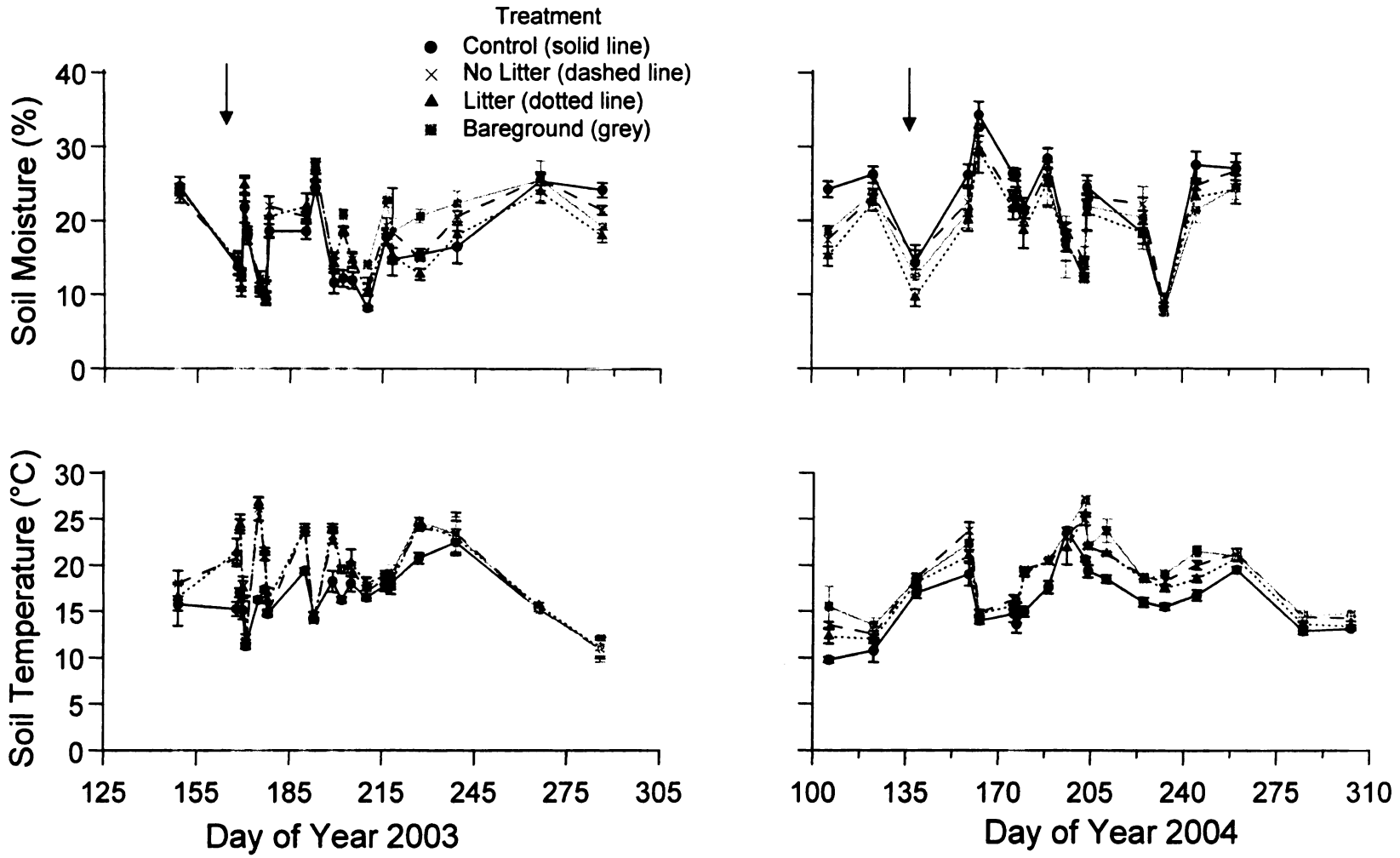

Figure 5.6. Cultivation effects in a previously uncultivated field on soil temperature and moisture in 2003 and 2004. No litter plots had all biomass removed prior to cultivation. Litter plots had all aboveground biomass incorporated with tillage. Bare-ground plots were weeded and contained root exclusion barriers. Arrows indicate cultivation dates. Bars are standard errors (n=4).

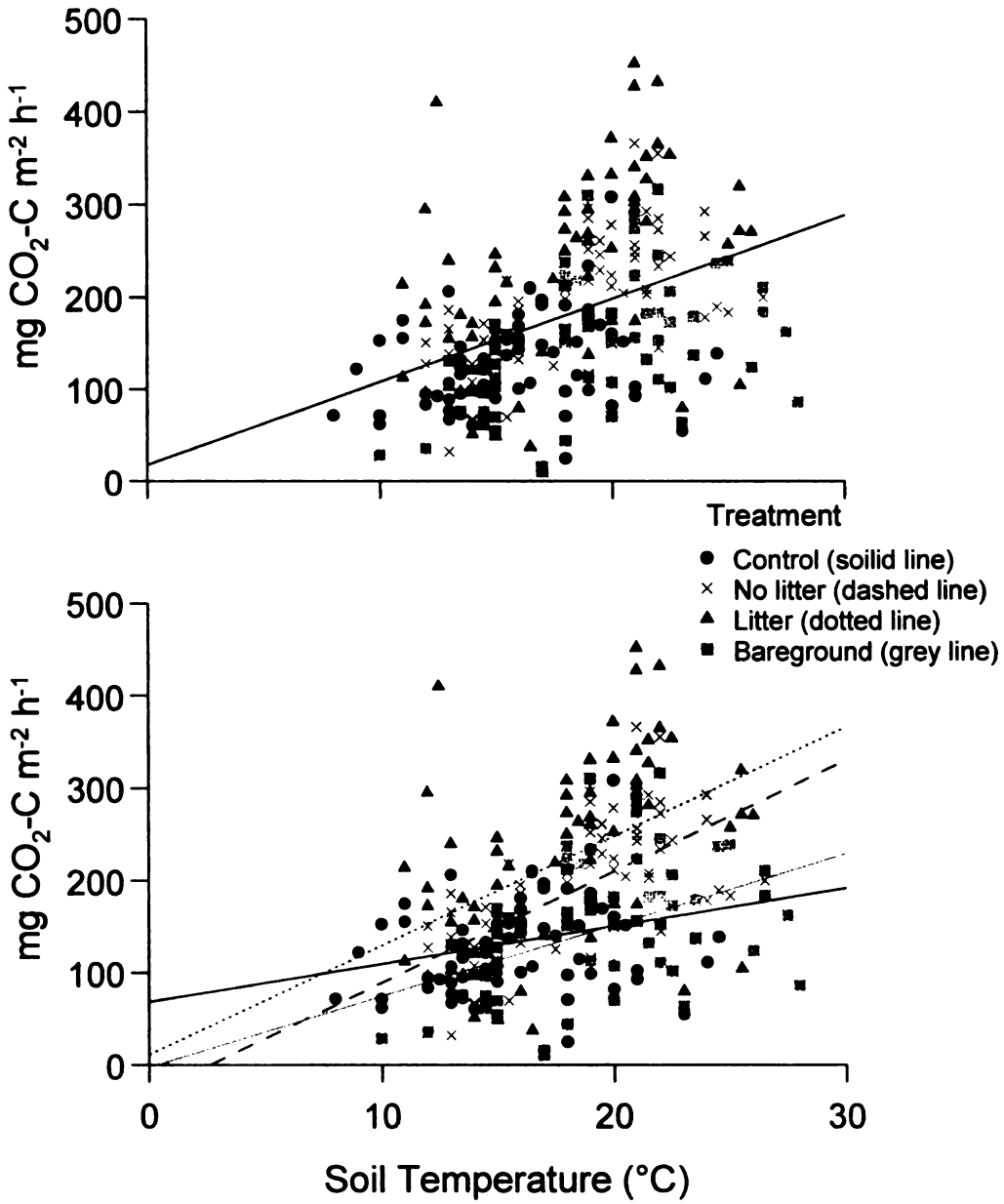

Figure 5.7. Relationship between soil temperature and CO_2 emissions in 2003 and 2004. Analysis of covariance (ANCOVA) results showed a significant temperature effect in 2003 ($p<0.003$): CO_2 flux = 14.9 + 0.026 (temperature), r2 = 0.22 ($p<0.000$). The line in 2003 thus represents all treatments. In 2004 there was a temperature by treatment interaction ($p<0.001$). Control plots: CO_2 flux = 13.3 + 0.020 (temperature), $r^2 = 0.08$ ($p<0.000$); no litter plots: CO_2 flux = 12.1 + 0.033 (temperature), $r^2 = 0.40$ ($p<0.000$); litter plots: CO_2 flux = 13.8 + 0.018 (temperature), $r^2 = 0.22$ ($p<0.000$); bare-ground plots: CO_2 flux = 15.5 + 0.026 (temperature), $r^2 = 0.20$ ($p<0.000$).

