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Responses of soil microbial processes and community structure to tillage events and implications for soil quality

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Abstract

The short-term responses of soil microbial processes and community structure to perturbation constitute one aspect of soil quality. Such responses are often associated with an increase in the emissions of greenhouse gases (i.e., CO_2, NO, or N_2O) and the accumulation and potential loss of nitrate by leaching. Here we describe our recent work on responses of soil carbon and nitrogen dynamics, microbial biomass, and microbial community structure to a tillage event in intensively managed vegetable crop systems in California. Our results indicate that CO_2 emission is high for the first day after tillage, but respiration declines or remains constant, suggesting that physical processes are responsible for the high flux from the soil surface. Net mineralization and nitrate accumulation increase for several days after tillage, and this can be accompanied by higher denitrification rates. Tillage causes immediate changes in microbial community structure, based on phospholipid fatty acid (PLFA) analysis, but little concomitant change in total microbial biomass. Tillage events contribute to decreased soil quality by increasing emissions of greenhouse gases, and increasing the potential for nitrate leaching to groundwater, and these negative aspects must be weighed against the benefits of tillage for increasing the health and productivity of some crops.

Keywords: Carbon dioxide flux; Denitrification; Nitrate; Nitrogen mineralization; Phospholipid fatty acid analysis

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1. Introduction

Soil quality is a broad concept that encompasses biological, chemical, and physical properties that sustain productivity, environmental quality, and support healthy organisms (Doran et al., 1996; van Bruggen and Semenov, 2000). Soil quality can be defined as 'the capacity of soil to function' (Karlen et al., 1997), but this is difficult to measure and quantify. Thus, many recent investigations have measured soil quality with a minimum data set that includes soil biological characteristics (e.g., assays for biological activity), soil chemical characteristics (e.g., concentrations of various fractions of soil organic matter), and physical characteristics (e.g., water infiltration rates or bulk density), as a means to examine suites of related properties with potential effects on biological productivity and environmental quality (Staben et al., 1997; Wander and Bollero, 1999; Campbell et al., 2001).

Short-term changes that occur in response to soil perturbation can be considered to be indicators of soil quality. Perturbations such as rewetting dry soil (van Gestel et al., 1993; Lundquist et al., 1999), fumigation (Macalady et al., 1998; Ibekwe et al., 2001), or tillage (Petersen and Klug, 1994; Calderón et al., 2000, 2001) alter soil microbiology, metabolic processes, biogeochemistry, and gaseous fluxes. Short-term disturbances are detrimental to soil quality if they increase the emissions of greenhouse gases (i.e., CO_2, NO, or N_2O), cause nitrate accumulation and leaching, or modify soil microbial community structure in a way that decreases the retention of organic C and N.

Many studies have documented that over the long term, frequently tilled soils undergo losses in soil organic matter and microbial activity, increases in net nitrate production, and deterioration of soil structure (Doran, 1982; Elliott, 1986; Woods, 1989). Tillage events are known to produce a temporary burst of CO_2 flux from the soil surface (Reicosky and Lindstrom, 1993; Reicosky et al., 1995, 1997; Rochette and Angers, 1999). Until recently, however, little was known about the concomitant biological, chemical, and physical responses of soil to tillage events that accompany this short-lived release of soil CO_2. Biogeochemical changes may be associated with changes in soil microbial community structure.

Here we summarize the results of three recent experiments on short-term responses (hours and days) to tillage events (Calderón et al., 2000, 2001; Calderón and Jackson, 2002). Our objectives were as follows: (1) to analyze factors that contribute to the immediate CO_2 flux after tillage; (2) to describe changes in inorganic nitrogen pools, microbial biomass N, and gaseous emissions of N; and (3) to assess the changes in soil microbial community structure that occur after tillage, and attempt to relate these changes to soil processes. Analysis of phospholipid fatty acids (PLFA) was used to describe microbial community structure. It provides a set of molecular markers for microbial taxa and indicators of microbial stress that can be used to track changes in composition of the soil microbial community, and it also gives a measure of the total viable microbial biomass (Vestal and White, 1989; Bossio and Scow, 1995; White et al., 1996). All experiments were conducted with soils under intensive vegetable production in California; one experiment included a grassland soil from the same soil type. Because intensive vegetable production typically uses more than 10 tillage passes

