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Microbial responses to simulated tillage in cultivated and uncultivated soils

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Abstract

Tillage is known to have long-term effects on organic matter and labile pools of nutrients in soil, but the short-term changes in microbial dynamics and activity after tillage are less well understood. We investigated the immediate effects of simulated tillage on microbial community structure as determined by phospholipid fatty acid (PLFA) profiles, microbial activity, and carbon (C) and nitrogen (N) pools. Intact cores were obtained from Chualar sandy loam soils under grassland and vegetable production. The top 15 cm of soil was sieved to simulate tillage, then the cores were incubated in the greenhouse. Sampling took place 1 day before the tillage simulation and throughout the next 2 weeks. In the grassland soil, multivariate analysis showed changes in PLFA profiles within hours, indicating rapid changes in microbial community structure. Specific PLFA markers indicated a reduction in microeukaryotic biomass as well as an increase in a microbial stress marker after sieving. Respiration (as determined by soil incubation in sealed containers) decreased immediately after sieving and continued to decline through the next 14 days. Sieving was followed by a continuous accumulation of nitrate. In the vegetable soil, the changes in PLFA profiles were slow and gradual. The PLFA stress indicator rose only slightly. Microbial activity and biomass were low, and only small changes occurred in most variables. A decline in respiration and an increase in nitrate occurred several days after sieving. In both soils, decreased soil moisture may have contributed to changes in soil responses after sieving. Short-term responses to tillage may be less pronounced in soils with a long history of cultivation because of a relatively resilient microbial community and/or because lower initial microbial biomass and nutrient pools preclude a strong response to disturbance.

Keywords: Denitrification; Nitrate; Nitrogen mineralization; Phospholipid fatty acids (PLFA)

1. Introduction

Tillage aerates the soil, breaks up plant and microbial cells, mixes biomass-rich top layers with deeper layers, affects the soil's temperature regime, and hastens soil drying (Doran, 1982; Larney and Bullock, 1994; Khan, 1996). Tillage can promote the release and subsequent degradation of previously protected organic matter (Rovira and Greacen, 1957; Adu and Oades, 1978), contributing to long-term decreases in soil microbial biomass and organic matter (Schimel et al., 1985; Elliott, 1986; de Luca and Keeney, 1994). Long histories of tillage diminish the capacity of the soil to retain nitrogen (N), promote the production of nitrate through nitrification, and decrease the capacity to immobilize N due to decreased C availability (Smith and Young, 1975; Doran, 1982; Follett and Schimel, 1989; Woods, 1989). These conditions favor the loss of N from agricultural systems, which can lead to atmospheric and groundwater contamination and poor soil quality.

The long-term effects of tillage have been well-characterized, but less information is available regard-

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ing the short-term effects of physical disturbances on soil nutrient cycling and microbial ecology. Significant alteration of the soil can occur rapidly after tillage. For example, CO_2 efflux from the soil surface may respond to physical disruption within a matter of hours and days (Roberts and Chan, 1990; Reicosky and Lindstrom, 1993; Reicosky et al., 1995, 1997; Rochette and Angers, 1999; Ellert and Janzen, 1999). Disturbance can also cause rapid changes in soil microbial community structure, as indicated by changes in soil phospholipid fatty acid (PLFA) profiles (Petersen and Klug, 1994); PLFA are cell membrane components that are rapidly degraded after cell death (White et al., 1996). Multivariate analysis of PLFA profiles can be used to study compositional changes of soil microbial communities caused by alterations of environmental conditions (Guckert et al., 1985; Bossio and Scow, 1995; Lundquist et al., 1999). Total PLFA content serves as an index of the viable microbial biomass.

Complex changes in environmental factors occur after soil disturbance. We hypothesized that tillage may cause temporary stress that limits the ability of soil microbes to assimilate nutrients and alters community structure. Alternatively, tillage may expose substrates and lead to a burst in microbial activity and growth. Our objective was to investigate short-term effects (hours and days) of simulated tillage on microbial community structure, microbial activity, and nutrient dynamics in soils from a cultivated and uncultivated soil that were expected to differ in soil organic C, microbial biomass, and N pools (Smith and Young, 1975; Woods, 1989; Ihori et al., 1995), and thus, in their response to disturbance.