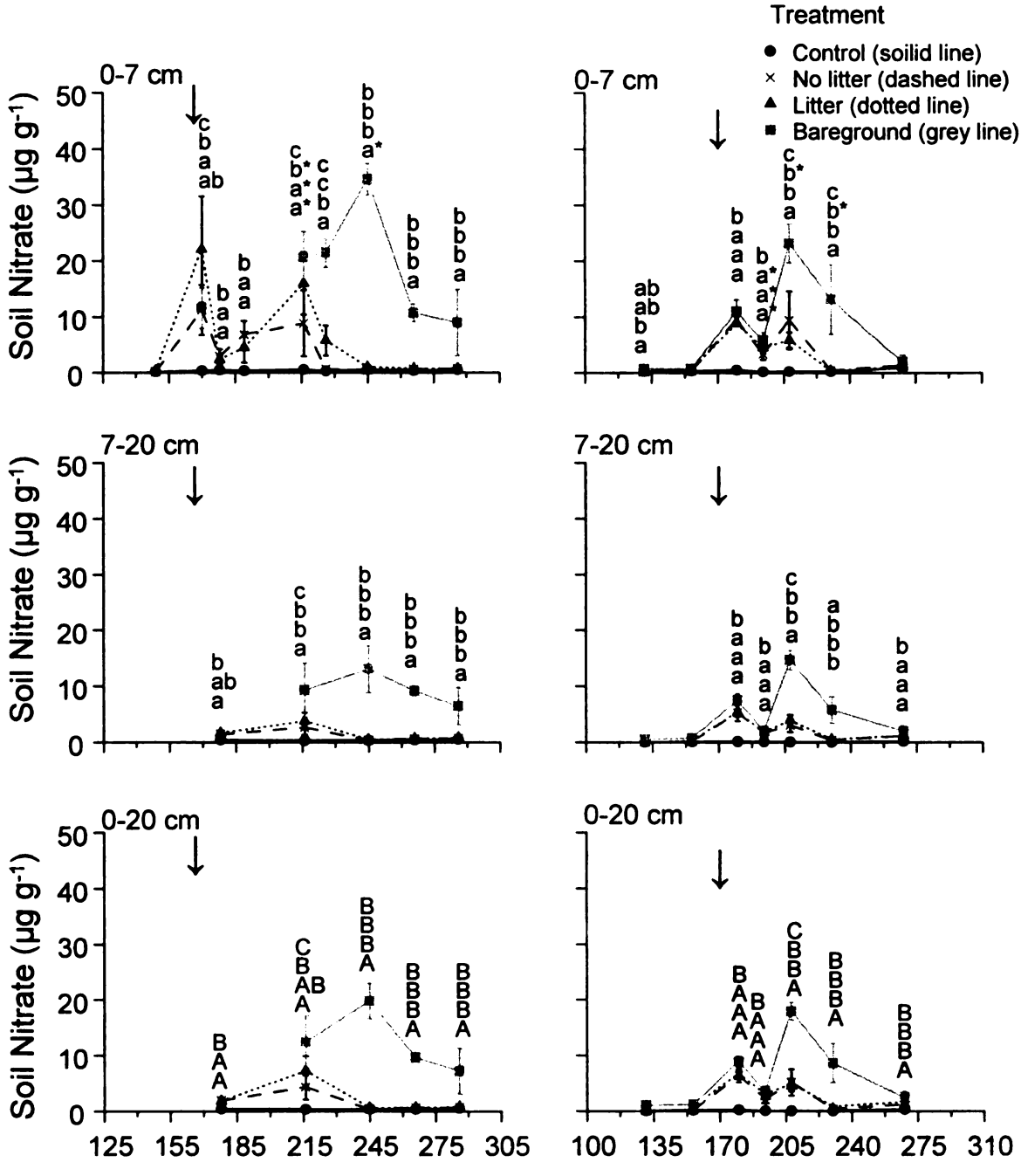

Figure 5.8. Cultivation effects in a previously uncultivated field on soil NO_3^- in 2003 and 2004 at 0-7, 7-20, and 0-20 cm depth. No litter plots had all biomass removed prior to cultivation. Litter plots had all aboveground bimass incorporated with tillage. Bare-ground plots were weeded and contained root exclusion barriers. Arrows indicate cultivation dates. Letters within a day of year are stacked in order: control, no litter, litter, bare-ground. Different letters indicate treatment differences within a day of year and soil depth. Asterisks indicate diffences between soil depths within a treatment and day of year. Bars are standard errors.

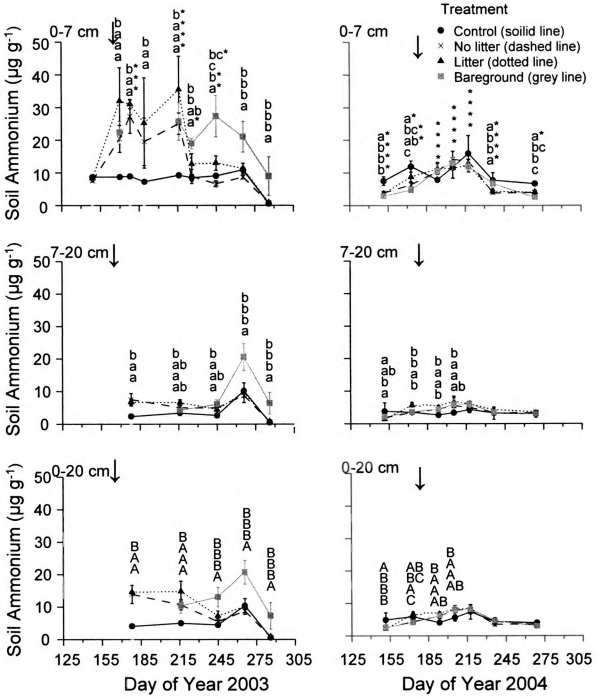

Figure 5.9. Cultivation effects in a previously uncultivated field on NH_4^+ in 2003 and 2004 at 0-7, 7-20, and 7-20 cm depth. No litter plots had all biomass removed prior to cultivation. Litter plots had all aboveground bimass incorporated with tillage. Bare-ground plots were weeded and contained root exclusion barriers. Letters within a day of year are stacked in order: control, no litter, litter, bare-ground. Different letters indicate treatment differences within a day of year and soil depth. Asterisks indicate diffences between soil depths within a treatment and day of year. Bars are standard errors.

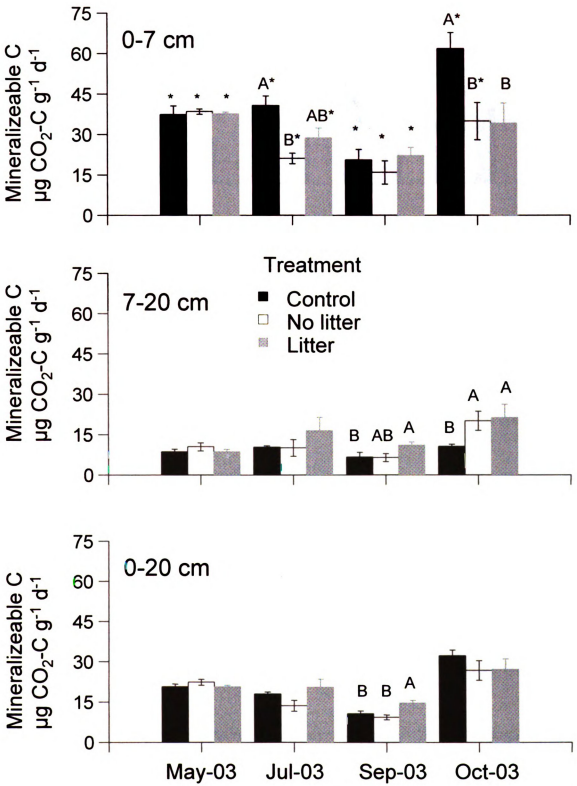

Figure 5.10. Cultivation effects in a previously uncultivated field on mineralizable C in 2003 at 0-7, 7-20, and 0-20 cm depth. No-litter plots had all biomass removed prior to cultivation. Bare-ground plots were weeded and contained root exclusion barriers.