per year, we were interested in determining how single events affected soil processes. Each experiment utilized a different approach for conducting tillage and monitoring the soil responses thereafter. Here we compare and integrate the results of these different approaches to develop a more robust description of the biological changes that occur after perturbation, and we relate these short-term data to other studies that examined more long-term responses to tillage practices. Particular aspects of each experiment are highlighted here, although more comprehensive data sets are presented in the original papers.

2. Materials and methods

2.1. Carbon dynamics following rototillage and disking

To compare soil responses to different types of tillage implements, a field experiment was conducted at a research station site for vegetable production at the University of California, Davis (Calderón and Jackson, 2002). The field was last tilled approximately 6 months before the experiment. The soil was a Yolo silt loam with 7.3 g organic C kg^{-1}, 0.95 g organic N kg^{-1}, and pH of 6.5 in the 0- to 15-cm layer.

Three treatments (rototilled, disk, and a not-tilled control) were replicated three times. Rototillage and disking tilled the top 0–15 cm of soil. A roller was attached to the disk implement. No rainfall occurred during the experiment. At 9 days after tillage, the field was sprinkler-irrigated for 0.5 h. Irrigation increased the soil moisture at 0–15 cm depth from approximately −35 to −5 kPa. No significant leaching occurred after irrigation although some water percolated below 15 cm in the disked treatment, which had an uneven surface. Soil cores (6.7 cm diameter, 15 cm deep) were composited from each of the treatment–block combinations. There were eight sampling times during a 2-week period. Most samplings were carried out between 6:00 and 8:00 am.

Microbial biomass C (MBC) was determined with the chloroform fumigation–extraction method (Vance et al., 1987; Yeomans and Bremner, 1988). For soil respiration, moist soil was placed in jars within 1 h of sampling, which was then sealed and incubated at 25 °C for 1 h. Carbon dioxide in the jar headspace samples was analyzed with an infrared gas analyzer (Horiba PIR-200; Horiba Instruments, Riverside, CA). Flux of CO_2 from the soil surface was obtained using the closed chamber method modified from Rolston (1986). Headspace samples were taken at 15 and 30 min after cap placement and were analyzed as described above.

We conducted a three-way ANOVA to test effects of tillage treatment, time, and block. Mean separations were with the least significant difference (LSD) test.

2.2. Nitrogen dynamics in cores taken from recently tilled and untilled soil

To examine a time course of N dynamics after tillage without changes in grower's practices and environmental conditions, we rototilled a fallow silt loam soil from an intensively managed vegetable field in the Salinas Valley, then removed intact soil

columns by pushing in PVC pipe (30.5 cm deep and 12.7 cm diameter) into tilled and control soils, and sampled them throughout a 2-week period in a growth chamber (Calderón et al., 2001). The soil was a Pico silt loam with 8.8 g organic C kg^{-1}, 1.2 g organic N kg^{-1}, and pH of 7.0 in the 0- to 15-cm layer.

Immediately after rototillage, cores for the first sampling time were processed and sampled in the field. The remaining cores were then transported to the University of California at Davis and incubated at 20 °C in a growth chamber until analyzed. No water was applied during the experiment. In the growth chamber, eight sampling times occurred during the 2-week period.

The chloroform fumigation extraction method (Brookes et al., 1985) was used for MBN, and the extracts were Kjeldahl-digested according to Wyland et al. (1994). The concentration of NH_4^+–N and NO_3^-–N in 2 N KCl extracts, and NH_4^+–N in the Kjeldahl digests of the MBN samples were determined colorimetrically using a Lachat Quick Chem II Flow Injection Analyzer (Zellweger Analytical, Milwaukee, WI).