Our approach was to follow a detailed time course of changes in PLFA, respiration, microbial biomass, and C and N pools after sieving intact soil cores to simulate the physical disruption caused by intense tillage. An unsieved control was not included because it was unfeasible to maintain similar soil moisture in sieved and unsieved soils in the greenhouse as tilled soils tend to dry more quickly (Khan, 1996). We made a comparison between an uncultivated grassland soil and a frequently-tilled vegetable production soil of the same sandy loam soil type from the Central Coast region of California. This study provides a demonstration of soil responses to the complex changes in the soil environment that occur after tillage, since moist soil was sieved at a soil matric potential that is typical of actual tillage events. It does not separate effects due to changes in specific environmental factors such as aeration and moisture.

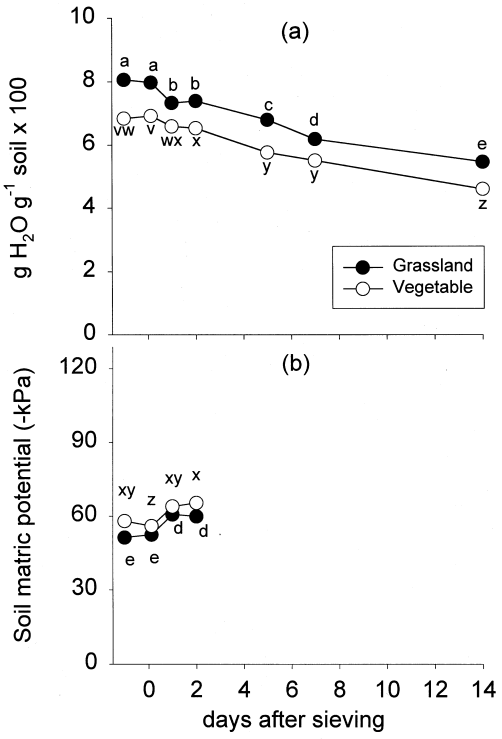

Fig. 1. Time course of gravimetric soil moisture and soil matric potential before and after sieving in the grassland and vegetable soils. Zero on the *x*-axis marks the time of sieving. Mean separations are shown separately for the two sites (*a*, *b*, *c*, and *d* for the grassland soil and *w*, *x*, *y*, and *z* for the vegetable soil). $n = 4$ except for time 14, when $n = 3$ for the grassland soil, and time 5 when $n = 3$ for the vegetable soil.

2. Materials and methods

2.1. Sites

Intact soil cores were collected in February 1997, from two sites of granite-derived Chualar sandy loam soils (fine-loamy, mixed, thermic Typic Argixeroll) in the Salinas Valley, California. The sites were ~10 km apart. Sites were: (1) a soil obtained from an uncultivated, non-irrigated grassland dominated by naturalized annual grasses (e.g., *Bromus hordeaceus* and *Avena barbata*) and (2) a soil under year-round cultivation, irrigation (~0.3–1 m $year^{-1}$), fertilization (~100–300 kg N ha^{-1} $year^{-1}$), and pesticide application for lettuce and cole crop (one to three crops per year) production for >50 years. The grassland soil was tilled for a brief period 30 years ago for hay production, while the vegetable soil has been tilled several times per year for >50 years. Productivity in annual grassland at nearby sites ranged from 200 to 550 g dry weight m^{-2} $year^{-1}$ (Stromberg and Griffin, 1996). In vegetable fields, production per crop is 300–1000 g dry weight m^{-2}, but between 60 and 80% is removed in crop harvest (Jackson et al., 1994; Wyland et al., 1996).

Soil characteristics of the <2 mm fraction of the 0–

15 cm depth were determined by the University of California, Division of Agriculture and Natural Resources Laboratory. The soil pH was determined from a saturated paste. Organic matter, total N, and total C were measured by combustion (Pella, 1990). Cation exchange capacity (CEC) was determined using the procedure of Janitzky (1986). Particle size analysis was done using with the hydrometer method (Gee and Bauder, 1986). The dry bulk density was measured from soil cores sampled before the tillage simulation. The two soils had nearly identical particle size distribution (Table 1), but organic C, organic N, CEC, and bulk density were higher in the grassland soil. The pH was lower in the grassland soil.