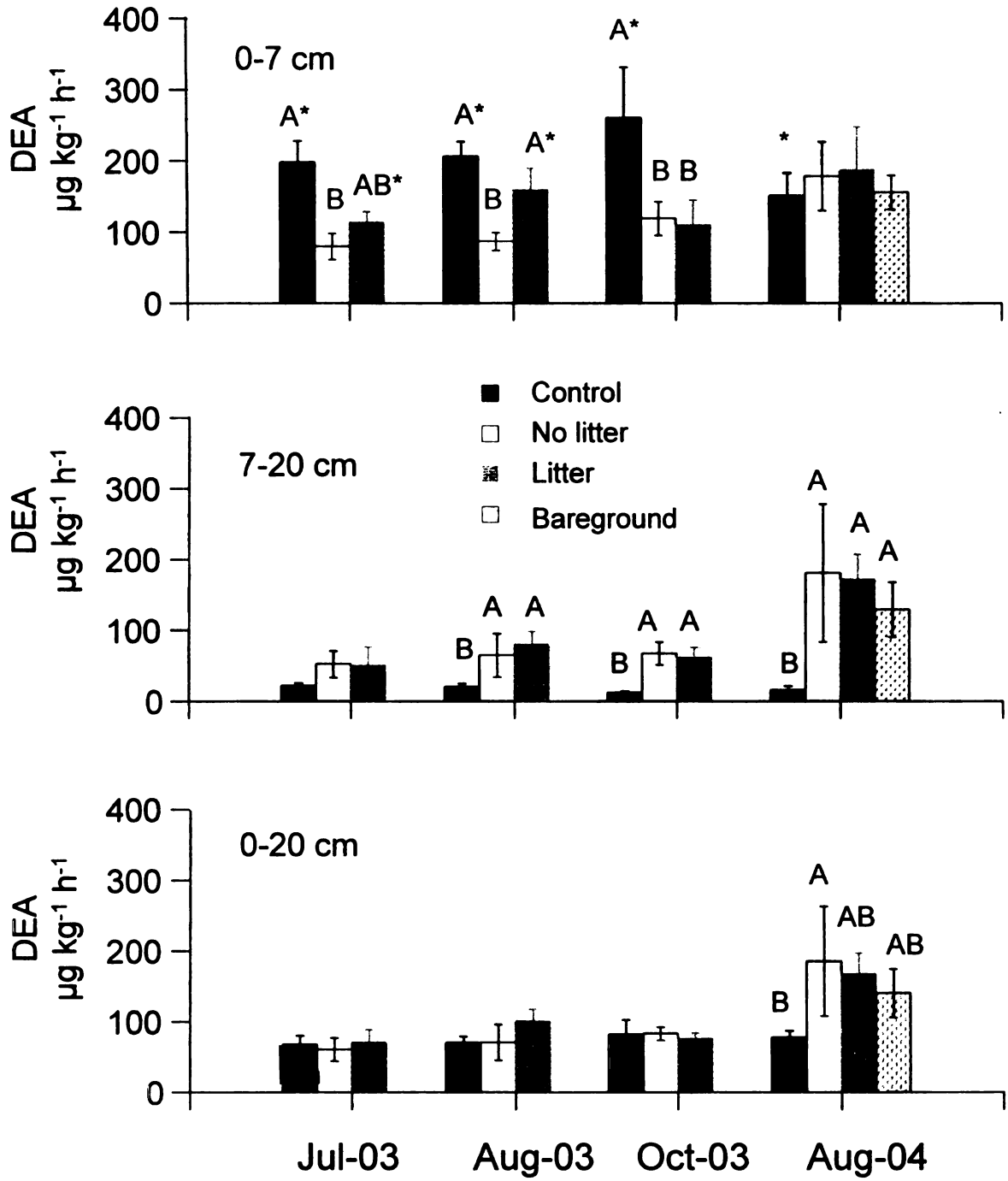

Figure 5.11. Cultivation effects in a previously uncultivated field on denitrification enzyme activity (DEA) in 2003 and 2004 at 0-7, 7-20, and 0-20 cm depth. No-litter plots had all biomass removed prior to cultivation. Bare-ground plots were weeded and contained root exclusion barriers.

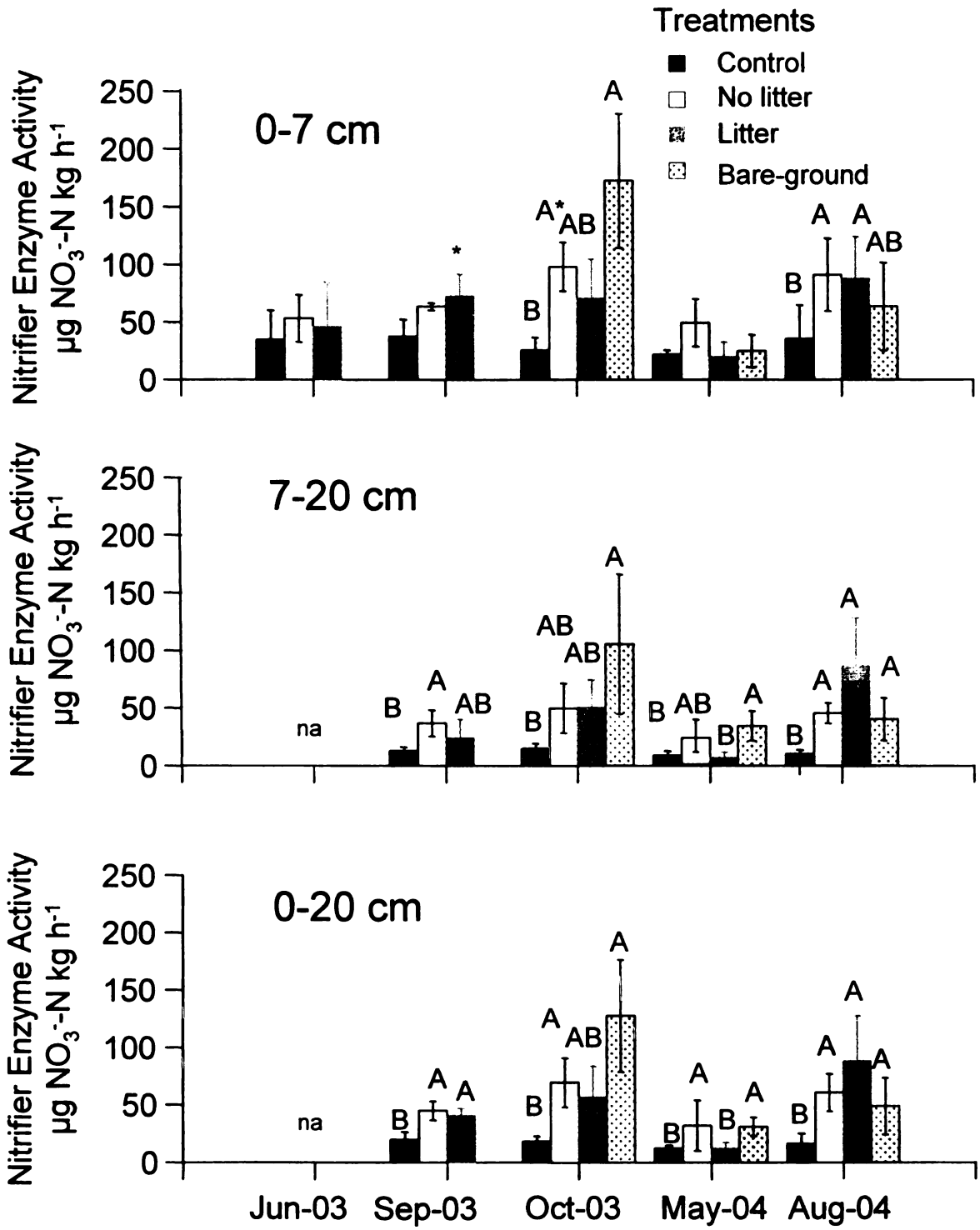

Figure 5.12 Cultivation effects in a previously uncultivated field on nitrifier enzyme activity in 2003 and 2004 at 0-7, 7-20, and 0-20 cm depth. No-litter plots had all biomass removed prior to cultivation. Bare-ground plots were weeded and contained root exclusion barriers.

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CHAPTER 6

Long-Term Trends in Nitrous Oxide Emissions, Soil Nitrogen, and Crop Yields of Till and No-Till Cropping Systems

ABSTRACT

No-till cropping can increase soil C stocks and aggregation but potential long-term changes in N_2O emissions, soil N availability, and crop yields still need to be resolved. We measured soil C accumulation, aggregation, soil moisture, N_2O emissions, soil inorganic N, and crop yields in till and no-till corn-soybean-wheat rotations between 1989 and 2002 in southwest Michigan and investigated whether tillage effects varied over time or by crop. Mean annual NO_3^- concentrations in no-till were significantly less in three of six corn years and during one year of wheat production. Yields were similar in each system for all but three of the 14 years during which yields were higher in no-till, indicating that soil NO_3^- reductions did not translate into yield losses. N_2O emissions were similar with till (3.27 ± 0.52 g N ha d^{-1}) and no-till (3.63 ± 0.53 g N ha d^{-1}) soil management. C accumulated in no-till soils at a rate of 26 g C m^{-2} y^{-1} over 12 yr; between 56 and 61 % of the reduction in CO_2 equivalents associated with this C sequestration was offset by N_2O emissions, however. After controlling for rotation and environmental effects by normalizing treatment differences between till and no-till systems we found no significant trends in soil N, N_2O emissions or yields through time. In these sandy-loam soils, no-till cropping enhances C storage, aggregation and associated environmental processes with no significant ecological or yield tradeoffs.

