

# Short-Term Dynamics of Nitrogen, Microbial Activity, and Phospholipid Fatty Acids after Tillage

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## ABSTRACT

Little is known about the short-term effects (hours to days) of tillage on labile pools of C and N, or microbial activity and community composition. We examined the effects of rototillage on microbial biomass C (MBC) and N (MBN), respiration (i.e., soil CO<sub>2</sub> production in 1-h incubations), CO<sub>2</sub> efflux from the soil surface, inorganic N, nitrification potential, denitrification rate, and phospholipid fatty acids (PLFA). A fallow silt loam soil was rototilled in the field and soil cores were immediately obtained from tilled and adjacent control soils. The soil cores were then incubated at constant temperature and sampled throughout a 2-wk period. Tilled soil had higher CO<sub>2</sub> efflux than the control soil. This increase occurred immediately after tillage and lasted for 4 d. Respiration was similar in both soils until the fourth day after tillage, and then declined in the tilled soil. Tilled soil showed increases in MBN, nitrate, and denitrification rates, suggesting that tillage makes available previously protected organic N. The overall reduction in respiration together with the lack of response in MBC, however, suggests that tillage did not make available significant amounts of readily decomposable C. These combined C and N dynamics suggest that low C/N ratio compounds may have been mineralized following tillage. Denitrification rates increased in the tilled soil even though the bulk of the soil had reduced respiration and bulk density. Tillage caused temporary changes in PLFA profiles, suggesting changes in soil microbial community structure. Phospholipid fatty acid 18:1 ω7t, which marks the presence of eubacteria, decreased in the tilled soil. In contrast, 19:0 cy, a marker for anaerobic eubacteria, increased in the tilled soil. Our results show that tillage causes short-term changes in nutrient dynamics that may potentially result in N losses through denitrification and nitrate leaching, as well as C losses through degassing of dissolved CO<sub>2</sub>. These changes are accompanied by concomitant shifts in microbial community structure, suggesting a possible relationship between microbial composition and ecosystem function.

**A**RABLE SOILS under a long-term regime of frequent tillage usually suffer from losses in organic matter, increased nitrification, and deteriorated soil structure, thus reducing agricultural sustainability (Doran, 1982; Elliott, 1986). However, little is known about the short-term effects (hours to days) of tillage on labile C and N pools, microbial activity and community structure, and nitrate that is prone to loss through denitrification and leaching.

Soil respiration is the production of CO<sub>2</sub> by metabolizing organisms within the soil matrix (Anderson, 1982). In contrast, CO<sub>2</sub> efflux is the movement of CO<sub>2</sub> from

soil to the atmosphere and it is measured at the soil surface (Rolston, 1986). Increases of soil CO<sub>2</sub> efflux are not always preceded by increases in soil respiration (Calderón et al., 2000). Tillage is known to induce short-lived increases in CO<sub>2</sub> efflux from soil (Reicosky and Lindstrom, 1993; Reicosky et al., 1995, 1997; Rochette and Angers, 1999; Ellert and Jantzen, 1999). It has been hypothesized that tillage may expose previously protected organic matter that may serve as a substrate for microbial growth (Rovira and Greacen, 1957). Yet, breaking up the soil structure may reduce or stress microbial populations and consequently reduce respiration (Petersen and Klug, 1994; Calderón et al., 2000). Thus, although tillage can increase the efflux of CO<sub>2</sub> from the soil surface, it may not always stimulate microbial activity. Degassing of dissolved CO<sub>2</sub> from the soil solution and pore space upon soil disturbance and aeration may partially explain the temporary efflux of CO<sub>2</sub> after tillage (Reicosky et al., 1997).

Changes in microbial activity and nutrient pools may be associated with simultaneous changes in microbial community structure. Analysis of PLFA is a useful assay because (i) the concentration of total PLFA can be used as an index of viable microbial biomass since phospholipids are rapidly degraded after cell death, (ii) multivariate analysis of the PLFA profiles can be used to detect changes in community composition, and (iii) certain fatty acids may be used as molecular markers for specific taxa and as indicators of microbial stress (Bossio and Scow, 1995; White et al., 1996; Vestal and White, 1989).

The complex interactions between C and N dynamics, microbial activity, and CO<sub>2</sub> efflux after tillage deserve further investigation. The purpose of this research was to measure the short-term (hours and days) effect of field rototillage on respiration (as measured by soil CO<sub>2</sub> production in sealed containers), CO<sub>2</sub> efflux from the soil surface, soil inorganic N, denitrification, microbial biomass C (MBC) and N (MBN) by chloroform fumigation-extraction, and microbial community structure (PLFA profiles). We tested the hypothesis that tillage makes available previously protected organic matter and as a result, causes changes in microbial composition as well as nutrient mineralization and, as a result, immobilization by microbes. A bare fallow silt loam soil from an intensively managed vegetable field was used. Our experimental approach was to till the soil in situ, then obtain soil cores of tilled and control soils, which were then sampled and analyzed throughout a 2-wk period under controlled environmental conditions.

## MATERIALS AND METHODS

### Site Characteristics

The experiment was carried out in April 1998 on an agricultural field in the Salinas Valley, California. The soil was a

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Pico silt loam (coarse loamy, mixed, thermic, Fluventic Haploxerolls). Typically, the site is tilled several times per year, and receives high levels of N fertilizer ( $>200 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) and pesticide applications. Cole crops, lettuce, and celery are the usual crops. Tillage and spring vegetable planting were delayed because of an unusually wet and prolonged winter, so the experimental plot was fallow with shaped 1-m-wide beds for eight months prior to the experiment. Weed density in the experimental plot was low, but we clipped weeds without disturbing the soil two weeks before initiating the experiment.

We obtained samples of the soil for pH, organic matter, total N, total C, and particle size distribution 4 d before the beginning of the experiment. Four samples from the 0- to 15-cm depth were sieved (2 mm) to exclude the coarse plant residue and rocks, which made up  $45 \text{ g kg}^{-1}$  of the soil. The soil characteristics were measured from the  $<2\text{-mm}$  fraction of soil. The pH was determined from a saturated paste. Organic matter was measured by combustion (Nelson and Sommers, 1982). Total N and C were measured according to Pella (1990). The particle size distribution was determined using the method of Gee and Bauder (1982). The soil was  $350 \text{ g kg}^{-1}$  sand,  $522 \text{ g kg}^{-1}$  silt, and  $128 \text{ g kg}^{-1}$  clay. The pH was 6.95. The soil had  $10.9 \text{ g kg}^{-1}$  organic matter. Total N was  $1.2 \text{ g kg}^{-1}$  and total C was  $8.8 \text{ g kg}^{-1}$ .