Five additional cores from each treatment were used repeatedly for denitrification measurements with the acetylene inhibition method (Mosier and Klemedtsson, 1994), using capped cores (Folorunso and Rolston, 1984) with a single acetylene supply probe inserted in each soil core. The N_2O concentration in the gas samples was determined using the method of Rasmussen et al. (1976). We used a Hewlett Packard 6890 Gas Chromatograph fitted with an electron capture detector. In addition to these cores that were supplied with acetylene, N_2O concentrations were measured in the headspace of untreated cores at every sampling time.

Main effects (tilled vs. control soil) and interactions of tillage and time were tested by two-way ANOVA. The LSD was used for mean separations.

2.3. Microbial community structure after simulated tillage

To track changes in soil microbial community structure after simulated tillage by sieving, intact soil cores were collected from two sites of granite-derived Chualar sandy loam soils in the Salinas Valley, California (Calderón et al., 2000). One was a site under intensive vegetable production, and the other was an annual grassland site. The soils had nearly identical particle size distribution. The vegetable production and grassland soils had the following characteristics in the 0- to 15-cm layer, respectively, 6.7 and 12.0 g organic C kg^{-1}, 0.8 and 1.2 g organic N kg^{-1}, and pH of 7.7 and 6.6.

PVC pipes (30.5 cm deep and 12.7 cm diameter) were driven into the soil, all vegetation was cut at ground level and the litter layer was removed, and cores were transported to a greenhouse to acclimate the cores under constant conditions at similar matric potential.

The soil moisture at the time of the tillage simulation was approximately −55 kPa. One set of cores was sampled before sieving. The top 15 cm of soil of the remaining cores was sieved through a 5-mm screen, pooled, mixed, and immediately repacked to 95% of the original bulk density. The two soils dried gradually at similar rates (data not shown), and no water was added during the experiment. There were seven sampling times during the 2-week experiment.

Duplicate samples for PLFA analysis from each cylinder were stored at −20 °C until the extraction. Total lipids were extracted using the procedure of Bligh and Dyer (1959). The PLFA were purified and then derivatized from the lipid extracts and analyzed by gas chromatography using the procedure, conditions, and terminology detailed by Bossio and Scow (1995). To compare microbial community structure between sites and with time, Canonical correspondence analysis (CCA) of CANOCO 4 (CANOCO, Microcomputer Power, Ithaca, NY) utilized the concentrations of the 26 different PLFA that were consistently quantified in all of the grassland and vegetable samples. In CCA, a total of 26 PLFA common to both data sets was used. A Monte Carlo permutation test tested for significant effects of time since sieving and/or site on the PLFA. For more details on analysis, see Calderón et al. (2000). Within each soil type, one-way ANOVA tested for differences between sampling times, and mean separations were with a Duncan's test.

3. Results

3.1. Carbon dynamics following rototillage and disking

In the field experiment comparing soil responses to different types of tillage implements, CO_2 flux from the soil surface was higher immediately after tillage than in the control, but this was a short-lived effect and lasted <12 h after tillage (Fig. 1). Highest flux was in the disked soil. After irrigation at 9 days after tillage, the CO_2 flux of all treatments increased. The largest response to irrigation occurred in the control soil, with a more than 10-fold increase that took 42 h to decline back to preirrigation levels. The CO_2 flux from the tilled soils was of lower magnitude and shorter duration.

No differences in soil respiration occurred between treatments during the course of the experiment (Fig. 1). Even though the disked soil had lower moisture throughout the experiment, no consistent differences occurred in respiration rates. Respiration increased in all treatments after irrigation, but the responses to irrigation were much lower than for CO_2 flux. Microbial biomass carbon in samples taken at 0–15 cm depth was similar between treatments throughout the experiment. It did not increase significantly in the sample taken at 12 h after irrigation. When samples were taken at 0–3 cm depth at the end of the experiment, however, MBC was reduced to 22% and 60% of the control treatment in the rototilled and disked soils (data not shown).