INTRODUCTION

Soil organic matter (SOM) losses are rapid following initial tillage of undisturbed soils (Martel and Paul, 1974; Balesdent et al., 1988; Davidson and Ackerman, 1993) and commonly approach or exceed 20-40% of the original soil C after twenty years (Davidson and Ackerman, 1993). Restoration of some portion of the 55 Pg C lost from arable soils globally is one of several short-term, high impact options for stabilizing global CO_2 levels (Caldeira et al., 2004; Cast, 2004; Lal, 2004). No-till cropping generally increases soil C stocks at an average annual rate of 30 - 60 g C m^{-2} y^{-1} (Davidson and Ackerman, 1993; West and Post, 2002) and is thus a prominent agricultural CO_2 mitigation strategy. Use of no-till soil management has been steadily increasing the past decade and between 1994 and 2004 the percentage of total cropland in no-till rose from 13.7 to 22.6%, or 9.5 million hectares (CTIC, 2004). Many more hectares are suitable for no-till but its deployment has been limited in part by the perception that accelerated rates of N leaching and immobilization may reduce plant N availability and decrease yields (Martens, 2001). Further, studies have suggested that increases in N_2O emissions following conversion to no-till might offset some portion of the CO_2 mitigation, making the long-term global warming potential (GWP) of the two systems similar (MacKenzie et al., 1997; Six et al., 2004).

Higher N_2O emissions in no-till than conventionally tilled cropping systems have been frequently reported (MacKenzie et al., 1997; Ball et al., 1999; Baggs et al., 2003) although some studies have found lower emissions in no-till soils or no difference between tillage systems (Robertson et al., 2000; Elmi et al., 2003), demonstrating the potential for responses to vary across cropping systems and soil types. In a recent review

Six et al. (2004) analyzed N_2O emissions data in a linear mixed-effect model and concluded that differences in N_2O fluxes between till and no-till change over time. In humid ecosystems N_2O fluxes are higher in no-till during the first decade but after 20 years no-till emissions are lower. In an assessment of potential European land-use changes on non-CO_2 greenhouse gases, Smith et al. (2001) calculated that eliminating tillage on all potential no-till lands could result in an increase in N_2O emissions equivalent to 20.5 Tg CO_2-C y^{-1}. The potential mechanisms explaining this include decreased O_2 availability associated with increased bulk density and water-filled pore space and increased soil organic matter decomposition in no-till soils (Linn and Doran, 1984; Guzha, 2004); alterations to the synchrony between plant N uptake and N availability (Six et al., 2004); and litter C accumulation at the soil surface (Arshad et al., 1999; Baggs et al., 2003).

Along with soil surface N_2O emissions, soil inorganic N cycling frequently changes following conversion to no-till and these changes may decrease yields (Rice et al., 1986; Niehues et al., 2004). Application of urea ammonium nitrate (UAN) fertilizer to the soil surface results in greater ammonia volatilization in no-till than in conventional till systems where fertilizer is incorporated into the soil (Keller and Mengel, 1986; Fox and Piekielek, 1993). Along with volatilization, immobilization of N in surface residues and decreased N mineralization rates can limit N availability and potentially decrease yields in no-till (Kaspar et al., 1987; Ismail et al., 1994). Reduced mineralization rates and other problems (e.g. delayed and uneven germination) due to cool and water-saturated soils may be particularly detrimental to yields in the northern corn belt (Kaspar

et al., 1987; Vetsch and Randall, 2000) and on fine-textured soils (Vyn and Raimbault, 1993; Beyaert et al., 2002).

As with N_2O emissions, soil N cycling and crop productivity responses to no-till may change over time. Vyn and Raimbault (1993) found that corn yields increased in no-till during the first eight years and then declined. Other studies have found that no-till has no effect on yields (Mehdi et al., 1999; Beyaert et al., 2002), decreases yields initially before a yield recovery (Rice et al., 1986), or is related to annual environmental conditions or other factors independent of time since initiation (Ismail et al., 1994). Additional data are needed describing long-term annual trends in soil N cycling, yields and, particularly, N_2O emissions to develop predictive models for long-term no-till cropping systems.

Our objectives in this study are to: 1) determine the effects of long-term no-till soil management in a corn-soybean wheat rotation on C sequestration and the extent to which increases in N_2O flux can offset the GWP impact of enhanced C storage; 2) determine whether changes in soil moisture content, N availability, and yields occur that could limit the deployment of no-till in our region (southwest Michigan); and 3) investigate whether responses to no-till vary over 14 y of no-till management. In a previous study Robertson et al. (2000) reported no difference in the mean annual N_2O flux from these treatments between 1991 and 1999. Here, we investigate soil moisture content, N_2O emissions, soil N concentrations, and crop yield by year to better understand the inter-annual patterns associated with no-till management between 1989 and 2002. We predict that N_2O emissions will initially be higher in no-till soils and then

decrease over time and that soil inorganic N concentrations will be lower in no-till soils, potentially lowering yields of corn and wheat in these northern corn belt soils.

MATERIALS AND METHODS

Experimental Site

Experimental plots were located at the W.K. Kellogg Biological Station Long-Term Ecological Research (LTER) Site (http://lter.kbs.msu.edu) in southwest Michigan (85° 24'W, 42° 24'N; 920 mm y^{-1} precipitation). Tillage treatments were established in 1989 in six replicated one ha plots organized in a randomized complete block design. Soils are Kalamazoo (fine-loamy) and Oshtemo (coarse-loamy) mixed, mesic, Typic Hapludalfs developed on glacial outwash (Crum and Collins, 1995). The two series co-occur in all ecosystems and differ mainly in their Ap horizon texture, though variation within a series can be as great as variation between a series (Robertson et al., 1997).

Agronomic Protocols

The crop rotation prior to 1995 consisted of corn followed by soybeans. In 1995, wheat was planted after soybean, initiating a corn-soybean-wheat rotation. Primary tillage from 1989 to 1998 consisted of spring moldboard plowing and spring chisel plowing from 1999 and 2002. Secondary tillage consisted of disking before wheat planting, field conditioner prior to soybean and corn planting, and inter-row cultivation for soy and corn. Fertilizer applications were identical in both systems. Detailed information about annual applications can be found on the KBS LTER website (http://lter.kbs.msu.edu). Side-dress NH_4NO_3 fertilizer was broadcast over corn in 1989

(112 kg N ha^{-1}), 1991 (112 kg N ha^{-1}) and 1993 (84 kg N ha^{-1}). In 1996 and 1999, corn received 28 kg N ha^{-1} UAN (28%) at planting and an additional 135 kg side-dress N ha^{-1} as $NH_4^+NO_3^-$. In 2002, corn received 29 kg N ha^{-1} starter fertilizer and an additional 124 kg side-dress N ha^{-1}. In April 1995 and 1998 wheat received 56 kg side-dress N ha^{-1} broadcast as $NH_4^+NO_3^-$. The 2001 wheat crop received 71 kg side-dress N as UAN. No fertilizer was applied to soybeans. Lime, P, and K were applied as needed according to Michigan State University best management recommendations.

Soil Sampling and Storage

Soil samples for aggregate size distribution and total C and N analysis were collected from five sites within each plot in June and July, 2001. At each of the five sample locations, two subsamples with a diameter of 7.6 cm were taken to a depth of 5 cm by gently hammering a PVC core into the ground to minimize compression and slicing of aggregates. One of the subsamples at each station was taken in the row and the other between the rows. All ten subsamples from each plot were combined to produce one representative sample. Four separate samples for bulk density analysis were taken at the same time as those for aggregate analysis, using a 8.0 cm diameter root corer.

Field-moist soil samples were put into a cooler (4°C) prior to being broken along natural fracture planes and passed through an 8 mm sieve within 72 h of sampling. After sieving, soils were air-dried in paper bags at 20°C prior to storage in plastic bags. Care was taken throughout the study to minimize disturbance of the samples that might influence aggregate structure. Total soil C and N concentrations were determined by dry

combustion methods in a CHNS analyzer (Costech ECS 4010, Costech Analytical Technologies, Valencia CA.).

Soil Aggregation

Aggregate distribution was determined on triplicate 35 g air-dried soil samples by hand-sieving in water through a series of sieves (2000 μm, 250 μm, and 53 μm). Mean weight diameter (WMD) of sand-free aggregates was determined by calculating the sum of the products of the mean diameter of each size fraction and the proportion of the total sample weight in that fraction (Kemper and Rosenau, 1986).

N_2O Gas Emissions

N_2O measurements were made using static chambers (Livingston and Hutchinson, 1995) at weekly to monthly intervals when soils were not frozen (Robertson et al., 2000). Single chambers were located in four of the six replicates of each treatment. Chamber lids were placed on semi-permanent aluminum bases removed only for cropping activities and accumulated headspace sampled four times over 120 min. All chambers were sampled on the same dates between 1991 and 2002, although no data are available for 1995. Samples were stored in 3 ml crimp-top vials and analyzed in the laboratory for N_2O with the flux for each chamber calculated as the linear portion of the gas accumulation curve for that chamber. N_2O was analyzed by gas chromatography using a ^{63}Ni electron capture detector (ECD).