### Experimental Design

Four transects were established along four adjacent parallel beds. Two transects were tilled using a BCS 745 rototiller (BCS S.p.A., Milano, Italy). The other two transects were left intact to be used as a non-tilled control. Each transect was 1 m wide and 20 m long. The tilled transects received two passes with a rototiller along the top of the fallow, raised beds. We measured the tilled layer to confirm that the depth of the tilled soil throughout the transects was uniformly 15 cm deep. Soil temperature (15 cm) at the time of tillage was  $20^\circ\text{C}$ . Pipes of polyvinylchloride (30.5 cm deep and 12.7-cm dia.) were driven at least 28 cm into the center of the raised beds and subsequently dug out taking care not to disturb the soil inside the cores. We obtained a total of 110 cores, 55 from the two tilled transects, and 55 from the two control transects. We analyzed five cores from each treatment and time combination. A total of 50 cores within each treatment were randomly assigned to one of the ten sampling times [0.04 (1 h), 0.83 (20.6 h), 1, 2, 3, 4, 7, 9, 11, and 14 d after tillage]. The remaining five cores from each treatment were assigned to the denitrification measurement (see below).

The cores for the first sampling time (1 hour after tillage) were processed and sampled in the field. The bottom of the cores for the rest of the sampling times were covered with a perforated plastic film to prevent contamination, while allowing some gas transfer. The cores were then transported to the University of California at Davis and incubated at  $20^\circ\text{C}$  in a growth chamber until analyzed. We incubated the soil cores in this controlled environment to avoid influences of weather and grower's practices that could mask the responses to tillage. Cores were not covered. Evaporative losses of moisture during the incubation were not replenished, since rewetting causes large changes in soil microbial activity (Lundquist et al., 1999).

The top 15 cm of the soil was removed from the cores at each sampling time and mixed gently to minimize the destruction of aggregates while achieving homogeneity of the sample. Each core was sampled immediately for PLFA, MBC and MBN, ammonium, nitrate, and gravimetric moisture. All sampling times, except the initial field sample, included measurements of nitrification potential.

### Measurements

Duplicate samples from each core (25 g moist soil) were analyzed for MBC and MBN by the fumigation extraction method (Brookes et al., 1985; Vance et al., 1987). Briefly, one moist soil subsample was extracted by shaking for 30 min with 60 mL of  $0.5 \text{ M K}_2\text{SO}_4$ . Another subsample was fumigated for 24 h with ethanol-free chloroform ( $\text{CHCl}_3$ ). The  $\text{CHCl}_3$  was removed, and the fumigated soil was extracted with the original stock  $\text{K}_2\text{SO}_4$  solution that was used to extract the fresh soil. The amount of organic C in the extracts was determined by oxidation with dichromate in concentrated sulfuric acid and phosphoric acid. The amount of unreacted dichromate was measured by manual titration with ferrous ammonium sulfate (Vance et al., 1987; Yeomans and Bremner, 1988). The total MBC was calculated by multiplying the flush of C by 2.64 (Vance et al., 1987). For MBN, the extracts were subjected to Kjeldahl digestion by the procedure of Wyland et al. (1994). The total ammonium in the digests was determined colorimetrically with a Lachat Quick Chem II Flow Injection Analyzer (Zellweger Analytical, Milwaukee, WI). The total MBN was calculated by multiplying the flush of inorganic plus organic N by 1.86 (Brookes et al., 1985).

The assay for soil respiration was conducted by placing 200 g of moist soil from each core into 250-mL jars, sealing the jars, and sampling the jar headspace for  $\text{CO}_2$  after 1 h. The soil was put into the jars with care to minimize further disruption of the soil. Each jar was then sealed with a cap fitted with a septum suitable for gas sampling, flushed with lab air, and incubated at  $25^\circ\text{C}$  for 60 min. Jars were then sampled with a needle attached to a 1-mL syringe. Respiration data for the first sampling time (0.04 d after tillage) were obtained by incubating soil at the University of California Extension facilities in Salinas and storing the gas sample in an evacuated container (Labco Exetainer System, Product Code 139R, Labco Ltd., High Wycombe, England). For all of the following sampling times, the samples were incubated in our laboratory at the University of California at Davis and the samples were analyzed without storage. Carbon dioxide was determined with an infrared gas analyzer (Horiba PIR-200, Horiba Instruments Inc., Riverside, CA). The amount of  $\text{CO}_2$  was calculated by comparison with a known  $\text{CO}_2$  standard.

We measured the  $\text{CO}_2$  and NO efflux from the soil surface of the cores using the closed chamber method (Rolston, 1986). The gas efflux sampling was carried out immediately before each of the cores was destructively sampled for the rest of the variables. Carbon dioxide was analyzed with a Hewlett Packard 5890A gas chromatograph (Hewlett Packard, Palo Alto, CA) with a 0.16-cm stainless steel column packed with Hayesep Q (Supelco, Bellefonte, PA) and connected to a TCD detector. Nitric oxide in the gas samples was measured by chemiluminescence (Sievers Instruments Model 270B Nitric Oxide Analyzer, Sievers Instruments, Boulder, CO).

Nitrification potential was measured by a modified chlorate inhibition method (Belser and Mays, 1980) on one sample from each core. Each sample (20 g moist soil) was placed in a 250-mL flask. One extra soil sample from each treatment was used as a blank. Then, 100 mL of phosphate buffer ( $0.136 \text{ g/L KH}_2\text{PO}_4$ ) was added to the blanks, and 100 mL of sample buffer [ $66.1 \text{ mg/L (NH}_4)_2\text{SO}_4$  in blank buffer] was added to the samples. Immediately, 3 mL of  $0.2 \text{ M}$  potassium sulfate was added to the blanks and 3 mL of  $0.2 \text{ M}$  potassium chlorate was added to the samples. The flasks were covered with perforated aluminum foil, and placed on a rotary shaker. Five hours after chlorate addition, the samples were centrifuged and the concentration of nitrite in the supernatant was determined colorimetrically with a Shimadzu UV-1601 spectrophotometer

(Shimadzu Scientific Instruments, Columbia, MD). We made a standard curve by adding nitrite to the blank supernatant.

One sample (30 g moist soil) was taken from each soil core and analyzed for inorganic N. The samples were mixed with 75 mL of 2 M KCl, shaken for 20 min, centrifuged, and the supernatants were stored at  $-20^{\circ}\text{C}$  until analysis. The concentration of ammonium and nitrate in the soil extracts was determined colorimetrically with a Lachat Quick Chem II Flow Injection Analyzer.