3.2. Nitrogen dynamics in cores taken from recently tilled and untilled soil

When intact cores of recently tilled soils were kept under controlled conditions, NO_3^-–N levels were consistently higher in tilled than control soil, beginning at 2 days after rototillage (Fig. 2). At 2 weeks after tillage, NO_3^-–N was two times higher in the tilled than in the control soil. The concentration of NH_4^+–N remained below 0.35 μg N g^{-1} throughout the experiment and was never significantly different between treatments (data not shown). Moisture content was similar in the tilled and control soils throughout most of the experiment. There was only a slight decline in moisture during the 2-week period. The

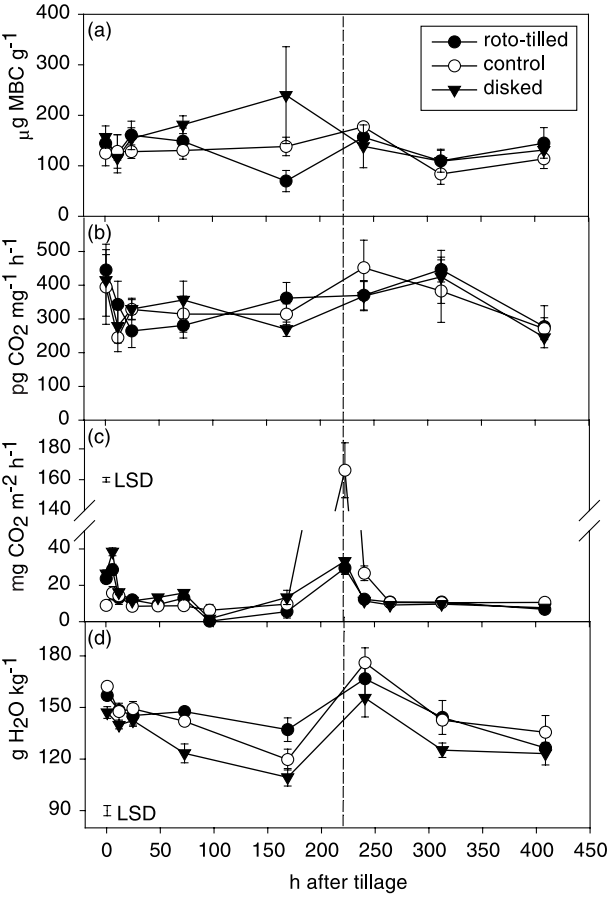

Fig. 1. Field experiment comparing Yolo silt loam soil in rototillage, disking, and control treatments. The soils were tilled at 0 h. The time of irrigation (222 h) is indicated by the dotted line. Soil samples were taken at 0–15 cm depth. Mean ± S.E. ($n=3$) for: (a) microbial biomass carbon, (b) respiration, (c) CO_2 flux, and (d) H_2O content. The least significant difference (LSD) between the treatments is shown. There were no significant differences in MBC or respiration at any point during the experiment. For reference, 110 g H_2O kg^{-1} is equivalent to −51 kPa.

tilled soil had an average dry bulk density of 1.02 g cm^{-3}, whereas the control soil averaged 1.09 g cm^{-3}.

Microbial biomass N increased briefly a few days after tillage (Fig. 2), but there was no concomitant increase in MBC (data not shown). After a pronounced increase in the tilled soil after day 2, MBN declined, and the tilled and control soil had similar MBN levels at the end of the 2-week experiment.