Inorganic Soil N

NH_4^+ and NO_3^- were extracted monthly from 1991-1995 and bimonthly from 1996-2002 from triplicate, field-moist, 10 g soil samples using a 1:10 soil/extractant ratio. Soil slurries were shaken for 1 minute, left to equilibrate overnight, and re-shaken >1 h prior to filtering. Extracts were filtered with a syringe filter using a one μm, glass fiber filter. Filtrates were stored in 7 ml scintillation vials and frozen until analysis for NH_4^+ and NO_3^- using an automated flow injection analyzer (NH_4^+ via diffusion colorimetry and NO_3^- via cadmium reduction and colorimetry).

Global Warming Potential

Global Warming Potential (GWP) calculations were made for soil C accumulation in no-till relative to till between 1989 and 2001 and N_2O emissions from both tillage systems. GWP for soil C accumulation in no-till was calculated according to Robertson et al. (2000) as follows:

$$X\,g\,CO_2 \cdot m^{-2} \cdot y^{-1} = \frac{(x_1 - x_2)\,kg\,C}{m^2 \cdot x_3 y} \times \frac{44\,kg\,CO_2}{12\,kg\,C} \times \frac{10^3\,g\,CO_2}{1\,kg\,CO_2}$$

where x_1 = soil C in no-till (kg C m^{-2}); x_2 = soil C in conventional till (kg C m^{-2}); and x_2 = years (y) of accumulation. GWP calculations for N_2O used an IPCC 20-year time horizon factor of 280 for N_2O:

$$X\,g\,CO_2 \cdot m^{-2} \cdot y^{-1} = \frac{x_1\,g\,N_2O\text{-}N}{ha \cdot d} \times \frac{44\,g\,N_2O}{28\,g\,N_2O\text{-}N} \times \frac{365\,d}{1\,y} \times \frac{1\,ha}{10^4\,m^2} \times \frac{280\,g\,CO_2}{1\,g\,N_2O}$$

where x_1 = average daily N_2O-N emission rate (g N ha^{-1} d^{-1}).

Statistical Analysis

Tillage effects were analyzed by Proc Mixed (Version 8.2, SAS Institute, 1999) using a randomized complete block design analysis of variance (ANOVA) with repeated measures. Year and tillage treatments were considered fixed effects. Where significant treatment by year interactions occurred, results were sliced to determine whether treatment means significantly differed within individual years. Additionally, to determine trends in N_2O flux, soil inorganic N and crop yields over time, differences between treatments within each block and year were normalized to the overall mean for that year:

$$\text{Normalized percent difference} = [((Y_c - Y_n) / Y_{annual})^* 100]$$

where Y_c and Y_n are the annual mean for each block within a year for conventional and no-till, respectively, and Y_{annual} is the overall mean for that year, averaged across treatments. Regression analysis was used to determine whether there were trends in the response of till relative to no-till sites across crops and years and also whether trends differed by crop. By normalizing the data prior to the regression analysis rather than using absolute differences we were able to isolate the relative effect of no-till over time and control for factors, such as the use of fertilizer or climatic differences, that might cause inter-annual variation in the magnitude of response in both treatments. The normalized differences were also analyzed by ANOVA using Proc Mixed with year as the fixed effect. Where there were significant year effects, indicating that the normalized difference varied with year, mean separation and ranking was carried out using a Tukey's test with the PDMix800 Algorithm (Saxton, 1998).

RESULTS

Soil Properties

In 2001, 12 years after the adoption of no-till, soil aggregate MWD was 55% higher in no-till soils and there was an additional 310 g C m^{-2} to a depth of 5 cm (Table 6.1). This change over 12 years represents an annual C increase to 5 cm of 26 g C m^{-2} y^{-1}. No bulk density differences were detected between the two treatments (Table 6.1).

There was a significant treatment by year interaction ($p<0.05$) for gravimetric soil moisture content (Figure 6.1). Soil moisture content was greater in no-till than till in 2000 but in all other years soil moisture content was similar in the two treatments. Regression analysis of the normalized difference between till and no-till treatments found no trend across all years or for particular crops.

There was a significant treatment by year interaction ($p<0.05$) for soil NO_3^- concentrations (Figure 6.2) indicating that the effects of tillage differed by year. No till reduced NO_3^- concentrations in 1991 (corn, 80%), 1996 (corn, 29%), 1999 (corn, 41%) and 2001 (wheat, 28%). There was not a significant linear trend in NO_3^- concentrations over all years, although there was a significant positive relationship between time and the normalized NO_3^- difference during wheat years (normalized NO_3^- difference = -15650 + 7.84(year); $r^2 = 0.44$; n = 3). This suggests that over 1995, 1998 and 2001 soil NO_3^- concentrations declined in no-till wheat plots relative to tilled plots. Mean separation showed that these three points were statistically equal and that 1991 was different from the other years because of higher NO_3^- concentrations in tilled soils.

Response of soil NH_4^+ concentration to no-till also varied by year ($p<0.05$; Figure 6.3). NH_4^+ concentrations were higher in tilled sites in 1991 (corn) and 2002 (corn) and

no-till sites in 2000 (soybean). Linear regression showed that there was not a significant trend in NH_4^+ concentrations across all the crops and years and there were no significant trends for individual crops. Mean separation of the normalized difference by year showed the potential for inter-annual variability in the response to tillage but also showed no consistent trends by crop.

N_2O Emissions and GWP

There was no detectable difference in N_2O emissions between tillage treatments averaged across years (conventional till = 3.27 ± 0.53; no-till = 3.63 ± 0.53 g ha^{-1} d^{-1}), but there was a significant year by treatment interaction (Figure 6.4) with higher emissions occurring in no-till plots in 1991 (corn, 300%) and 2000 (soybean, 300%). Linear regression showed that there was no significant relationship between time and the normalized difference in N_2O flux across years and crops but indicated that for the soybean crop N_2O emissions increased in no-till plots relative to till plots over 4 growing seasons between 1992 and 2000 (normalized N_2O difference = 31210 – 15.7 (year); r^2 = 0.48; n = 4 years). Tukey's test ($p<0.05$) showed that normalized differences in N_2O emissions were similar across years. In the no-till system C storage between 1989 and 2001 reduced soil CO_2 fluxes by 95 g CO_2 m^{-2} y^{-1} but 56-61% of that mitigation was offset by N_2O emissions of 53-58 g CO_2 equivalents m^{-2} y^{-1} (Table 6.2).

Crop Yields

There was a significant ($p<0.05$) treatment by year interaction for crop yields (Figure 6.5). Crop yields were significantly greater in no-till sites in 1992 (soybean),

1996 (corn) and 1997 (soybean). There was not a significant linear relationship between crop yield and time across years. There was a significant positive relationship between time and wheat yields in no-till relative to conventional till (normalized yield difference = -5980 + 3.00(year); $p < 0.05$; $r^2 = 0.40$; $n = 3$ years) for three growing years between 1995 and 2001. Mean separation indicated that the normalized mean difference was statistically equal for these three wheat years.

DISCUSSION

N_2O and GWP

Although we measured significantly greater N_2O fluxes from no-till plots in 1991 and 2000 such differences were not sustained throughout the study, and averaged across years there was not a significant effect of tillage (Figure 6.4). Grant et al. (2004) predicted that across all of Canada adoption of no-till would reduce N_2O emissions by an average of 17% but in Eastern Canada greater precipitation and soil water-filled pore space would increase N_2O emissions. Grant et al.'s predictions are consistent with Six et al. (2004) that in humid climates no-till systems will have higher N_2O emissions for a decade or more after conversion. MacKenzie et al. (1997) found in eastern Canada that no-till increased N_2O emissions from 17 to 60%. In Saskatchewan, Canada, Aulakh et al. (1984) found that no-till doubled N_2O fluxes.

The mechanisms proposed to explain higher N_2O fluxes in no-till soils are related mainly to C accumulation at the soil surface, increased bulk density, and decreased crop yields altering the availability of C, N and O_2 (Ball et al., 1999; Smith et al., 2001). While some no-till soils may be more susceptible to O_2 depletion following precipitation

(Doran, 1980; Martens, 2001), the 50% greater aggregate MWD and statistically equal bulk density that we measured in no-till suggest that drainage and hence O_2 availability are similar or higher than those in tilled soils. Although these measurements were made 12 yr after no-till conversion and may not represent soil structural dynamics during the first few years after adoption of no till, they are confirmed by measurements made on samples taken in 1995 (Six et al., 2000) showing statistically greater aggregation in no-till. Other studies have shown that no-till increases aggregation (Liebig et al., 2004), and that these changes can occur within the first few years after adoption (Rhoton, 2000), and have a positive impact on water dynamics and crop yields (Diaz-Zorita et al., 2004).