At each sampling time, gravimetric moisture was determined after drying approximately 50 g of soil at  $105^{\circ}\text{C}$  for 48 h. Before the experiment, we compared matric potential with concurrent measurements of gravimetric moisture to produce a soil-drying curve on undisturbed soil cores collected 4 d before tillage occurred. The results of the drying curve allowed us to estimate the water potential in the experimental soil.

In addition to the cores in the main experimental blocks, a separate set of five cores per tillage treatment was sampled repeatedly for  $\text{CO}_2$  efflux and denitrification using the acetylene inhibition method (Mosier and Klemmedtsson, 1994). These cores were placed in a separate  $20^{\circ}\text{C}$  growth chamber. We decided against measuring denitrification on the cores sampled for all other variables because of the inhibitive effect of acetylene on nitrification. The same cores were used repeatedly throughout the experiment for the soil efflux measurements. The efflux of  $\text{N}_2\text{O}$  after the acetylene block was measured from capped cores (Folorunso and Rolston, 1984) with a single acetylene supply probe inserted in each soil core. The acetylene supply probe was a plastic tube (5-mm-internal dia.) with holes drilled throughout the underground length of the tube and was set to a depth of 20 cm. A soil gas probe was inserted vertically from the soil surface into each core at a depth of 15 cm to monitor the concentration of acetylene and make sure that it remained above the threshold level of 10%. In addition to acetylene, we measured the  $\text{N}_2\text{O}$  and  $\text{CO}_2$  concentrations in the soil gas. The soil gas probe was a 35 cm piece of plastic tubing (2-mm dia.) with a septum at the top and a 6 cm perforated plastic tube (3-mm dia.) glued to the bottom end. Acetylene was dispensed to the eight cores at a rate of 70 to 85 mL/min for 60 min before each sampling. Immediately after the acetylene flow was turned off, the cores were capped with an airtight lid of known internal volume fitted with a gas sampling port. Gas samples (5 mL) were taken from the headspace as well as the soil gas at 30 and 60 min. The  $\text{N}_2\text{O}$  concentration in the gas samples was determined by the method of Rasmussen et al. (1976). We used a Hewlett Packard 6890 Gas Chromatograph fitted with an electron capture detector. The gas chromatograph had a one-meter long, 0.16 cm diameter stainless steel precolumn with HayeSep T packing, and a two meter 0.16 cm diameter column with HayeSep Q packing. Sampling times for denitrification were concurrent with the destructive samplings for the rest of the variables.

At least three soil samples (8 g dry weight) were analyzed for phospholipid fatty acids at each sampling time. Several months had elapsed since the field had been planted, but any coarse fragments of plant residue were discarded from the soil samples. Total lipids were extracted by the procedure of Blich and Dyer (1959). The PLFA were purified from the lipid extracts and analyzed by gas chromatography using the MIDI Microbial Identification System (MIDI, Newark, DE) as detailed by Bossio and Scow (1995). Fatty acid terminology utilizes A:B $\omega$ C where "A" indicates the total number of carbon atoms, "B" the number of unsaturations, and " $\omega$ " precedes "C," the number of carbon atoms between the closest unsaturation and the aliphatic end of the molecule. The suf-

fixes "c" and "t" indicate cis and trans geometric isomers. The prefixes "i" and "a" refer to iso and anteiso methyl branching. Hydroxy groups are indicated by "OH," preceded by the number of hydroxyl groups in the molecule. Cyclopropyl groups are denoted by a "cy" suffix. A methyl group on the tenth carbon from the carboxylic end of the fatty acid is referred to as "10 Me." Unidentified fatty acids are termed "unk."

### Statistical Analyses

Main effects (tilled vs. control soil) and interactions of tillage and time were tested by ANOVA using the GLM procedure of SAS version 6.11. The Least Significant Difference (LSD) based on a Student's *t*-test was used to illustrate the differences between the tilled and control soils on the figures (SAS Institute Inc., Cary, NC).

The Canonical Correspondence Analysis (CCA) of CANOCO 4 (Microcomputer Power, Ithaca, NY) was used to analyze the PLFA profiles. The average PLFA concentration ( $\text{ng g}^{-1}$ ) from each sampling time was used for the analysis. A total of 39 compounds were consistently quantified in each of the treatment and time combinations and thus were included in the analysis. Canonical Correspondence Analysis is useful for illustrating the relationships between the PLFA profiles of the different treatment combinations. The biplot generated with CCA expresses the pattern of variation between the response variable (PLFA profiles) and an environmental variable (tilled or control). The eigenvalue of each axis in the biplot is a measure of the importance of the axis in explaining the variation in the PLFA profile data. Biplot scores for the individual PLFA are positive when the PLFA increases along the axis and negative when the PLFA decreases. The biplot scores are thus valuable in illustrating which variables are responsible for determining the distribution of the samples in the CCA biplot. A Monte Carlo permutation test was used to test the significance of tillage in explaining variation among the PLFA profiles. A discussion of these multivariate procedures can be found in ter Braak (1987, 1990).

## RESULTS

### Moisture, Bulk Density, Carbon and Nitrogen Dynamics

The tilled and control soils had similar moisture content throughout most of the experiment, except at the initial sampling time (Table 1; Fig. 1). Initially, moisture was slightly higher in the tilled soil ( $192 \text{ g H}_2\text{O kg}^{-1}$  for the tilled soil vs.  $173 \text{ g H}_2\text{O kg}^{-1}$  for the control soil). The mean gravimetric moisture ranged from  $155 \text{ g H}_2\text{O}$

**Table 1. ANOVA results for the tilled and control soils.**

Variable	Time	Tillage	Time $\times$ Tillage
Ammonium ( $\mu\text{g NH}_4\text{-N g}^{-1}$ )	*	NS	NS
Bulk Density ( $\text{g cm}^{-3}$ )	NS	*	NS
$\text{CO}_2$ efflux ( $\text{mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ )	*	*	*
Denitrification ( $\mu\text{g N m}^{-2} \text{ h}^{-1}$ )	*	*	*
Microbial Biomass C ( $\mu\text{g C g}^{-1}$ )	NS	NS	NS
Microbial Biomass N ( $\mu\text{g N g}^{-1}$ )	*	*	*
Moisture ( $\text{g H}_2\text{O g}^{-1} \times 100$ )	*	NS	*
Nitrate ( $\mu\text{g NO}_3\text{-N g}^{-1}$ )	*	*	NS
Nitrification potential ( $\mu\text{g NO}_2\text{-N g}^{-1} \text{ h}^{-1}$ )	*	NS	NS
$q\text{CO}_2$ ( $\text{mg CO}_2\text{-C g}^{-1} \text{ MBC h}^{-1}$ )	*	*	NS
Respiration ( $\text{pg CO}_2\text{-C mg}^{-1} \text{ h}^{-1}$ )	*	*	*
Total PLFA ( $\mu\text{g g}^{-1}$ )	*	*	NS

\* Indicates significance at  $P < 0.05$ ; NS, indicates nonsignificance.

kg<sup>-1</sup> to 192 g H<sub>2</sub>O kg<sup>-1</sup> (-11.0 to -22.8 kPa) in the tilled soil, and from 153 g H<sub>2</sub>O kg<sup>-1</sup> to 173 g H<sub>2</sub>O kg<sup>-1</sup> (-16.7 to -23.4 kPa) in the control soil (Fig. 1). Tillage caused a significant reduction in the soil bulk density (Table 1). The tilled soil had an average dry bulk density of 1.02 g cm<sup>-3</sup>, while the control soil averaged 1.09 g cm<sup>-3</sup>.