Denitrification with the acetylene block increased significantly after tillage, beginning at day 2 and lasting until a week after tillage (Fig. 2). During the 14-day period, the total N denitrified is estimated to be 155 g ha^{-1} for the tilled soil, and 48 g ha^{-1} for the control soil, based on extrapolations of measured values from hourly to daily rates. In the

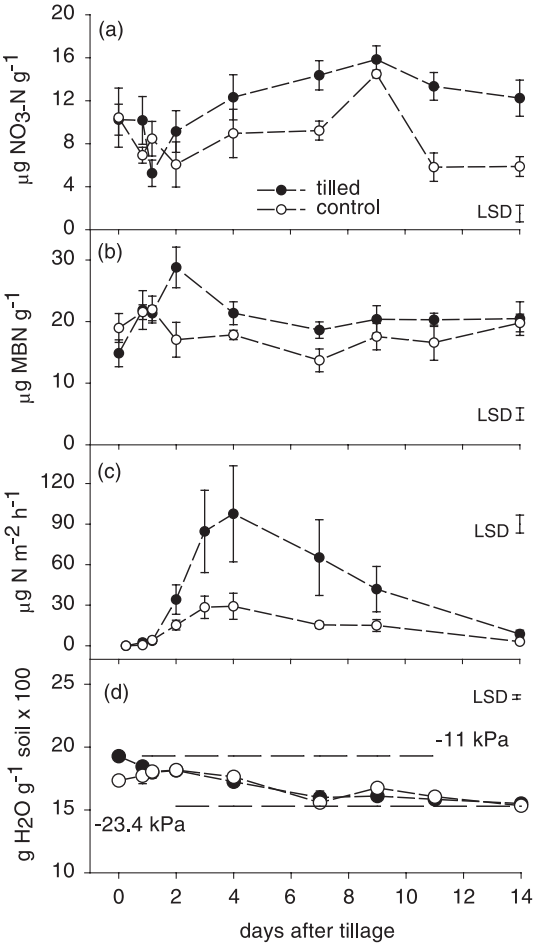

Fig. 2. Microcosm experiment in which intact cores of Pico silt loam were transported to a controlled environment after rototillage. The sample at 0.04 days was obtained in the field immediately after tillage, while later samples were taken from intact cores incubated in the 20 °C growth chamber. Soil samples were taken at 0–15 cm depth. Mean ± S.E. ($n = 5$) for rototilled and control soils: (a) NO_3–N, (b) microbial biomass N, (c) denitrification rate with acetylene block, and (d) H_2O content. Day zero is the time of tillage. The least significant difference (LSD) between tilled and control soil is shown.

acetylene-free cores that were sampled concurrently, N_2O flux was never above ambient (data not shown), indicating that N_2 loss by denitrification was the main source of N_2O during the acetylene block assay.

3.3. Microbial community structure after simulated tillage

Sieving had little effect on the concentration of total PLFA, which is considered to be a measure of the total microbial biomass, in either the vegetable or the grassland soil during

the first week after sieving, except for a brief decline between 3 and 24 h (Fig. 3). In the second week, the total PLFA remained constant in the vegetable soil, but decreased markedly in the grassland soil. Microbial biomass C, as measured by chloroform fumigation extraction, was also much higher in the grassland soil, and showed a generally similar temporal response as the total PLFA (data not shown). Thus, sieving had longer-term effects on microbial biomass in the noncultivated compared to the frequently tilled soil.

Changes occurred in some specific PLFA, indicating temporal changes in the relative abundance of certain microbial groups (Fig. 3). A fungal marker (18:2 ω6; Vestal and White, 1989) tended to decrease in the grassland soil during the second week of the experiment. In the vegetable soil, concentrations of this marker were always lower than in the grassland soil, and changed little throughout the 2-week period. In both soils, a marker for microeukaryotes (Vestal and White, 1989; Zelles, 1997) declined immediately after sieving, but concentrations recovered to initial values in the vegetable but not the grassland soil. This molecule has been found in the biomass of protozoans, as well as in *Mycelia sterilia* and Pythiacious fungi (Gandhi and Weete, 1991; Stahl and Klug, 1996). Sieving caused a gradual increase in the ratio of 19:0 cy to 18:1 ω7 in both soils, which is an indicator of stressful conditions for bacteria, such as stationary growth, acidic

