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Initial cultivation of a temperate-region soil immediately accelerates aggregate turnover and CO_2 and N_2O fluxes

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Abstract

The immediate effects of tillage on protected soil C and N pools and on trace gas emissions from soils at precultivation levels of native C remain largely unknown. We measured the response to cultivation of CO_2 and N_2O emissions and associated environmental factors in a previously uncultivated U.S. Midwest Alfisol with C concentrations that were indistinguishable from those in adjacent late successional forests on the same soil type (3.2%). Within 2 days of initial cultivation in 2002, tillage significantly ($P = 0.001$, $n = 4$) increased CO_2 fluxes from 91 to 196 mg CO_2-C m^{-2} h^{-1} and within the first 30 days higher fluxes because of cultivation were responsible for losses of 85 g CO_2-C m^{-2}. Additional daily C losses were sustained during a second and third year of cultivation of the same plots at rates of 1.9 and 1.0 g C m^{-2} day^{-1}, respectively. Associated with the CO_2 responses were increased soil temperature, substantially reduced soil aggregate size (mean weight diameter decreased 35% within 60 days), and a reduction in the proportion of intraaggregate, physically protected light fraction organic matter. Nitrous oxide fluxes in cultivated plots increased 7.7-fold in 2002, 3.1-fold in 2003, and 6.7-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 rates and fivefold greater nitrifier enzyme activity, likely contributed to increased NO_3^- concentrations. Our results demonstrate that initial cultivation of a soil at precultivation 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 occasional cultivation in order to preserve sequestered C.

Keywords: aggregates, trace gases, CO_2, enzymes, light fraction, N_2O, nitrogen, NO_3^-, soil moisture, soil temperature, tillage

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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} yr^{-1} (CAST, 2004) and conversion to no-till annual crops has the potential to sequester C at about half this rate (West & 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 & Socolow, 2004). Some models predict that soils with sequestered C may rapidly lose C and N following cultivation (Baisden & 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 & Ackerman, 1993; West & Post, 2002; Miller *et al*., 2004).

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N_2O emissions are also affected by tillage *per se*. Although models and measurements suggest that long-term tilled soils can have similar (e.g. Grandy *et al*., 2006), somewhat lower (e.g. Smith *et al*., 2001), or somewhat higher (e.g. Six *et al*., 2004) emission rates compared with long-term no-till soils, there is nonetheless potential for large differences immediately following initial cultivation. The combination of greater microbial activity, increased N availability, and concomitant changes in soil moisture and temperature could substantially accelerate N_2O emissions for some period of time. In a very short-term study, Pinto *et al*. (2004) found that from 12 to 16 days after cultivating a 17-year-old, unfertilized perennial pasture that cumulative N_2O emissions were increased by 23 mg N_2O-N m^{-2} relative to uncultivated sites. They attributed increases to accelerated SOM turnover's producing excess inorganic N that was subsequently used by N_2O-producing nitrifiers and denitrifiers. The persistence of this effect and its magnitude in other soils is unknown.

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). Mechanistic 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 & Conen, 2004), and alters microbial community structure and function (Cavigelli & Robertson, 2000; Buckley & Schmidt, 2001). Over time, these changes lead to 30–60% declines in SOM (Davidson & Ackerman, 1993; Buyanovsky *et al*., 1997; West & 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; Six et al., 1999). Evidence for protection of SOM by aggregates includes low interior-aggregate oxygen concentrations that limit respiration rates, small intraaggregate 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 & 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 reductions in soil aggregation occur after initial cultivation, in conjunction with changes in soil temperature and other environmental controls over decomposition, then SOM turnover, microbial activity, and trace gas fluxes will rapidly increase. We further hypothesize that these accelerated processes will persist throughout the growing season and into the following spring. If so, we also predict that subsequent cultivation will primarily serve to prevent the recovery of aggregation and nutrient cycling to precultivation levels, rather than leading to additional declines in soil function. Our first objective, therefore, is to measure changes in CO_2 and N_2O emissions for 3 years following cultivation of a previously uncultivated field at precultivation 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 mid-successional grassland community that has since been mowed each fall, with mown biomass left in place to decompose *in situ*. Soils at the site are Kalamazoo (fine-loamy) and Oshtemo (coarse-loamy) mixed, mesic, Typic Hapludalfs formed on glacial outwash (Crum & Collins, 1995). The two series co-occur at our site with variation within a series often as great as variation between series. Before cultivation, soils contained 31.8 ($\pm$ 1.4) g C kg^{-1}, 2.59 ($\pm$ 0.11) g N kg^{-1}, and soil pH averaged 5.93 ($\pm$ 0.11); the same soil C levels had been measured 3 and 12 years before this study and are statistically indistinguishable from C concentrations 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 3×6 m plots to which we assigned four 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 four 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 before cultivation. Primary