Tillage significantly increased the concentration of nitrate in the soil (Table 1; Fig. 2a). Nitrate levels in the tilled soil started to rise above that in the control soil 2 d after tillage and remained high throughout the rest of the experiment. Two weeks after tillage, nitrate concentration in the tilled soil was more than twice that in the control soil. The increase in nitrate of the tilled soil was not accompanied by an increase in nitrification potential (Table 1; Fig. 2b). Tillage had no effect on the ammonium concentration of the soil, which remained below 0.35 µg N g<sup>-1</sup> throughout the experiment in both treatments (data not shown).

Tillage significantly increased the soil MBN (Table 1; Fig. 2c). The effect was delayed as the MBN levels of the tilled soil started to rise 2 d after tillage and did not decrease until after 9 d after tillage. The difference was most pronounced at Day 2, when MBN was 69% higher in the tilled than the control soil. Two weeks after tillage, the tilled and control soil had similar MBN levels. The time × tillage interaction (Table 1) apparently resulted from the slightly higher initial MBN of the control soil.

Tillage significantly increased the denitrification rate of the soil (Table 1; Fig. 2d). Denitrification with the acetylene block started to increase at Day 2 in both treatments and peaked 4 d after tillage. At this point, the denitrification rate of the tilled soil was more than three times the rate of the control soil. The total N denitrified during the 14-d period after tillage amounted to 155 g ha<sup>-1</sup> for the tilled soil, and 48 g ha<sup>-1</sup> for the control soil, based on extrapolations of measured values from hourly to daily rates. In the acetylene-free cores that were sampled concurrently with the main set of

cores used for soil variables and rates of denitrification with an acetylene block, the levels of N<sub>2</sub>O or NO efflux in the acetylene-free cores were never above ambient (data not shown), indicating that N<sub>2</sub> loss by denitrification was the main source of N<sub>2</sub>O during the acetylene block assay.

Tillage had a significant but delayed negative effect on soil respiration, as measured by 1-h incubations of soil in sealed containers (Table 1). The tilled and control soils had initially similar respiration rates. The samples for the first sampling time (one hour, i.e., 0.04 d after tillage) were processed differently than the rest of the sampling times and were not included as part of the

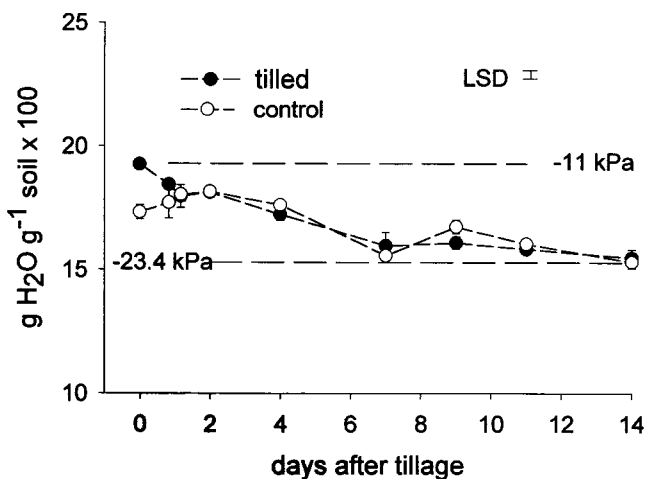


Fig. 1. Mean gravimetric moisture content of the rototilled and control Pico silt loam soils. The error bars are the standard error of the mean (n = 3). The dashed lines show the soil matric potential of the highest and lowest values.

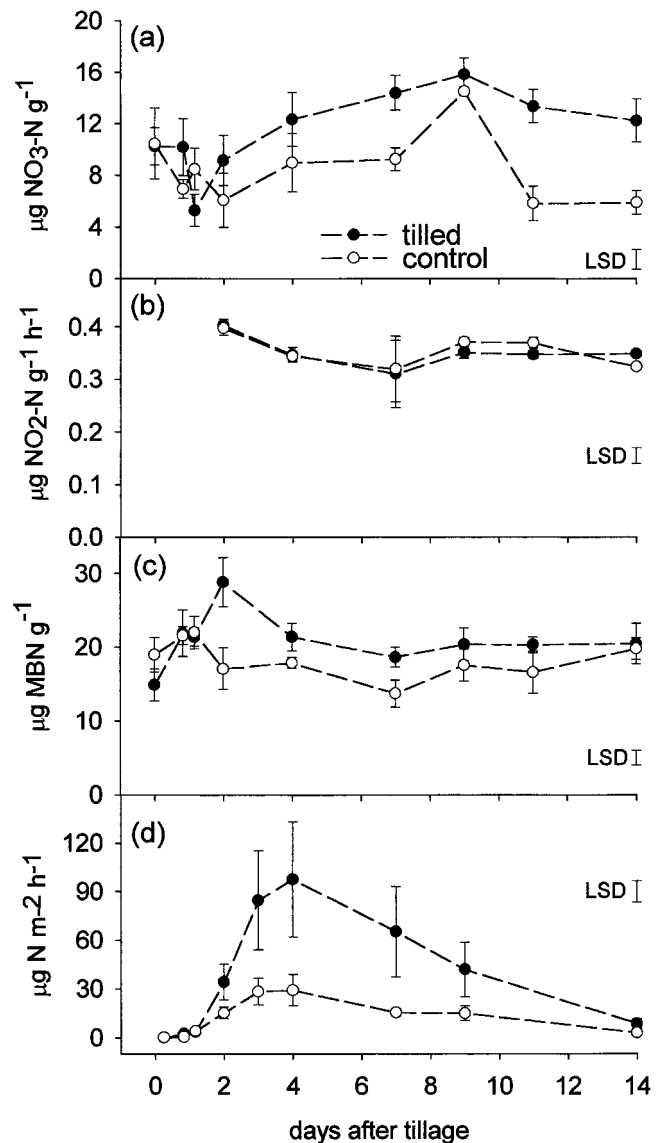


Fig. 2. Mean values (n = 5) for the nitrogen pools and rates of nitrogen transformations of the rototilled and control Pico silt loam soils. (a) NO<sub>3</sub>-N, (b) nitrification potential, (c) MBN, and (d) denitrification rate with acetylene block. Day zero is the time of tillage. The sample at 0.04 d was obtained in the field immediately after tillage, while later samples were taken from intact cores incubated in the 20°C growth chamber. The error bars are the standard error of the mean. The Least Significant Difference (LSD) based on a Student's *t*-test between tilled and control soil is shown.