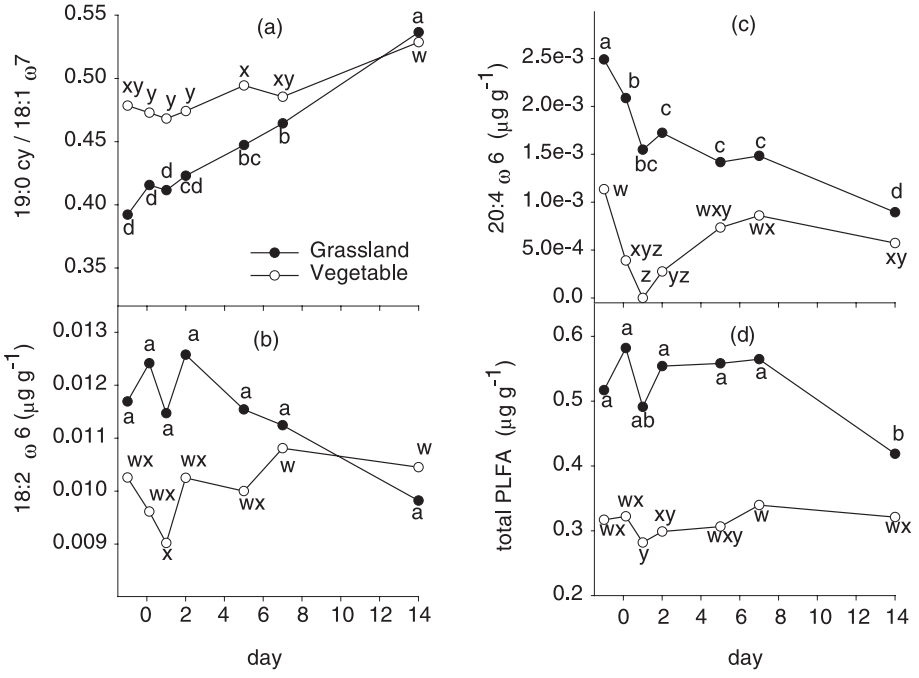

Fig. 3. Concentrations of selected phospholipid fatty acid (PLFA) during the time course following sieving of vegetable production and grassland soils of similar parent material and texture. Soil samples were taken at 0–15 cm depth. Mean ± S.E. ($n = 4$). Mean separations for the vegetable production soil are shown with *w*, *x*, *y*, and *z*, and for the grassland soil with *a*, *b*, *c*, and *d*. Note differences in the scales of the *y*-axis.