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cultivation consisted of moldboard plowing to a depth of 19 cm and secondary cultivation was carried out with a disc harrow. Both methods are commonly used today 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

Soil samples for aggregate and total soil C and N analysis were collected to 20 cm before initial cultivation on 18 June 2002, and post initial cultivation on 24 August 2002, 27 May 2003, and 21 October 2003. An additional soil sample for total C analysis was taken 24 September 2004. Aggregate-associated light fraction (LF) organic matter and whole soil particulate organic matter (POM) were determined on the samples collected 24 August 2002 and 21 October 2003. Five 3.8 cm soil cores were taken from each plot to a depth of 20 cm for aggregate, LF, POM and total C and N analysis, placed in plastic bags, and refrigerated (<7 days) before 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 at each time of trace gas sampling for gravimetric soil moisture determination. Cores were passed through an 8 mm sieve and homogenized before analysis. Soil water content is reported for the 0–7 cm soil layer. Inorganic soil N analysis and nitrifier enzyme activity were determined periodically throughout the study, either on a subsample of the soil used for gravimetric soil moisture determination or, for days where trace gas measurements were not made, on a soil sample collected and prepared in the same manner as those used to determine moisture content. Samples used for inorganic N and nitrifier enzyme analysis were stored at 4 °C for 24 h (inorganic N analysis) or <4 days (nitrifier enzyme activity).

Soil NO_3^- and NH_4^+ concentrations were determined for the 0–7 cm soil layer 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 for the 7–20 cm soil layer on three dates in 2003 and on 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. Changes in nitrifier enzyme activity following cultivation were determined for the 0–7 and 7–20 cm soil layers on 13 October 2003 (DOY 286) and 17 August 2004 (DOY 230).

Plant and litter C estimates before initial cultivation were estimated by drying and analyzing litter and plant samples collected on 21 June 2002 (DOY 172) from two 25 × 25 cm quadrats in each plot. Changes in the litter layer after cultivation were estimated on 15 September 2003 from two 25 × 25 cm 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, USA).

Trace gas fluxes

Gas fluxes were determined using a single 25 cm diameter static PVC chamber (5500 cm^{-3}) located within each plot (Livingston & Hutchinson, 1995). Before 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). Open vials were capped and flushed with 40 mL chamber headspace immediately before sample collection. Gas sampling was generally performed between 09:00 and 14:00 hours and all plots were sampled on each sampling day. Within 48 h of collection CO_2 was analyzed using an infrared gas absorption analyzer (EGA CO_2 Analyzer, Analytical Development Company, Hoddesdon, Herts, UK) and N_2O analyzed using a gas chromatograph (Hewlett Packard 5890 Series II, Rolling Meadows, IL, USA) 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 and mean CO_2 and N_2O fluxes over the measurement period.

POM density distribution

Cultivation effects on the distribution of POM 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 by shaking for 48 h on a rotary shaker set at 190 rpm. Dispersed samples were

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poured through a 53 μm sieve and rinsed thoroughly with deionized (DI) water. Sand and POM remaining on the sieve were transferred to filter paper and then backwashed into a 100 mL beaker with 40 mL 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 of <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, and <1.3 g cm^{-3}). Clay plus silt associated C was determined by difference between total soil C and POM C.

Aggregate and LF separation

Aggregate distribution was determined by hand on triplicate 35 g air-dried soil subsamples (0–20 cm soil depth) by initial saturation followed by wet-sieving in water through a series of 2000, 250, and 53 μm sieves (Grandy & Robertson, 2006). 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 & Rosenau, 1986).

The method we used to separate inter- and intraaggregate LF is based on previously published protocols (Six *et al*., 1998; Gale *et al*., 2000). Before LF analysis, 8 g aggregate subsamples were prewetted to minimize aggregate slaking during LF separation. Subsamples were 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. Aggregates were slowly wetted by capillarity after four mL of DI water was trickled onto the paper filters. After 16 h, aggregates were then transferred from the membrane filters to 100 mL beakers 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. Preliminary investigations showed that the final density of the sodium polytungstate was 1.60 g cm^{-3} following equilibration with the water contained in aggregates.

LF was aspirated from the surface of the sodium polytungstate after 24 h and then rinsed on a hardened, ashless filter paper (Whatman 541) with at least 600 mL DI H_2O. We refer to this LF pool as interaggregate LF. After removal of the interaggregate LF, we aspirated the remaining sodium polytungstate. Aggregates were then dispersed, as described previously for POM samples, to release the intraaggregate LF using sodium hexametaphosphate and resuspended in NAPT (density = 1.62 g cm^{-3}). The intraaggregate LF was aspirated from the surface of the NAPT.