data in Fig. 3a. For the first sampling time, the tilled and control soils had statistically similar respiration ( $232.0 \text{ pg CO}_2\text{-C mg}^{-1} \text{ h}^{-1}$  for the tilled soil and  $202.7 \text{ pg CO}_2\text{-C mg}^{-1} \text{ h}^{-1}$  for the control soil). Thereafter, respiration fluctuated in both treatments during the first 2 d in the growth chamber (Fig. 3a). Respiration in the control soil stabilized after this initial increase. The respiration of the tilled soil, however, decreased between 6 and 9 d after tillage and was lower than the control soil at the end of the experiment.

Microbial biomass C was unaffected by either tillage or the time of incubation of the soil cores (Table 1).

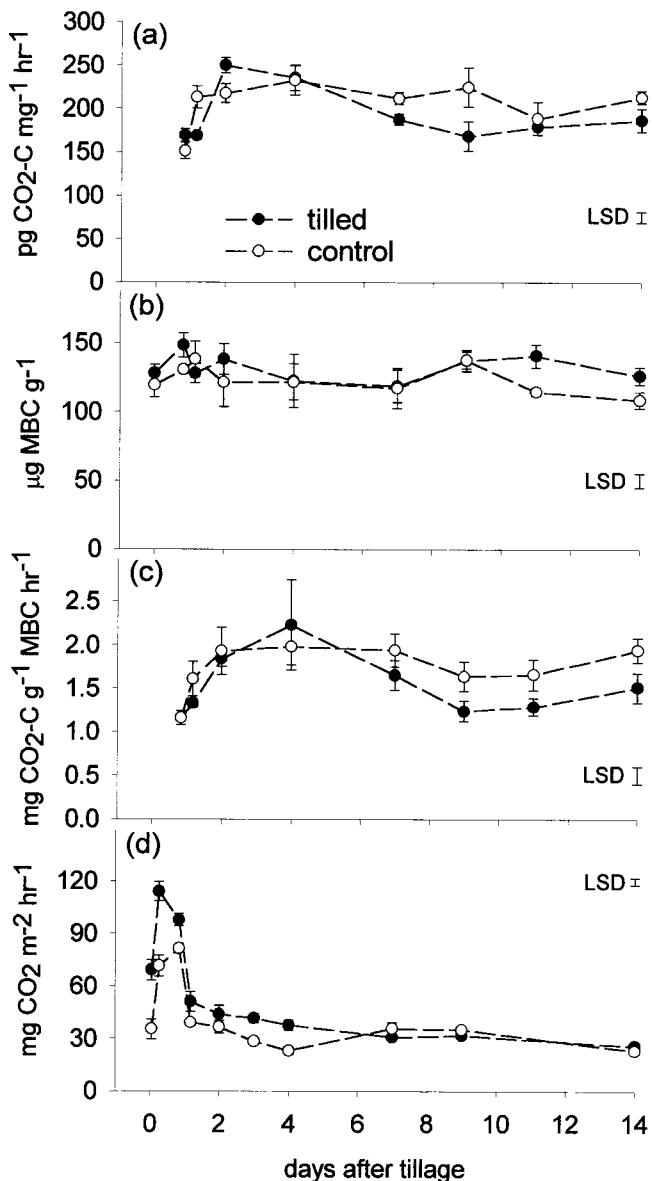


Fig. 3. Mean values ( $n = 5$ ) for the carbon pools and rates of C transformations of the rototilled and control Pico silt loam soils. (a) respiration rate as measured by 1-h soil incubation, (b) MBC, (c)  $q\text{CO}_2$ , and (d)  $\text{CO}_2$  efflux from the soil surface. The sample at 0.04 d was obtained in the field immediately after tillage, while later samples were taken from intact cores incubated in the  $20^\circ\text{C}$  growth chamber. The error bars are the standard error of the mean. The Least Significant Difference (LSD) based on a Student's  $t$ -test between tilled and control soil is shown.

The values for the MBC of the tilled and control soils ranged from 109 to  $148 \text{ mg C g}^{-1}$  (Fig. 3b). The values of the MBC and respiration were used to calculate the metabolic quotient ( $q\text{CO}_2$ ;  $\text{mg CO}_2\text{-C g}^{-1} \text{ MBC h}^{-1}$ ). Tillage had a delayed but significant negative effect on the  $q\text{CO}_2$  (Table 1; Fig. 3c). The tilled soil had lower  $q\text{CO}_2$  than the control soil at the latter sampling times. This was a result of the reduced respiration in the tilled soil (Fig. 3a).

Carbon dioxide efflux into the headspace of capped soil cores was significantly higher for the tilled soil than for the control soil (Table 1). In the initial field sample at 1 h after tillage,  $\text{CO}_2$  efflux was nearly two times higher from the tilled than from the control soil (Fig. 3d). Both the tilled and control soils had increasing effluxes right after placement in the incubation chambers and during acclimation to the incubation conditions (Fig. 3d). The  $\text{CO}_2$  efflux from the tilled soil, however, was 44% higher on average than the control soil through the first 4 d of the experiment. After the fourth day, tilled and control soils had similar efflux values.