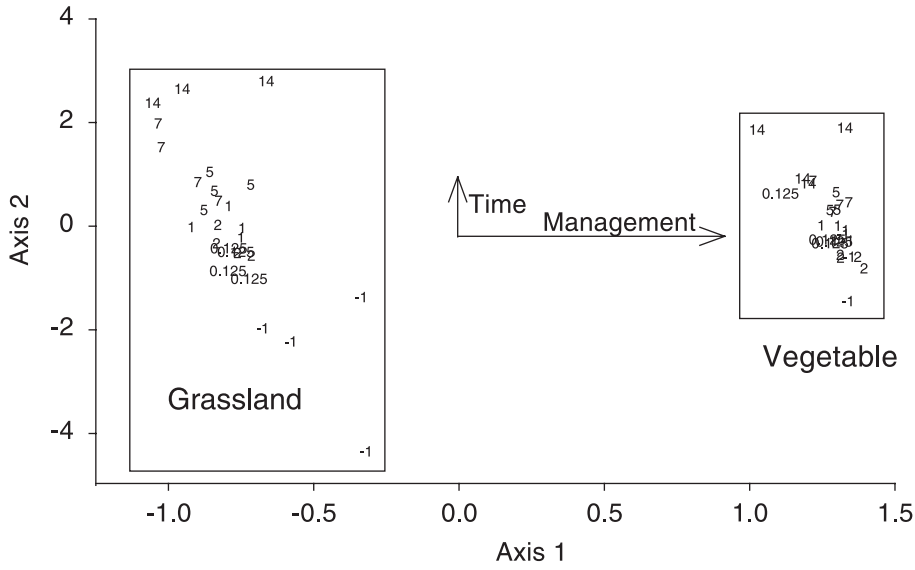

Fig. 4. Canonical correspondence analysis (CCA) ordination biplot for the phospholipid fatty acid (PLFA) profiles of the vegetable production and grassland soils. Soil samples were taken at 0–15 cm depth. The arrows are the scores of the environmental variables on the biplot. Management type (vegetable production vs. grassland) is significantly correlated with axis 1 and explains 73% of the total variation. Time since sieving is significantly correlated with axis 2, and explains 5% of the total variation.

conditions, low oxygen, and high temperature (Guckert et al., 1986). The increase was much more pronounced in the grassland compared to the vegetable soil (Fig. 4).

Multivariate analysis showed that the grassland and vegetable soils had distinctly different PLFA composition, and that PLFA composition was significantly related to time since sieving. Thus, microbial community structure was different between the sites, and changed significantly with time in both soils. There were greater relative temporal differences, however, in PLFA profiles in the grassland soil than in the vegetable soil. In fact, from before sieving and within 1 day after sieving, the grassland soil had already developed a different microbial community structure. In both soils, greater chronological separation occurred during the first week after sieving than in the second week.

4. Discussion

These experiments demonstrate that short-term responses to tillage, that is, emission of CO_2 from the soil surface, net nitrate production, and changes in soil microbial community structure, constitute aspects of soil quality that should be considered in evaluating soil management practices. For each of the three experiments, more comprehensive data sets were collected, and they corroborate the results that are presented here (Calderón et al., 2000, 2001; Calderón and Jackson, 2002). Soil respiration rate never increased after tillage or sieving, supporting the idea that CO_2 flux immediately after tillage is due to physical

processes. Nitrate accumulation always occurred, but at higher amounts in the grassland than vegetable soil, and in the silt loam compared to the sandy loam soils. PLFA profiles in the rototilled silt loam changed most rapidly during the first week after tillage, and were nearly identical to the untilled soil after 2 weeks, corroborating that soil microbial community structure responds quickly to disturbance in intensively managed agricultural soils, but does not experience prolonged changes. In this soil, increased denitrification after rototillage was accompanied by an increase in a PLFA marker for anaerobic eubacteria, 19:0 cy, suggesting that tillage created anaerobic microsites. In previously undisturbed grassland soil, however, sieving resulted in large changes in soil microbial community structure and PLFA profiles did not return to pre-disturbance status within a 2-week period. The results of these and other studies (Petersen and Klug, 1994; Reicosky et al., 1995, 1997; Rochette and Angers, 1999) suggest that tillage events cause temporary stress conditions for soil microbes, decrease their ability assimilate nutrients, alter community structure, and increase the potential for loss of C and N from the soil, even in soils that experience frequent tillage.

In terms of soil quality, the largest impact of a tillage event may be net nitrate production and loss, especially in fine-textured soils (Silgram and Shepherd, 1999). Our studies and others have shown that net mineralization of N after soil disturbance is greater in fine- than coarse-textured soils, probably due to release of more previously protected organic matter (Hassink, 1992; Cabrera and Kissel, 1988). Because N mineralization is typically greater than C mineralization after soil disturbance, the previously protected organic matter that is exposed by tillage apparently has a lower C/N ratio than the rest of the organic matter (Hassink, 1992; Calderón et al., 2001). As in our experiments, the flush of N mineralization is typically ephemeral, and only lasts from a few days to a few weeks after tillage (Silgram and Shepherd, 1999).