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. Soil inorganic N was determined by extracting NH_4^+ and NO_3^- 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, USA). 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, USA). Soil inorganic N concentrations were corrected for the gravimetric moisture content in the sample and are expressed on a per gram dry soil basis.

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 nonlimiting 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 four 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 as this is usually not part of the aggregate structure. Carbon content of SOM fractions was calculated on an area basis by correcting for bulk density and soil depth. Where changes in soil processes because of cultivation are presented as a percentage, the difference between cultivated and control plot means was determined relative to the mean value for the control plots. Tillage effects on trace gas fluxes, soil moisture, temperature and inorganic N were analyzed by Proc Mixed (SAS 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 × treatment interactions, differences between treatments on separate days were

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determined using the slicing command in SAS. Cultivation effects on aggregation and SOM pools were similarly analyzed but without repeated measures. Analysis of covariance (ANCOVA; Goldberg & Scheiner, 2001) was used to examine the relationship between soil temperature, moisture and CO_2 emissions 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} h^{-1} (Fig. 1, top). We estimate that within the first 33 d following cultivation, 85 g C m^{-2} was lost owing to tillage. During the next 30 days, tillage effects on CO_2 flux were somewhat lower, resulting in a daily average loss of 1.4 g C m^{-2} day^{-1} over the 60 day measurement period (Table 1).

Before the second cultivation in 2003, CO_2 fluxes were again higher in the cultivated plots ($P = 0.003$, $n = 4$). In 2003, the tillage-induced CO_2 response was measurable for 80–120 days (Fig. 1) and average daily CO_2-C loss owing to cultivation was 1.9 g m^{-2} day^{-1} for the 138 day sampling period (Table 1).

Before cultivation in 2004, there were no significant differences in CO_2 flux between treatments (Fig. 1). 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 owing to cultivation were 1.0 g C m^{-2} day^{-1} over the 198 sampling period in 2004 (Table 1).

ANCOVA (Table 2) showed that soil moisture and temperature (Table 1; Fig. 1) were related to CO_2 flux differently in different years. In 2002, there was no temperature effect but there was a significant soil moisture $\times$ treatment interaction, indicating that the relationship between CO_2 flux and soil moisture differed between treatments (Table 2). The CO_2 response to moisture was greater in control than cultivated plots and a higher proportion of the variability in CO_2 emissions was explained by soil moisture differences in control plots. Moisture effects were not significant in 2003 or 2004. In 2003 and 2004 there were significant temperature effects on CO_2 emissions.

N_2O fluxes in 2002 increased in response to cultivation with a time lag of 30 days when fluxes in cultivated plots were as high as 87 μg N_2O-N m^{-2} h^{-1} (Fig. 1, bottom). 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 emissions were 2.87 in control plots and 22.2 μg N_2O-N m^{-2} h^{-1} in cultivated plots (Table 1); in 2003, the average N_2O fluxes were 3.72 in control plots and 11.5 μg N_2O-N m^{-2} h^{-1} in cultivated plots; in 2004, control plots emitted 11.4 μg N_2O-N m^{-2} h^{-1} compared with 76.1 μg N_2O-N m^{-2} h^{-1} in cultivated sites (Table 1). In 2004, there were extremely high fluxes owing to cultivation on DOY 163 (217 ± 153 μg N_2O-N m^{-2} h^{-1}) and DOY 189 (1290 ± 573 μg N_2O-N m^{-2} h^{-1}).

Soil aggregation

In 2002, the MWD of aggregates measured 60 days after tillage was 35% lower in cultivated than in control plots ($P = 0.009$, $n = 4$; Fig. 2). These differences were still evident before tillage in 2003 ($P = 0.011$, $n = 4$). In October 2003 (DOY 294), the reduction in MWD associated with tillage was 37% ($P = 0.022$, $n = 4$; Fig. 2), similar to the difference found in 2002. The fraction of 2000–8000 μm aggregates declined to 19% from 34% in control plots by DOY 236, 2002 (Fig. 3). In 2003, 2000–8000 μm aggregates remained lower in cultivated plots while aggregates in the 53–250 and <53 μm size classes increased.

SOM 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} ($P = 0.001$, $n = 4$) 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). 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, although there was a trend ($P = 0.073$, $n = 4$) toward more POM with a density of 1.3–1.6 g C m^{-2} in cultivated plots (Table 3).

The proportion of intraaggregate to total LF in 2000–8000 μm aggregate size class declined from 28% to 16% within 60 days of the first cultivation in 2002 (Fig. 4). In 2003 there was a trend towards a reduced proportion of intraaggregate LF associated with 2000–8000 μm aggregates in cultivated (21%) compared with control (27%) plots ($P = 0.054$, $n = 4$). In 2002, interaggregate and total LF increased in the 250–2000 μm size class and in 2003 the total LF in this size class increased. In the 53–250 μm size class there was an increase in interaggregate and total LF in both years and also an increase in intraaggregate LF in 2003.