### Phospholipid Fatty Acid Analysis

A total of 54 different PLFA were detected in the soil extracts. Of these, 39 PLFA were consistently present and were used for the multivariate analysis. The CCA biplot shows the patterns of variation in PLFA profiles among treatments and sampling times (Fig. 4). There was a clear distinction between tilled and control soils along Axis 1, especially between Day 0.8 and Day 9. The Monte Carlo test showed that tillage had a significant effect ( $P < 0.01$ ) on PLFA profiles. The overall separation of the two tillage treatments along Axis 1 indicates that tillage triggered a change in microbial community structure. Axis 2 primarily separates the PLFA profiles of the last sampling time (14 d after

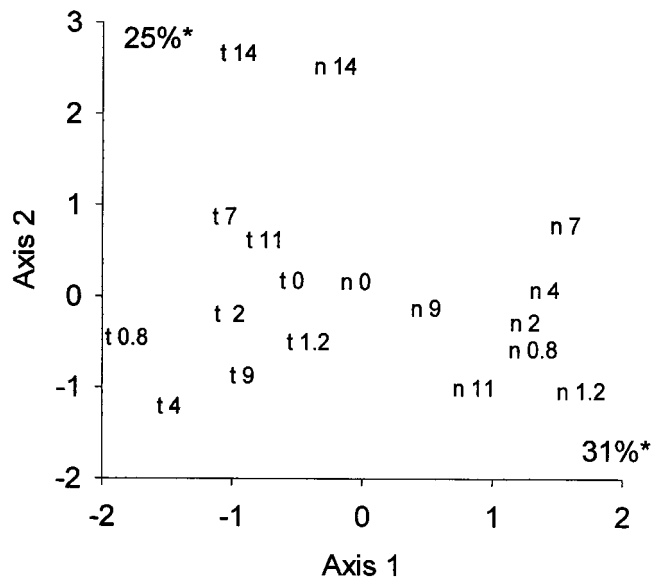


Fig. 4. CCA ordination biplot of the PLFA profiles in the study of short-term tillage responses on the Pico soil. Tilled soil is designated  $t$  and the control soil is designated  $n$ . The number of days after tillage follows the  $t$  or  $n$  designation. Axis 1 has an eigenvalue of 31%, while Axis 2 has an eigenvalue of 25%.

**Table 2.** Biplot scores of the PLFA used for the CCA analysis. The highest and lowest three scores for each axis in Fig. 4 are shown.

Molecule	Score	Specificity as a Marker†
<b>Axis 1</b>		
16:1 $\omega$ 11	-0.337	Non-specific
19:0 cy	-0.0983	Eubacterial anaerobes, gram-negative bacteria
18:3 $\omega$ 6c	-0.0537	Fungi
il3:0	0.0631	Gram-positive bacteria
15:0 3OH	0.863	Non-specific
18:1 $\omega$ 7t	0.2648	Eubacteria, cyanobacteria, actinomycetes
<b>Axis 2</b>		
19 cy‡	-0.0952	Eubacterial anaerobes, gram-negative bacteria
18:3 $\omega$ 6c	-0.0935	Fungi
19:0 cy	-0.0661	Eubacterial anaerobes, gram-negative bacteria
a 19:0	0.094	Gram-positive bacteria
16:1 $\omega$ 11	0.1459	Non-specific
il3:0	0.1587	Gram-positive bacteria

† From Vestal and White (1989).

‡ This is an unresolved mixture of fatty acids containing a 19 carbon fatty acid with a cyclopropyl group.

tillage) and the earlier sampling times, indicating an overall similarity in PLFA profiles by the end of the 2-wk experiment.

Table 2 shows the highest biplot scores for the axes of the CCA biplot in Fig. 4. Three PLFAs (16:1  $\omega$ 11, 19:0 cy, and 18:3  $\omega$ 6c) have the most negative biplot scores along axis 1. These molecules are found in higher concentrations in the tilled soil. Conversely, PLFA 18:1  $\omega$ 7t has the highest positive biplot score for Axis 1, indicating that this molecule declined in the tilled soil. Table 2 indicates that PLFAs such as 19:0 cy and 18:3  $\omega$ 6c exhibited negative biplot scores along Axis 2, which showed some separation between the last sampling time and the rest of the samples, and these molecules declined in both soils at the end of the experiment.

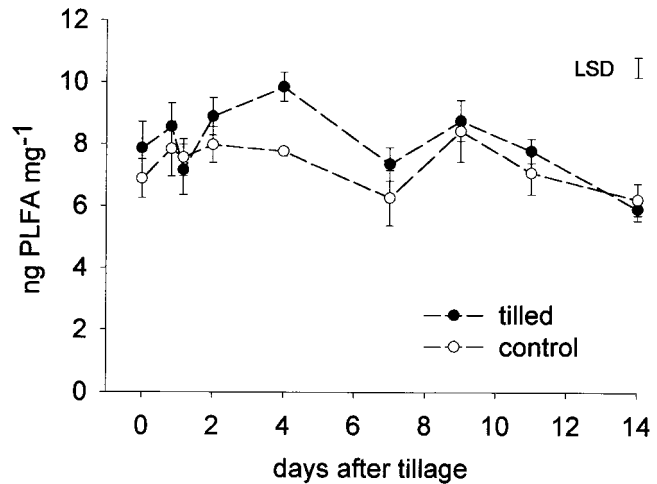
Tillage had a significant effect on the total PLFA (Table 1; Fig. 5), which is an indicator of the total microbial biomass. The difference between the tilled and control soil was highest 4 d after tillage, when the tilled soil had 27% higher total PLFA than the control soil (Fig. 5).

## DISCUSSION

Our hypothesis that tillage would stimulate C and N mineralization and immobilization was partially supported by our results. Increases in MBN, nitrate production, and denitrification support the idea that tillage of this Pico silt loam exposed N-rich substrates that changed the N cycling dynamics in the soil. However, tillage was followed by a decline in respiration and did not affect MBC, suggesting that newly exposed organic matter was not sufficient to produce an overall increase in microbial metabolism or biomass C. The responses in nutrient dynamics were accompanied by changes in the microbial community structure, suggesting a relationship between community structure and function.

### Nitrogen Dynamics

Disruption of the soil structure increased the amount of nitrate in this silt loam soil within a few days of disturbance. This agrees with other studies that show increases in soil inorganic N within days after diking



**Fig. 5.** Mean total PLFA of the rototilled and control Pico silt loam soils. The error bars are the standard error of the mean.  $n = 3$ . The Least Significant Difference (LSD) based on a Student's *t*-test between tilled and control soil is shown.

or plowing in the field (Reicosky et al., 1997). In our study, however, potential nitrification was not affected by tillage, indicating that nitrate accumulation was not due to an increased population of nitrifiers in the tilled soil. Net nitrate production was concomitant with a period of net N immobilization, as indicated by increased MBN. A high demand for ammonium by nitrifiers and immobilizers may have caused the high turnover rate of a small ammonium pool. Tillage may have exposed microbes to labile substrates of low C/N ratio, stimulating net N mineralization and nitrification without increasing the soil respiration rate (see below).