The association between decreased crop yields and increased N_2O flux in no-till has been attributed partly to an asynchrony between crop N uptake rates and soil N availability (Six et al., 2004). Resulting changes in temporal patterns of soil inorganic N availability coupled with greater water-filled pore space and C availability stimulate N_2O production. In our system, no-till did not significantly affect crop yields averaged over the entire experiment although in 1992 (soybean), 1996 (corn), and 1997 (soybean) there were higher yields in no-till, suggesting that this asynchrony did not differ between treatments.

Our results demonstrate that N_2O emissions are a critical component of the GWP of agricultural ecosystems (Table 6.2). We found that 56-61% of the reduction in GWP associated with C sequestration in the top 5 cm was offset by N_2O emissions from no-till. These results are consistent with Robertson et al. (2000) who found that 51% of the soil C storage to 7 cm in no-till was offset by N_2O emissions between 1991 and 1999 in these plots. Over time, C sequestration rates will slow down in the no-till soils as they

approach a new C equilibrium (West and Post, 2002) and, concomitantly, the proportion of annual C sequestration that is offset by N_2O emissions will increase.

Robertson et al. (2000) found that conventional, no-till, low-input, organic, and continuous alfalfa cropping systems at the KBS LTER produced statistically similar N_2O emissions between 1991 and 1999 and that only successional communities, where soil N concentrations were substantially lower, had lower N_2O emission rates. In our soils, increases in N_2O emissions from no-till are nil or a small proportion of the total N_2O flux from agricultural soils receiving supplemental anthropogenic N (Table 6.2). Asynchrony between N availability and crop uptake in systems receiving supplemental N results in excess soil inorganic N and enhanced N_2O production (MacKenzie et al., 1998; Grant et al., 2004). Efforts to mitigate N_2O emissions from agricultural cropping systems should thus focus on improving N use efficiency in cropping systems (Mosier et al., 1998; Mosier, 2001) rather than on tillage differences. Management practices designed to better synchronize plant uptake with N availability have been described in detail elsewhere (Mosier et al., 1998; CAST, 2004) and include using plant and soil sampling to determine crop N requirements, split N fertilizer applications, and slow-release fertilizers.

Changes in Yields and Soil Properties

In the northern corn belt of the U.S., cold soils, compaction, and decreased N availability can be particularly problematic in no-till soils, resulting in yield declines (Mehdi et al., 1999; Vetsch and Randall, 2004). We found that that there was no yield cost for no-till corn, soybean or wheat. In fact, yields were similar in each system for all

but three of the 14 years during which yields were higher in no-till, indicating that soil NO_3^- reductions did not translate into yield losses.

The use of starter N fertilizer, side-dress N, and a two to three year rotation in our experiment likely contributed to the persistence of equal or greater yields in no-till. These management strategies reduce N limitation due to low mineralization rates or fertilizer N immobilization in no-till. Vetsch and Randall (2000) reported an increase in continuous corn yields of 0.5 Mg ha^{-1} when starter fertilizer was used in no-till systems and Vetsch and Randall (2002) concluded that optimizing no-till corn-yields following soybean also required the use of starter fertilizer. Starter fertilizer enhances early season crop growth and increases corn yields in no-till soils that may be susceptible to spring N deficiencies (Jokela, 1992; Bullock et al., 1993). Side-dressing N after corn emergence further optimizes crop N use efficiency by targeting the crop when it is actively removing soil N (Fox et al., 1986; Mosier, et al., 1998; Vetsch and Randall, 2004). In a review of N cycling in no-till, Martens (2001) suggested that N limitation may not be as great when corn is grown in rotation with other crops with differing N requirements.

Increased aggregation and soil C content indicates that, over time, no-till may have become a better habitat for crop growth. Increased aggregate MWD will improve aeration and drainage and make the system more resilient to compaction. Increased soil C concentrations will improve soil structure and erosion resistance and potentially increase the N mineralization potential during the growing season when soils are warm enough to support active decomposition.

Trends over Time

Predicting N_2O emissions and crop yields in no-till systems depends on understanding short and long-term dynamics following transition to no-till. The potential for N cycling, crop yield and N_2O emissions in no-till to be dependent on the time since conversion has been demonstrated by other studies (Rice et al., 1986; Vyn and Raimbault, 1993; Six et al., 2004). We did not detect a trend through time in the difference between till and no-till for soil moisture, N_2O emissions, soil nitrate and ammonium, or crop yields. Analysis of variance and mean separation of the normalized differences by year further showed that while there was some inter-annual variability, different years were generally very similar. Corn yields in both treatments were lower in 1996, 1999 and 2002 than in previous years due to localized, seasonal droughts during critical stages of corn development.

There was some evidence of changes in N cycling shortly after the adoption of no-till. Relatively high rates of N_2O flux were measured in 1991 and although these differences were not sustained, they do corroborate other results showing the potential for increased N_2O production after no-till conversion (Ball et al., 1999; Yamulki and Jarvis, 2002; Baggs et al., 2003). Additionally, there seemed to be a trend towards decreased soil N concentrations in no-till corn over time. In 1989, the NO_3^- concentrations were the same in both treatments but in 1991, 1996, 1999, and 2001 there was lower NO_3^- in no-till sites. However, linear regression, even with the anomalous 1991 results removed (data not shown), did not show a significant relationship between time and normalized NO_3^- difference.

Long-term responses to no-till may vary considerably with soil type, climate and management practices and their effects on litter accumulation and soil bulk density. Following conversion to no-till, increased N immobilization is likely where litter C accumulates rapidly at the soil surface (Baggs et al., 2003), and N_2O production should increase where increased bulk density alters soil pore-space dynamics (Linn and Doran, 1984; MacKenzie et al., 1998). McConkey et al. (2002) reported that N cycling and grain yield responses to no-till varied considerably with soil type. On a sandy-loam soil, the apparent N balance (applied N minus N removed in grain) was 26% greater in no-till than minimum till sites over 13 years of continuous wheat; on a clay soil the apparent N balance was 78% greater in no-till than continuous till sites suggesting that there was greater immobilization or gaseous losses of N on fine-textured soils. Several studies reporting increased N_2O emissions following no-till conversion have also been conducted on fine-textured soils including Ste. Rosalie clay and Ormstown silty clay loam (MacKenzie et al., 1997; 1998); imperfectly drained clay loam (Ball et al., 1999); clay-loam (Aulakh et al., 1984); and silt loam (Baggs et al., 2003). In contrast, Elmi et al. (2003) found that no-till did not have higher N_2O emissions than conventional till on a sandy-loam soil in Quebec, Canada.

Over time, increases in soil aggregation and associated soil physical properties and increased yields might decrease N_2O production in fine-textured, no-till soils. Rapid increases in N_2O emissions following cultivation of long-term no-till soils provides evidence for the benefits of long-term no-till (Estavillo et al., 2002; Pinto et al., 2004). In our system with relatively low clay contents and low rates of litter accumulation due to

modest productivity and high decomposition rates, the negative effects of no-till are nil or short-lived over the first 12 years.

CONCLUSIONS

At the KBS LTER Site no-till increased soil C in the top 5 cm from 0.69 to 1.00 kg C m^{-2} and increased aggregate MWD from 1.32 to 2.04 mm. N_2O emissions were higher in no-till in two out 10 years but there was no significant effect of tillage averaged across years. N_2O emissions offset between 56 and 61% of the CO_2 reduction associated with C sequestration in no-till. In the three years where significant tillage effects on yield occurred, no-till had higher yields than conventional till, indicating that decreases in N availability with no-till did not reduce yields. There were no trends in soil N cycling, yields, or N_2O emissions across all years. No-till effects on fine-textured soils are likely stronger than those on sandy loam and other coarse-textured soils with high sand concentrations. Our results demonstrate that the adoption of no-till can increase soil C storage and physical structure without increased N_2O emissions or yield trade-offs.

Table 6.1. No-till soil management effects on soil aggregation, bulk density, and C sequestration in 2001 after 12 y at the KBS LTER Site.[†]

	Aggregate MWD	Bulk Density	Organic C	$\Delta C^{\ddagger}$
	mm	g cm^{-3}	kg m^{-2}	g m^{-2} y^{-1}
Conventional till	1.32 (0.14)*	1.37 (0.01)	0.69 (0.04)*	0
No till	2.04 (0.11)	1.36 (0.03)	1.00 (0.04)	26

* Significant at the $p<0.05$ probability level

[†] 0-5 cm sampling depth

[‡] MWD: mean weight diameter.

[§] Robertson et al. (2000) reported a C accumulation rate of 30 g C m^{-2} to 7 cm.

Table 6.2. Global warming potential (GWP) contributions in CO_2 equivalents from soil C storage and N_2O emissions in conventional and no-till systems between 1991 and 2002 at the KBS LTER Site.

	Conventional till	No till
	CO_2 Equivalents (g m^{-2} y^{-1})	
Soil C Storage[†]	0	-95
N_2O Emissions	53	58
GWP (N_2O + CO_2)	53	-37

[†] 0-5 cm sampling depth.