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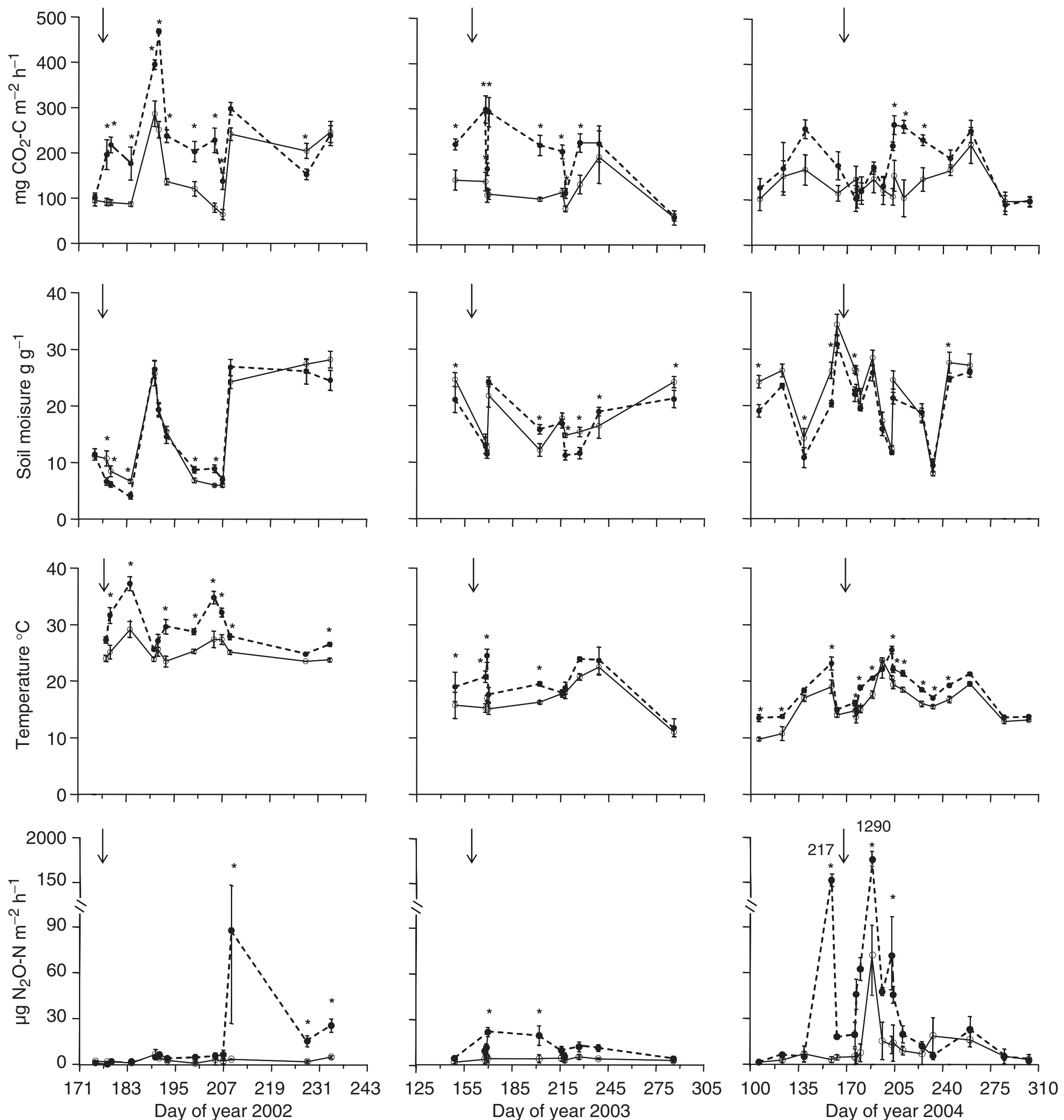

Fig. 1 Changes in soil CO_2 flux, moisture, temperature, and N_2O flux following cultivation of a previously never tilled soil in southwest Michigan. Open symbols with solid lines represent uncultivated control plots; closed symbols with dashed lines, cultivated plots. Arrows indicate cultivation dates for 2002 (left panel), 2003 (middle panel), and 2004 (right panel). Note different *x*-axis ranges, reflecting different durations of sampling. Soil moisture was determined for the 0–7 cm soil layer and soil temperature measured at 7 cm soil 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.