Nitrate accumulates after tillage and becomes susceptible to loss. More nitrate was leached from conventionally tilled compared to conservation tillage or no tillage corn production (Drury et al., 1993). Nitrate leaching losses were greater when autumn cereal crops were grown on ploughed soils compared to direct drilling (Goss et al., 1993). Flux of N_2O from the soil surface tended to increase with the degree of soil disturbance by tillage implements in a wheat–fallow rotation (Kessavalou et al., 1998a). Further work is needed to understand the factors that cause denitrification to increase after rototillage (Calderón et al., 2001). Most studies show that N mineralization and nitrate accumulation increase in tilled soils compared to no-till soils (Silgram and Shepherd, 1999). In no-till agricultural soils, however, higher rates of potential nitrification and denitrification activity have been measured than in conventionally tilled soils, but this has been explained by the accumulation of readily mineralizable organic matter through time, and its stratification in the surface layer of no-till soils (Staley et al., 1990; Rasmussen and Collins, 1991; Kandeler and Böhm, 1996), rather than to higher in situ rates of N mineralization.

Our measurements of the initial burst of CO_2 flux from the soil surface after tillage were lower than in grain and corn cropping systems from the central and eastern United States and Canada (Reicosky et al., 1997; Ellert and Janzen, 1999; Kessavalou et al., 1998b; Rochette and Angers, 1999), and much lower than after incorporation of wheat stubble (Reicosky and Lindstrom, 1993). In our implement experiment, CO_2 flux from tilled soil after rewetting was lower than in a wheat–fallow system (Kessavalou et al., 1998b),

although both studies showed that the most disruptive tillage methods released the least amount of CO_2 following wetting, possibly because prior tillage had already caused the partial pressure of CO_2 in the soil atmosphere to decline. Rewetting events, however, also increase soil respiration, and rates of microbial CO_2 production are strongly related to the size of the soil organic C and microbial biomass pools (Lundquist et al., 1999). In intensive California vegetable production, little crop residue is returned to the soil, and organic matter amendments are not usually applied. Thus, soils may likely have less readily decomposable organic matter than in grain cropping systems. This could result in lower partial pressure of CO_2 in the soil atmosphere so that a tillage event does not produce such a large burst of CO_2 by degassing, as well as less substrate for microbial decomposition in the subsequent period after tillage has occurred. Further work in soils from other regions and cropping systems is needed to confirm our limited findings in California vegetable and grassland soils that tillage events cause temporary stress for soil microbes, rather than lead to a burst of microbial activity, and that changes in soil microbial community structure may be associated with the ability of microbes to retain soil C and N.

In our 2-week experiments, tillage caused a short period of increased CO_2 flux from the soil surface. Soil profile degassing of CO_2 immediately after tillage, however, can be followed by increased soil respiratory metabolic activity for a longer period of time, especially when temperatures are warm, and readily decomposable organic matter or crop residue is present (Dao, 1998; Rochette and Angers, 1999). A comparison of tilled and no-till soils showed that tilled soils had higher amounts of cumulative CO_2 flux over a growing season, as well as higher microbial biomass, activity, and respiration, and that these differences could largely be explained by higher soil temperature in the tilled soils (Dao, 1998). Soils under long-term cultivation typically have higher porosity, lower bulk density, and lower pore connectivity and continuity, so that water holding capacity and saturated hydraulic conductivity decrease (Silgram and Shepherd, 1999). These soil characteristics result in slightly warmer temperatures and lower water content in tilled than no-till soils. Because microbial processes follow a Q_{10} response pattern, such that a doubling in activity occurs with a 10 °C increase in temperature, changes in soil physical properties in tilled soils may trigger the onset of a longer-term increase in C mineralization rates. Changes in soil microbial community structure may occur in response to altered soil physical properties that affect the soil microenvironment, with possible effects on the efficiency of C conservation by microbes. A readily mineralizable source of organic matter would enhance responses of soil microbial processes to changes in the soil environment, for example, warmer temperatures, caused by the tillage regime. Thus, even though our findings indicate that a single tillage event does not immediately stimulate microbial activity, changes in soil physical properties and availability of soil organic matter may increase rates of C mineralization and CO_2 flux over the long term in tilled soils.

Acknowledgements

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