Although net N immobilization by microbes increased, no significant C immobilization occurred after tillage. The increase in nitrate and MBN after tillage suggests that previously protected organic matter acted as a significant source of available N but not C after soil disturbance. Rovira and Greacen (1957) hypothesized that organic matter that accumulates in minuscule pores will be out of reach of microbes unless a physical disturbance brings the organic matter and microbes together. In fact, soil microaggregates may protect labile organic matter of low C/N that becomes available for mineralization when the soil aggregates are disturbed (Beare et al., 1994). In sandy soils with little clay protection of soil organic matter, tillage may not create much of an increase in availability of labile substrates. In fact, in a related study, both MBN and MBC remained fairly constant after disturbance of an arable sandy loam soil, and less nitrate accumulated (Calderón et al., 2000).

Denitrification was stimulated by tillage, and the increase was concomitant with the increase in nitrate accumulation. These parallel patterns suggest that denitrification was nitrate-limited before tillage occurred. After tillage, however, nitrate concentration increased significantly, suggesting that early increases in nitrate supply met the demands by denitrifiers. The results of the respiration and MBC analyses do not support the idea that tillage exposed new C sources, so the increases in denitrification after tillage were not likely due to increased

C availability to denitrifiers. Denitrification was higher in the tilled soil despite its lower bulk density and possibly increased aerobicity; however, physical disturbances may redistribute denitrifiers, nitrate, and C substrates in a way that promotes the formation of denitrifying microsites (Parkin and Tiedje, 1984), even though the bulk of the tilled soil may have higher aerobicity. Christensen et al. (1990) observed measurable denitrification in aerobic soils with low microbial activity. They hypothesized that soils may have "hot spots" of oxygen depletion and denitrification, even when respiration in the bulk soil is low. Since denitrification did not occur immediately after tillage, but instead increased gradually during the first 4 d after tillage, it is unlikely that higher denitrification in the tilled soil was due to lower bulk density allowing greater gaseous efflux. In addition, soil N gas samples obtained from the 15-cm depth were higher in tilled than in control cores, and followed a similar temporal pattern as gaseous efflux (data not shown).

### Carbon Dynamics

Rototilling this silt loam soil resulted in decreased respiration as determined by soil incubation. These results support previous observations of reduced respiration shortly after soil disturbance (Petersen and Klug, 1994; Calderón et al., 2000). In contrast, Rovira and Greacen (1957) showed the opposite effect: disturbing the soil structure enhanced oxygen consumption. We hypothesize that factors such as the intensity of soil disruption, the amount of initial microbial biomass, and the quantity of previously protected C sources in soil pores may determine the impact of tillage on microbial activity. In addition, soils with different microbial compositions may respond differently to tillage. Frequent tillage may favor selection of microbial populations that are able to withstand physical disruption (Calderón et al., 2000).

Tillage reduced respiration without affecting MBC, and thus reduced the  $q\text{CO}_2$ . Either C availability to microbes decreased during the post-tillage period and/or a change in microbial community structure resulted in a lower inherent  $q\text{CO}_2$ . Organisms may vary in their ability to conserve C during succession after a disturbance (Odum, 1969).

It seems contradictory that  $\text{CO}_2$  efflux was higher from the surface of tilled soil, given that respiration rates were equal or lower in the tilled compared to the control soil. A plausible explanation is that immediately after tillage, the  $\text{CO}_2$ -rich soil atmosphere is flushed with air of relatively low  $\text{CO}_2$  content. This causes a reduction in the  $\text{CO}_2$  partial pressure in the soil air, which is followed by the degassing of dissolved  $\text{CO}_2$  from the soil solution (Reicosky et al., 1995, 1997; Rochette and Angers, 1999; Ellert and Jantzen, 1999). The lower bulk density of the tilled soil also facilitates  $\text{CO}_2$  diffusion out of the soil atmosphere, resulting in a short-term burst of  $\text{CO}_2$  efflux from the soil surface.

This study monitored the effect of tillage on bare soil that had been fallow for several months, unlike several

previous studies on short-term effects of tillage on  $\text{CO}_2$  efflux which have dealt with instances where crop residues were incorporated at the time of tillage (Reicosky et al., 1997). The incorporation of plant material during tillage increases  $\text{CO}_2$  efflux as a result of the mineralization of freshly incorporated plant material (Prior et al., 1997). We have shown in this study that tillage may affect  $\text{CO}_2$  efflux even when fresh plant residue is not incorporated.

### PLFA and Microbial Community Structure

The total PLFA concentration is indicative of the total microbial biomass in soil samples (White et al., 1996). Unlike MBC, total PLFA increased temporarily after tillage, suggesting that although tillage did not stimulate significant accumulation of carbon in the biomass, the active microbial biomass increased momentarily. Microbes may thus be able to respond very rapidly to tillage, as they do to rewetting of dry soil (Lundquist et al., 1999). The lack of correspondence of the MBC and total PLFA data underscores the fact that they describe different aspects of the microbial biomass. MBC is determined from the flush of C that is rendered water-soluble by fumigation with chloroform. Substances such as riboses, nucleic acids, proteins, cell wall components, and sugars are extracted in increased amounts from the fumigated soil (Badalucco et al., 1992). PLFA measures the amount of phospholipids in intact microbial membranes. Factors such as cell size may affect the amount of phospholipid per unit of microbial biomass (White et al., 1996). Therefore, we suggest that a close correlation between MBC and total PLFA should not always be expected.

Changes in N pools, MBN, and processes such as mineralization and denitrification, were concurrent with changes in PLFA profiles, suggesting that changes in biogeochemical processes may be related to changes in the functional diversity of the soil bacterial community. For example, 19:0 cy is thought to be a marker for anaerobic eubacteria (Vestal and White, 1989). This compound increased in the tilled soil, with a pattern consistent with the hypothesis that tillage created anaerobic microsites. Denitrification is a process that is carried out under anaerobic conditions by facultative anaerobes, so an increase in the anaerobicity of the soil might in turn have favored increased denitrification. Physical disruption of the soil can be specifically detrimental to fungi, since their filamentous nature makes them particularly susceptible to the break up of soil aggregates (Petersen and Klug, 1994). However, fungal PLFA markers such as 18:2  $\omega$ 6 and 18:3  $\omega$ 6c did not decrease after tillage, suggesting that fungi were not negatively affected relative to pre-tillage conditions. Other PLFAs that distinguish the tilled and control soils were 16:1  $\omega$ 11 and 18:1  $\omega$ 7t. The 18:1  $\omega$ 7t, which marks the presence of eubacteria (Vestal and White, 1989), decreased in the tilled soil relative to the control soil, suggesting that some groups of eubacteria may have been particularly affected by tillage.

The PLFA profiles of both soils at the last sampling

time are more similar to each other than to those observed at the other sampling times. Two weeks after tillage, the microbial community of the tilled soil may have returned to a composition similar to that of the intact soil. For example, phospholipid fatty acid 19:0 cy declined toward the end of the experiment in both treatments. At 14 d after tillage, tilled soil had 41% less 19:0 cy and the control soil had 24% less 19:0 cy relative to the first sampling time. This suggests that eubacterial anaerobes may have been responding similarly in tilled and control soils at the end of the experiment.