Soil inorganic nitrogen

Cultivation in 2002 increased extractable soil NO_3^- concentrations to 3.18 µg NO_3^--N g^{-1} (compared with 0.41 µg NO_3^--N g^{-1} in control plots) after 24 days (Fig. 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

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Table 1 Initial cultivation effects on mean hourly CO_2 and N_2O emissions and soil temperature and moisture in 2002, 2003, and 2004*†

	2002				2003				2004			
	CO_2-C ($mg\,m^{-2}\,h^{-1}$)	N_2O-N ($\mu g\,m^{-2}\,h^{-1}$)	Moisture (%)	Temperature (°C)	CO_2-C ($mg\,m^{-2}\,h^{-1}$)	N_2O-N ($\mu g\,m^{-2}\,h^{-1}$)	Moisture (%)	Temperature (°C)	CO_2-C ($mg\,m^{-2}\,h^{-1}$)	N_2O-N ($\mu g\,m^{-2}\,h^{-1}$)	Moisture (%)	Temperature (°C)
Control	153 (7.87)	3.07 (0.49)	15.4 (0.65)	25.3 (0.57)	115 (11.6)	4.72 (2.07)	18.1 (0.49)	16.8 (0.27)	134 (18.7)	11.9 (4.31)	23.1 (0.96)	16.2 (0.33)
	170	**2.87**			**123**	**3.72**			**140**	**11.4**		
Cultivated	235 (11.51)	13.4 (4.74)	14.6 (0.68)	29.4 (0.30)	202 (9.00)	10.7 (1.52)	16.5 (0.58)	19.7 (0.48)	171 (7.37)	105 (19.4)	20.1 (0.45)	18.4 (0.25)
	227	**22.2**			**202**	**11.5**			**180**	**76.1**		
ANOVA *F*-tests												
Source							*P* Values					
Cultivation	0.004	0.020	0.510	0.007	0.009	0.093	0.110	0.010	0.224	0.011	0.042	0.013
Day of year	0.001	0.001	0.001	0.010	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Cult × DOY	0.001	0.001	0.001	0.034	0.001	0.001	0.001	0.034	0.001	0.001	0.034	0.001

Means are shown with standard errors in parentheses ($n = 4$ replications). Bold values below the mean hourly fluxes are averages of hourly interpolated emission rates.

*Cultivation occurred for the first time on 25 June 2002 and again in the same plots on 15 June 2003 and 20 June 2004.

†Differences in CO_2 emissions between control and cultivated plots were equal to 1.4 $g\,m^{-2}\,day^{-1}$ in 2002, 1.9 $g\,m^{-2}\,day^{-1}$ in 2003, and 1.0 $g\,m^{-2}\,day^{-1}$ in 2004.

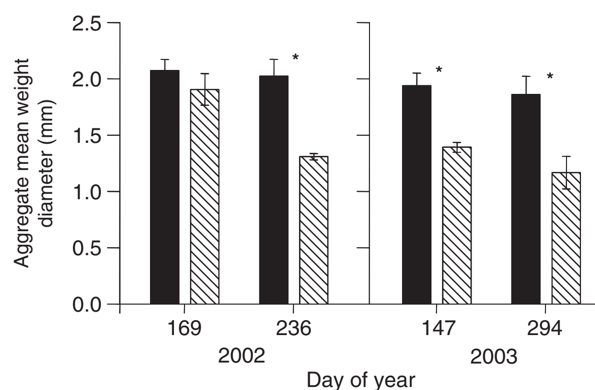

Fig. 2 Mean weight diameter (MWD) of soil aggregates. Black bars represent control plots; patterned bars, cultivated plots. Tillage occurred on day of year (DOY) 176 in 2002 and DOY 166 in 2003. Aggregation was determined for the 0–20 cm depth. Treatment means are shown ± standard error ($n = 4$); * indicates statistically significant ($P < 0.05$) differences between control and cultivated treatments within a single DOY.

differences on the other sampling dates (Fig. 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 (Fig. 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.

Mean nitrifier enzyme activity (Fig. 6) in 2003 was fivefold higher in cultivated than uncultivated treatments (25.4 vs. 127 $\mu g\,N\,kg^{-1}\,soil\,h^{-1}$) in the 0–7 cm soil layer and threefold higher in the 7–20 cm soil layer (14.9 vs. 45.2 $\mu g\,N\,kg^{-1}\,soil\,h^{-1}$). Similar trends were observed in 2004 when control plots had lower mean nitrifier enzyme activity at 0–7 cm (35.5 vs. 109 $\mu g\,N\,kg^{-1}\,soil\,h^{-1}$) and 7–20 cm (10.5 vs. 45.7 $\mu g\,N\,kg^{-1}\,soil\,h^{-1}$) soil depths.