[‡] N_2O emissions were statistically equal in till (3.27 ± 0.52 g N ha d^{-1}) and no-till (3.63 ± 0.53 g N ha d^{-1}). N_2O production thus offset between 56 and 61% of the CO_2 stabilization associated with soil C increases in no-till.

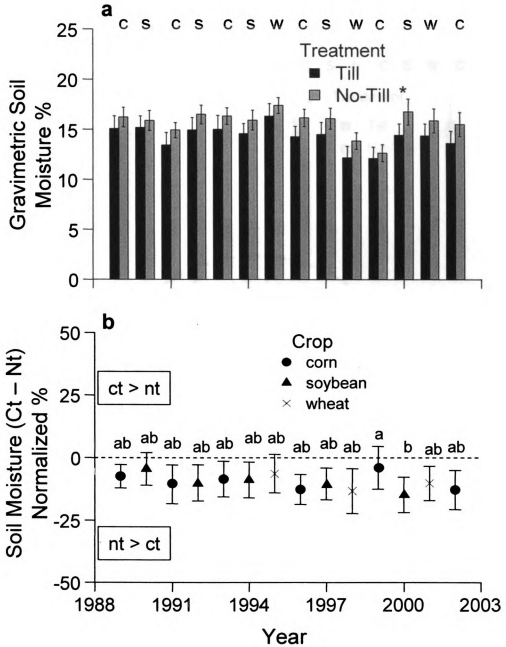

Figure 6.1. (a) Gravimetric soil moisture content in till and no-till treatments between 1989 and 2002. Letters indicate the crop harvested in that year: c = corn; s = soybean; w = wheat. * indicates statistically different responses within a year ($p<0.05$) determined by slicing of the treatment by year interaction. (b) Difference between conventional till (Ct) and no-till (Nt) normalized by the overall mean soil moisture content for that year. Points greater than zero indicate a till response greater than a no-till response. Year means were significantly different ($P<0.05$) and separated using Tukey's test. Linear regression analysis showed no trends in soil moisture concentration ($p<0.05$) across all crops and years or for specific crops over time.

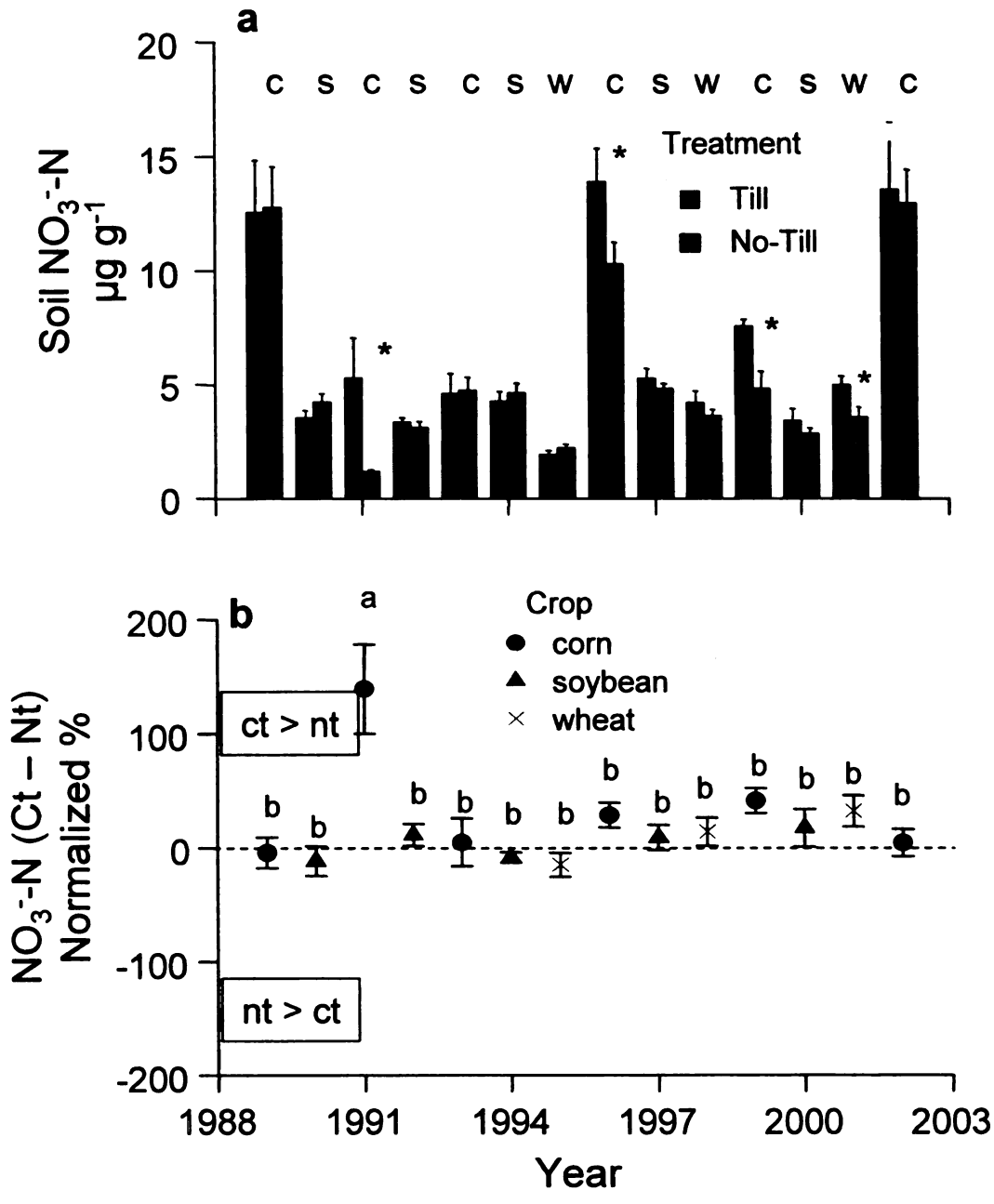

Figure 6.2. (a) Soil NO_3^- concentration in till and no-till treatments between 1989 and 2002. Letters indicate the crop harvested in that year: c = corn; s = soybean; w = wheat. * indicates statistically different responses within a year ($p<0.05$) determined by slicing of the treatment by year interaction. (b) Difference between conventional till (Ct) and no-till (Nt) normalized by the overall mean NO_3^- concentration for that year. Points greater than zero indicate a till response greater than a no-till response. Year means were significantly different ($P<0.05$) and separated using Tukey's test. Linear regression analysis showed no trend in NO_3^- concentration ($p<0.05$) over across all crops and years; NO_3^- concentrations in wheat significantly increased in till relative to no-till over time [normalized NO_3^- difference = -15650 + 7.84 (year); $r^2 = 0.44$; n = 3 years]. Mean ± S.E.