The observed increases in net nitrate production and denitrification after tillage have agronomic and environmental implications, since they result in potential and direct losses of N from agricultural systems. Short-term losses of C as CO<sub>2</sub> efflux demonstrate that tillage contributes to atmospheric CO<sub>2</sub> and accelerates the loss of C from soil. Our study shows that tillage also affects soil microbial biomass and community structure as well. Factors such as the time of year, the presence of plant residues, the quality of the residues, and the type of tillage implement affect the magnitude of the short-term CO<sub>2</sub> efflux after tillage (Reicosky et al., 1995; Reicosky et al., 1997; Rochette and Angers, 1999; Ellert and Jantzen, 1999), and would be expected to affect microbial biomass and community structure.

#### ACKNOWLEDGMENTS

This project was funded by the Kearney Foundation of Soil Science Grants 96-10-D, and 97-D-24, USDA-NRI Soils and Soil Biology Grant 96-35107-3128, and the USEPA Center for Ecological Health Research (R819658). We thank Tanimura and Antle Inc. for allowing us access to the experiment site. Thanks to Dianne Louie and Amy Clymo for their help during the field sampling and the gas flux analyses.

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## Effects of Oxygen on Denitrification Inhibition, Repression, and Derepression in Soil Columns

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### ABSTRACT

Although it is known that O<sub>2</sub> inhibits and represses denitrification, few studies have examined the effect of O<sub>2</sub> on NO production. Our objectives were to measure O<sub>2</sub> inhibition, repression, and derepression of NO and N<sub>2</sub>O production by denitrifying microorganisms in soil columns continuously purged by N<sub>2</sub> or various constant (0.074–15%) O<sub>2</sub>-N<sub>2</sub> mixtures. Net rates of NO and N<sub>2</sub>O production were measured under successive anaerobic, partially aerobic, and anaerobic conditions. Oxygen inhibition was rapid and reversible. Within 5 min after exposure to >5% O<sub>2</sub>, NO production was reduced to ≈50 to 58% and N<sub>2</sub>O rates to ≈29 to 32% of their maximum anaerobic rates. Maintaining O<sub>2</sub> at ≥5% in soil without added C or at >10% O<sub>2</sub> in C-amended soil decreased (repressed) NO production rates by a factor of ≈1.5 to 1.8 d<sup>-1</sup>. Rates of N<sub>2</sub>O repression remained constant at ≈0.07 d<sup>-1</sup> for all C and O<sub>2</sub> treatments. Restoration of anoxic conditions following the aerobic phase reversed inhibition; within 5 min, NO production rates by the nonrepressed denitrifiers increased to 55 to 101% of their respective anaerobic rates and N<sub>2</sub>O production rates increased to 26 to 62%. The rates of NO and N<sub>2</sub>O production then increased more slowly (derepression) during this anaerobic period. This research supports previous observations for O<sub>2</sub> effects on N<sub>2</sub>O production and apparently is the first systematic study of O<sub>2</sub> inhibition, repression, and derepression of NO production.

OXYGEN is usually considered to be the most critical proximal regulator of microbial denitrification (Hutchinson, 1995; Tiedje, 1988). The reductive enzymatic steps in the process are most active under anaerobic conditions, but many organisms are known to respire O<sub>2</sub> and denitrify simultaneously (Ottow and Benckiser, 1994; Robertson and Kuenen, 1991). Similarly, many species from different genera can nitrify and denitrify simultaneously (Robertson and Kuenen, 1991). Competition for O<sub>2</sub> limits aerobic processes in soil and depletion of O<sub>2</sub> results in the formation of local anaerobic microsites (Parkin, 1987) that stimulate denitrifier production of gaseous NO, N<sub>2</sub>O, and N<sub>2</sub>.

Production and consumption of NO and N<sub>2</sub>O by different denitrifying bacterial species may exhibit different enzyme kinetic properties (Conrad, 1995). Although the regulation of enzyme synthesis depends mainly on O<sub>2</sub> and N substrate concentrations, the patterns of regulation and sensitivity to O<sub>2</sub> may differ (Conrad, 1996

and references therein). This may have a major bearing on the NO/N<sub>2</sub>O/N<sub>2</sub> ratio produced with variation in O<sub>2</sub> availability.

To obtain a complete description of the function and concentration distribution of O<sub>2</sub> within the soil would require an accurate model of soil structure, porosity, O<sub>2</sub> diffusion, and rates of major microbial processes. Several attempts to generate useable models of soil anaerobiosis in aggregated soil have been made (Sierra and Renault, 1996). The results emphasize the complexity of the problem and the need to obtain direct quantitative data regarding the effect of O<sub>2</sub> on specific steps in processes such as denitrification in soil.

Adding to the complexity, O<sub>2</sub> not only inhibits denitrifying enzyme activity, but also represses denitrifying enzyme synthesis (Smith and Tiedje, 1979; Knowles, 1981; Tiedje, 1988; McKenney et al., 1994). Few studies have examined the kinetics of these effects, particularly in relation to specific intermediate reactions in the process. Studies using pure cultures of nitrifying or denitrifying organisms (Kester et al., 1997 and references cited therein) have greatly increased our understanding of O<sub>2</sub> effects at the cellular level. How inhibition, repression, and derepression are expressed in soils rather than in pure microbial cultures is of special importance, but it remains difficult to quantitatively relate field NO and N<sub>2</sub>O emissions to the chemostat investigations (Conrad, 1996).

Studies by Dendooven and Anderson (1994) and Smith and Tiedje (1979) provided considerable insight regarding derepression or de novo synthesis of denitrifying enzymes following O<sub>2</sub> depletion in soil. Kramer and Conrad (1991) studied the overall influence of O<sub>2</sub> on production and consumption of NO in an agricultural soil containing both nitrifying and denitrifying microbes and in a forest soil that contained only denitrifiers. However, quantitative evaluation of inhibition and repression of net NO and N<sub>2</sub>O production and derepression of net NO production by denitrification in soils apparently have not yet been examined. Such processes are undoubtedly related to the very large spatial and temporal variability observed in field-scale studies (Parkin, 1993; Groffman, 1991).

Many factors influence O<sub>2</sub> availability to soil organisms, and the effect can be rapid and significant. For example, O<sub>2</sub>-consuming microbial respiration, which is strongly dependent on C availability, is a very important controller of denitrification in partially aerobic soil (Tiedje, 1988). The emissions of NO and N<sub>2</sub>O from

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