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 $g\,C\,m^{-2}\,day^{-1}$ in 2002, 2003, and 2004, respectively, equivalent to 84 $g\,C\,m^{-2}$ over the 60 days following cultivation in 2002, 262 g C over the 138 days sampling period in 2003, and 198 g C over the 198 d sampling period in 2004. N_2O emissions also markedly increased during our measurement period following cultivation: 7.7-fold in 2002, 3.1-fold in 2003, and 6.7-fold in 2004. Based on IPCC calculations using a 100-year time horizon for N_2O, these emission

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Table 2 Significant relationships between soil surface CO_2 emissions and soil temperature and moisture in control and cultivated plots between 2002 and 2004, determined by analysis of covariance (ANCOVA)*†

Year	Significant effect	P value	R^2	Regression line equation
2002	Cultivation × Moisture	0.024	0.74	Control: $\log_{10}$ CO_2 flux = 0.28 + 0.77 ($\log_{10}$ moisture)
			0.21	Cultivated: $\log_{10}$ CO_2 flux = 0.98 + 0.33 ($\log_{10}$ moisture)
2003	Temperature	0.042	0.42	$\log_{10}$ CO_2 flux = −0.61 + 1.40 ($\log_{10}$ temperature)
2004	Temperature	0.028	0.16	$\log_{10}$ CO_2 flux = 0.05 + 0.89 ($\log_{10}$ temperature)

*Soil moisture was determined for the 0–7 cm soil layer and soil temperature was determined at a depth of 7 cm.
†The soil temperature main effect ($P = 0.678$) and its interaction with cultivation ($P = 0.278$) were not significant in 2002; the temperature × cultivation ($P = 0.151$), moisture ($P = 0.099$), and moisture × cultivation ($P = 0.767$) effects were not significant in 2003; the temperature × cultivation ($P = 0.633$), moisture ($P = 0.544$), and moisture × cultivation ($P = 0.533$) effects were not significant in 2004.

Table 3 Cultivation effects on the distribution of soil organic matter (0–20 cm soil layer) among different density fractions in 2002 (DOY 236) and 2003 (DOY 294)*†

		Particulate organic matter density ($g\,cm^{-3}$)			
	Mineral-associated C	>1.9	1.6–1.9	1.3–1.6	<1.3
2002			$g\,C\,m^{-2}$		
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)

Values are means ($n = 4$) with standard errors in parentheses.
*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 $kg\,m^{-2}$).

differences are equivalent to 22.5, 9.1, and 75.4 g CO_2-C equivalents $m^{-2}\,yr^{-1}$ for 2002, 2003, and 2004, respectively, for a 3-year average of 35.7 g CO_2-C m^{-2}. These C and C-equivalent losses can be compared with an average annual C gain of ca. 30 g C $m^{-2}\,yr^{-1}$ under no-till systems at KBS (Robertson *et al*., 2000) and in the U.S. Midwest (Franzluebbers & Steiner, 2002).

Soil structure and SOM

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 and that these changes are persistent. MWD differences measured 60 days after tillage were largely attributable to declines in 2000–8000 μm aggregates (Fig. 4). In cultivated sites, 19% of the soil was in the 2000–8000 μm size class, compared with 34% in the control plots, identical to adjacent agricultural fields on the same soil type that have been tilled for more than 50 years (data not shown). Before tillage in 2003, aggregation in cultivated sites remained substantially lower, demonstrating that cultivation effects persisted throughout the winter and following spring. There was no measurable additional decline in soil structure following cultivation in 2003, further demonstrating that cultivation effects occur immediately and are persistent.

In previously cultivated soils, soil CO_2 emission responses to cultivation may occur for only hours or days, suggesting that physical phenomena such as diffusion rates are largely responsible for these 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, likely due primarily to increased substrate availability. These

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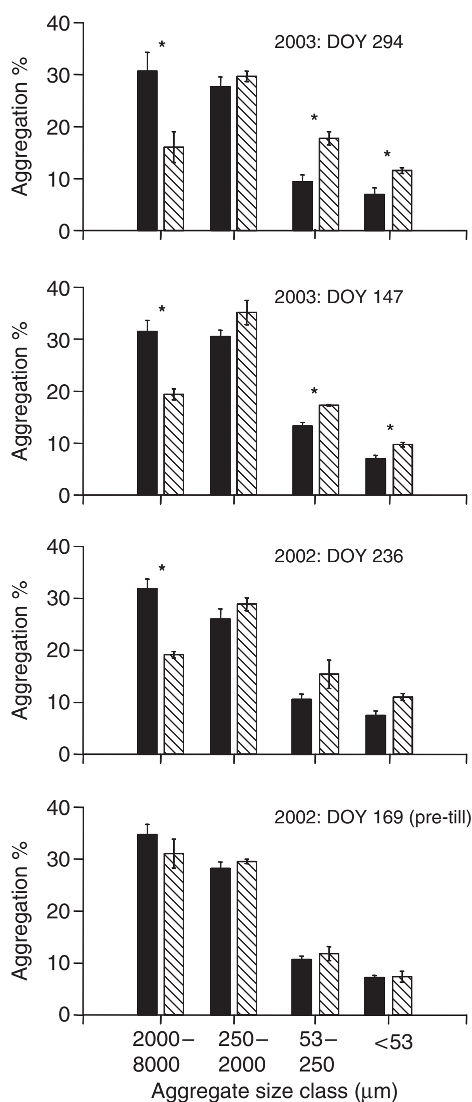

Fig. 3 Soil aggregate distribution in four size classes. Black bars represent control plots; patterned bars, cultivated plots. Tillage occurred on day of year (DOY) 176 in 2002 and DOY 166 in 2003. Aggregation was determined for the 0–20 cm depth. Treatment means are shown ± standard error ($n = 4$); *indicates statistically significant ($P < 0.05$) differences between control and cultivated treatments within a size class and DOY.