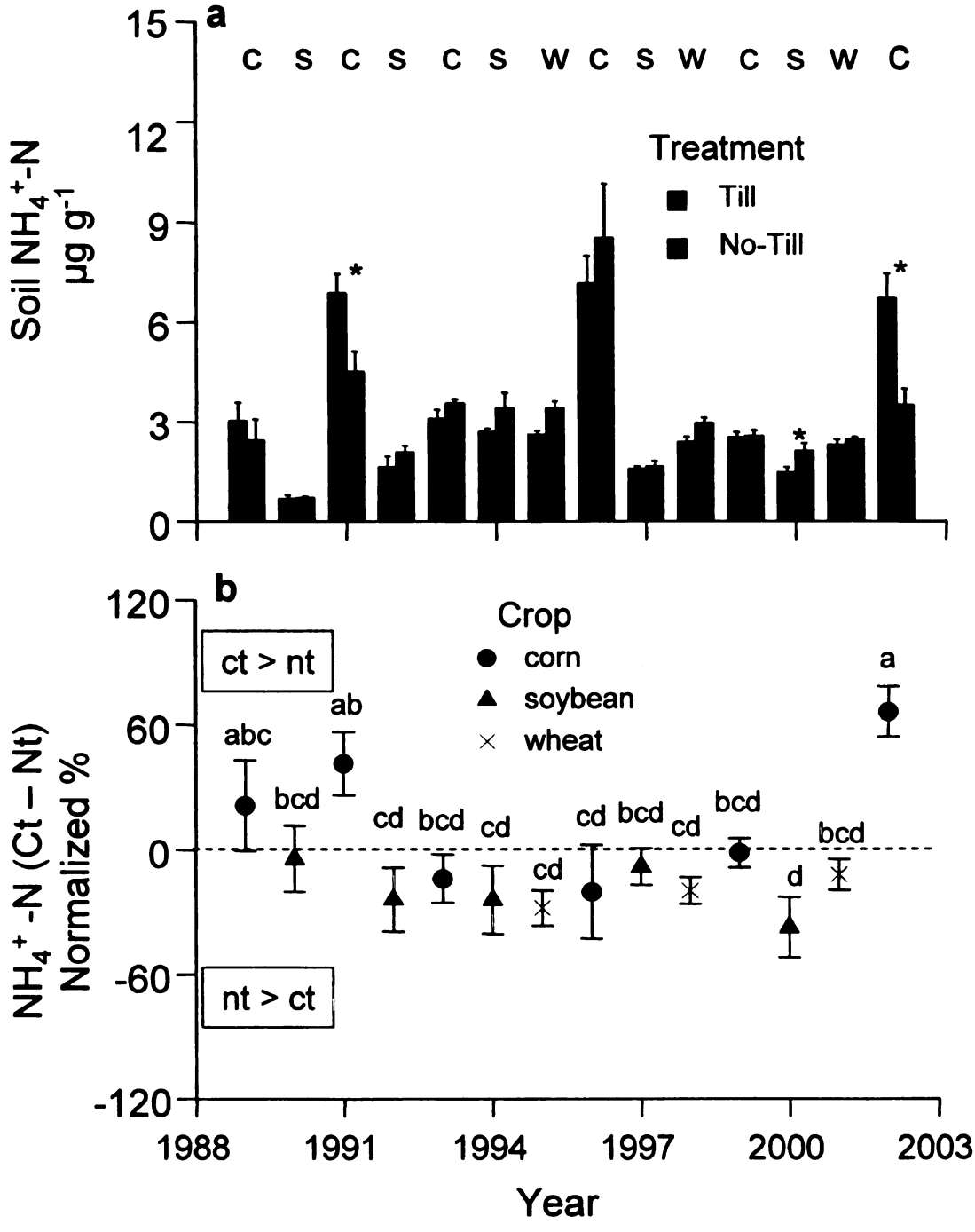

Figure 6.3. (a) Soil NH_4^+ concentrations in till and no-till treatments between 1989 and 2002. Letters indicate the crop harvested in that year: c = corn; s = soybean; w = wheat. * indicates statistically different responses within a year ($p<0.05$) determined by slicing of the treatment by year interaction. (b) Difference between conventional till (Ct) and no-till (Nt) normalized by the overall mean NH_4^+ concentration for that year. Points greater than zero indicate a till response greater than a no-till response. Year means were significantly different ($P<0.05$) and separated using Tukey's test. Linear regression analysis showed no trends in soil NH4+ concentration ($p<0.05$) across all crops and years or for specific crops over time.

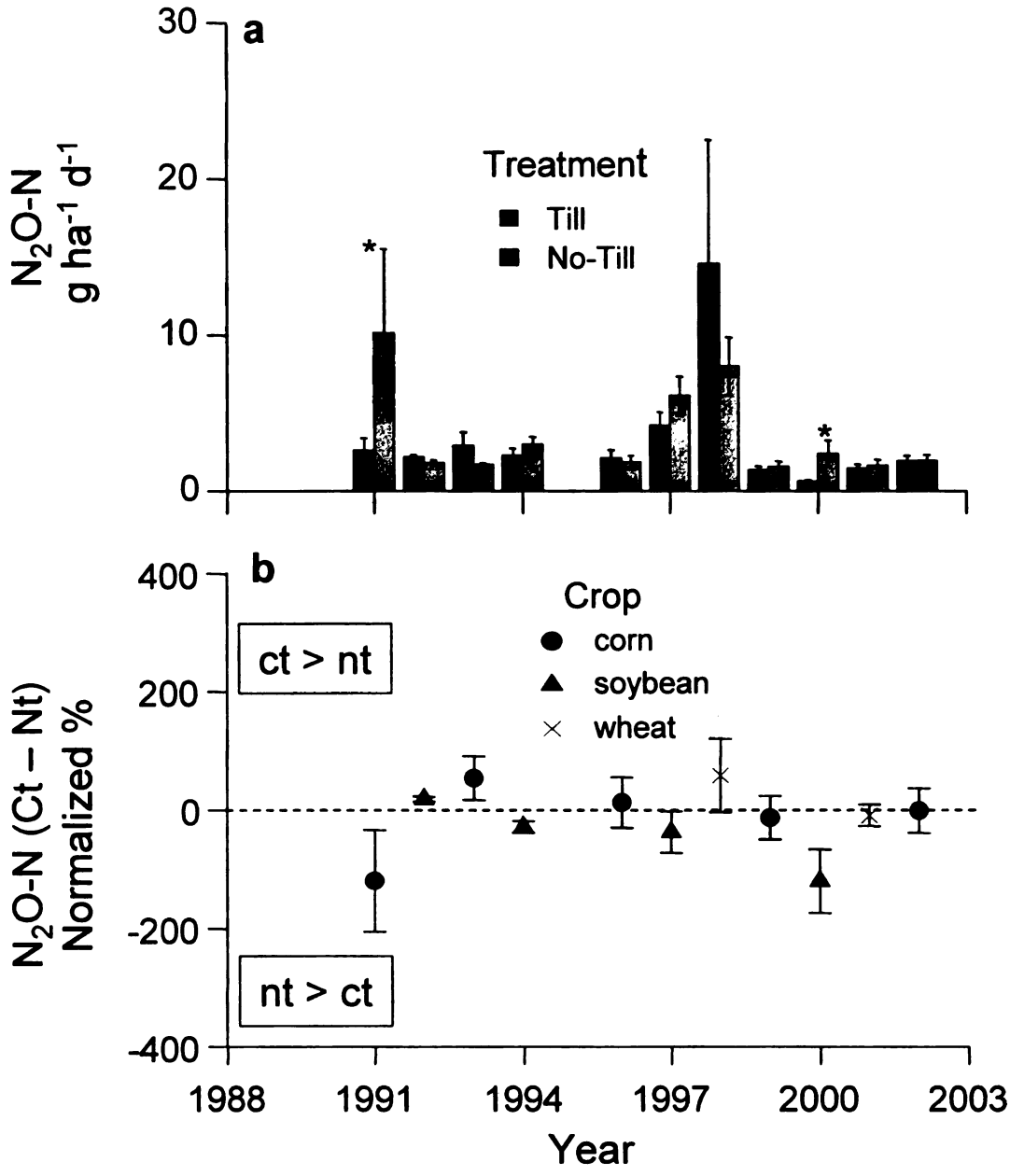

Figure 6.4. (a) Soil N_2O emissions in till and no-till treatments between 1991 and 2002 (no data available for 1995). Letters indicate the crop harvested in that year: c = corn; s = soybean; w = wheat. * indicates statistically different responses within a year ($p<0.05$) determined by slicing of the treatment by year interaction. (b) Difference between conventional till (Ct) and no-till (Nt) normalized by the overall mean N2O emissions for that year. Points greater than zero indicate a till response greater than a no-till response. There was no detectable difference between treatments based on a Tukey's test. Linear regression analysis showed no trend in N_2O emissions ($p<0.05$) across all crops and years; emissions from soybean significantly increased in till relative to no-till over time [normalized N_2O difference = 31210 – 15.7 (year); $r^2 = 0.48$; n = 4 years]. Mean ± S.E.

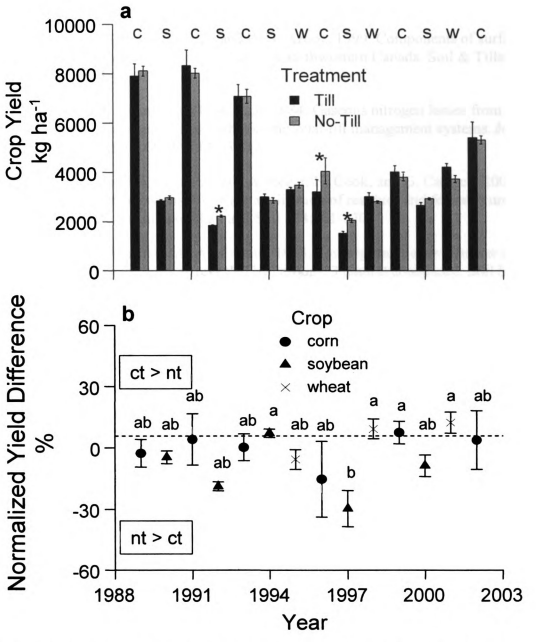

Figure 6.5. (a) Crop yields in till and no-till treatments between 1989 and 2002. Letters indicate the crop harvested in that year: c = corn; s = soybean; w = wheat. * indicates statistically different responses within a year ($p<0.05$) determined by slicing of the treatment by year interaction. (b) Difference between conventional till (Ct) and no-till (Nt) normalized by the overall mean crop yield for that year. Points greater than zero indicate a till response greater than a no-till response. Year means were significantly different ($P<0.05$) and separated using Tukey's test. Linear regression analysis showed no trend in crop yields ($p<0.05$) across all crops and years; wheat yields significantly increased in till relative to no-till over time [normalized yield difference = -5980 + 3.00 (year); $r^2 = 0.40$; n = 3 years]. Mean ± S.E.

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