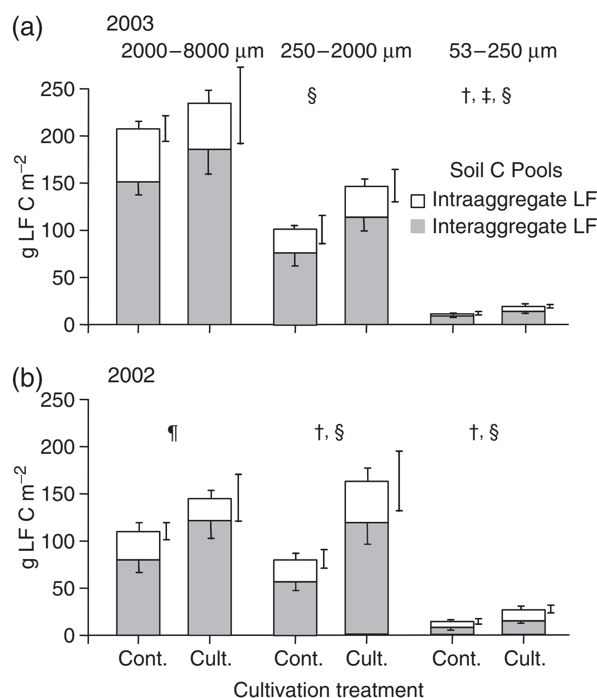

Fig. 4 Distribution of inter and intraaggregate light fraction (LF) organic matter in control and cultivated plots in 2002 and 2003. The 0–20 cm soil layer was analyzed for samples collected on 24 August 2002, day of year (DOY) 236 and 15 June 2003 (DOY 166) and wet-sieved into aggregate size classes. †, ‡, §, and ¶ indicate significant differences ($P < 0.05$) within an aggregate size class between cultivation treatments for interaggregate LF, intraaggregate LF, total LF, and the proportion of total LF within aggregates, respectively. In 2003 there was trend towards a decreased proportion of intraaggregate LF ($P < 0.054$) in 2000–8000 μm aggregates. Bars above the intraaggregate LF boxes are positive standard errors for intraaggregate LF; bars within the interaggregate LF boxes are negative standard errors for interaggregate LF; bars to the right of each column are standard errors (positive and negative) for total LF.

substrates included LF released from large aggregates in addition to aboveground C that entered low-density, unprotected, interaggregate POM pools (Table 3; Fig. 4), which are rapidly oxidized following disturbance (Arrouays & Pelissier, 1994; Six *et al.*, 1999; DeGryze *et al.*, 2004). In 2002, tillage incorporated $142 \pm 30\,\mathrm{g\,C\,m^{-2}}$ of litter and $228 \pm 11\,\mathrm{g\,C\,m^{-2}}$ of plant biomass. Other tillage studies have demonstrated that incorporating aboveground plant residues into soil increases its decomposition rate by 50–100% (Burgess *et al.*, 2002; Lupwayi *et al.*, 2004). LF pools consisting of recently deposited C have been shown to be correlated with soil surface respiration rates (Janzen *et al.*, 1992; Alvarez & Alvarez, 2000) and its depletion represents a major portion of C loss in cultivated soils (Cambardella & 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 4 weeks for significant plant community 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

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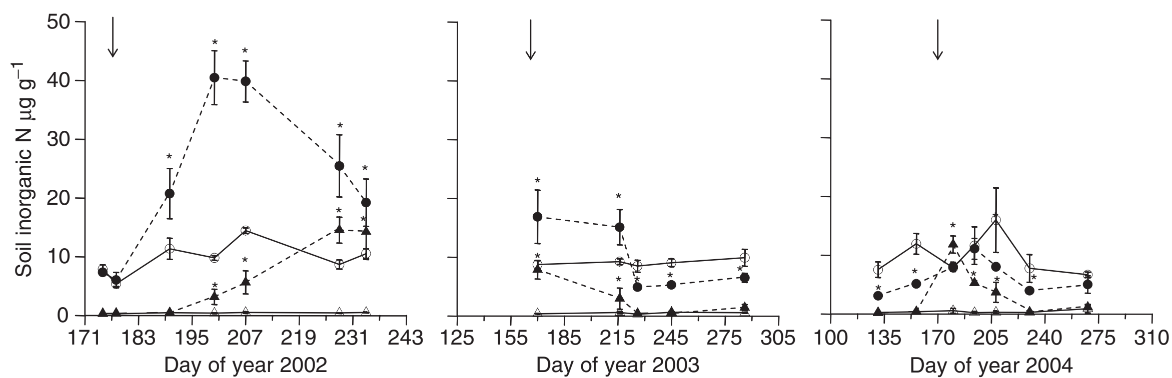

Fig. 5 Changes in inorganic N concentration (NH_4^+-N and NO_3^--N) following initial cultivation. NH_4^+ (circles) and NO_3^- (triangles) in control plots (open symbol, solid line) and cultivated plots (closed symbol, dashed line). Arrows indicate cultivation dates for 2002 (left panel), 2003 (middle 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) where there was a significant treatment × DOY interaction.

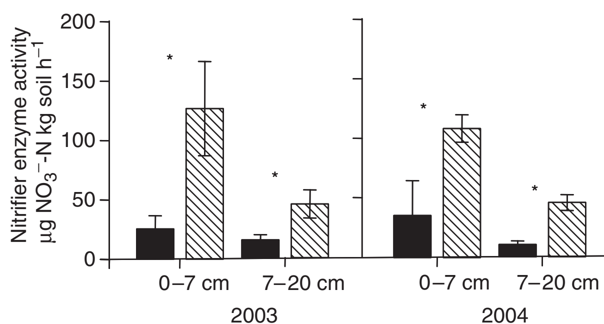

Fig. 6 Changes in nitrifier enzyme activity following cultivation on 13 October 2003 (DOY 286) and 17 August 2004 (DOY 230). Black bars represent control plots; patterned bars, cultivated plots. Treatment means are shown ± standard error ($n = 4$); *indicates statistically significant ($P < 0.05$) differences between control and cultivated treatments within a day of year (DOY) where there was a significant treatment × DOY interaction.

because of 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

ANCOVA 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 $g\,C\,m^{-2}\,day^{-1}$), whereas at higher soil moisture contents, the CO_2 flux difference was only 21% (5.84 vs. 7.08 $g\,C\,m^{-2}\,day^{-1}$). 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 & Schimel, 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 vs. control plots in 2003 and 2004 (Table 1) 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). 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 certain pools of C are more 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

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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 days after cultivation, likely due to low soil NO_3^- concentrations and moisture: not until then did cultivated soils exhibit both a high soil moisture content (>20%) and substantially elevated soil NO_3^- concentrations (Figs 1 and 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^- concentrations following an initial lag period.

In the unfertilized soils studied here, changes in N 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, over time, 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 increased plant N uptake. Additional changes in the synchrony between N 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 (MacKenzie *et al*., 1998; Grant *et al*., 2004; Six *et al*., 2004; Grandy *et al*., 2006; Grandy and Robertson, 2006). 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 that sizeable and persistent N_2O-N losses can occur following cultivation of no-till ecosystems, and corroborate measurements by Pinto *et al*. (2004) showing rapid increases in N_2O emissions 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 in agricultural systems near our study site and on the same soil type, however, have found low or undetectable emissions (G. P. 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 & Beauchamp, 1991; Koponen & Martikainen, 2004). In our soils, with clay contents generally <20% (Crum & 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 precultivation levels of native C. Following a single tillage event, aggregates in the 2000–8000 μm size class declined to levels found in adjacent agricultural fields with a long history of intensive, annual tillage. These changes persisted throughout the winter and following spring and additional cultivation did not lead to additional declines in soil structure. Aggregate destruction appeared to release light fraction SOM from within intraaggregate microsites and limit the incorporation of POM originating from aboveground C pools into aggregates. The ensuing increases in interaggregate, unprotected C and decreases in the proportion of intraaggregate LF that we measured likely enhanced substrate availability, which, along with changes in temperature, would have contributed to higher oxidation rates. As a result, cultivated plots lost an additional 1.4, 1.9, and 1.0 $g\,C\,m^{-2}\,day^{-1}$ in 2002, 2003 and 2004, respectively. Cultivation also increased nitrifier enzyme activity fivefold, soil NO_3^--N, which approached 15 $\mu g\,N\,g^{-1}$ in cultivated plots, and N_2O emissions, which increased 7.7-fold in 2002, 3.1-fold in 2003, and 6.7-fold in 2004. These increases in N_2O emission rates, expressed as C equivalents, are alone similar to the average annual C gain under no-till systems in the U.S. Midwest (30 $g\,C\,m^{-2}\,yr^{-1}$). Our results demonstrate that successional communities at precultivation 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 occasional plowing.

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