2.2. Core removal and sampling sequence

To extract the cores, PVC pipes (30.5 cm deep and 12.7 cm diameter) were driven into the soil and subsequently dug out with care not to compress or disturb the soils. Following the coring, all vegetation was cut at ground level and the litter layer was removed. The cores were stored at 4°C for 3 weeks, then 1 week before the experiment, the cores were taken to the greenhouse. The purpose of these pre-treatments was to acclimate the cores under constant conditions with minimal moisture loss.

The soil moisture at the time of the tillage simulation (Fig. 1) was typical for tillage events conducted by farmers in the region (Wyland et al., 1996). The moisture lost from the cores was not replaced during the experiment because rewetting soil has profound effects on microbial activity (Soulides and Alison, 1961; Sparling and Ross, 1988; Lundquist et al., 1999). In the greenhouse, moist cheesecloth was placed above the soil in the cores to diminish water loss while allowing for adequate ventilation. The maximum air temperature during the experiment was 26.9°C, and the minimum temperature was 14.7°C. Mean daily air temperature was approximately 20°C.

Four cores from each site management treatment were randomly assigned to each sampling time (1 day before sieving, and then at 3 h, and 1, 2, 5, 7, and 14 days after sieving). Each site management treatment thus had a total of 28 cores. Cores were placed on a greenhouse bench in a randomized complete block design. The initial sampling of four intact cores was done by gently mixing the soil, as described in more detail below. On the next day, the top 15 cm of soil of all the remaining cores was sieved through a 5 mm screen in the greenhouse. The sieved soil from all the cores within each site was pooled and mixed to minimize differences in non-uniformity between experimental units. All coarse mineral and organic fragments >5 mm, including roots, were removed from the soils during sieving to avoid increased availability of plant residue during the experiment, and focus the study on soil responses to tillage. The cores were immediately re-packed to 95% of the original bulk density. The sieving, mixing, and re-packing of the whole set of cores was carried out in <2 h. At each sampling, the top 15 cm of the soil was removed from the re-packed cores, mixed gently for approximately 1 min, and sampled immediately. We obtained samples for PLFA, microbial biomass C (MBC) and N (MBN), respiration, dissolved organic C (DOC) and dissolved organic N (DON), ammonium, nitrate, and gravimetric moisture. A separate set of four cores was sampled for denitrification using the acetylene block technique.

2.3. Soil assays

We obtained duplicate samples for PLFA analysis from each cylinder and placed them 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). Fatty acid terminology utilizes "A:B ω C" where "A" indicates the total number of carbon atoms, "B" the number of unsaturations, and "ω" precedes "C", the number of carbon atoms between the closest unsaturated and the aliphatic end of the molecule. The suffixes "c" and "t" indicate cis and trans geometric isomers. The prefixes "i" and "a" refer to

Table 1
Soil characteristics of the 0–15 cm depth (<2 mm fraction) at the two field sites where intact soil cores were collected

Site	Annual grassland	Vegetable production
pH	6.6	7.7
Cation exchange capacity (meq 100 g dry soil^{-1})	12.5	11.5
Bulk density (Mg dry soil cm^{-3})	1.54	1.44
Organic carbon (g kg^{-1})	12.0	6.7
Organic nitrogen (g kg^{-1})	1.2	0.8
Sand (%)	58	59
Silt (%)	29	28
Clay (%)	13	13

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. The 10 Me refers to a methyl group on the tenth carbon from the carboxylic end of the fatty acid.

Triplicate 25 g samples from each core were taken for MBN and MBC analysis using the fumigation–extraction method (Brookes et al., 1985; Vance et al., 1987). One soil subsample was shaken for 30 min with 60 ml of 0.5 M K_2SO_4, and another subsample was fumigated for 24 h with ethanol-free chloroform ($CHCl_3$). We used a 2.4:1 ratio of K_2SO_4:soil as opposed to the typical 4:1 ratio (Vance et al., 1987) to increase the concentration of organic C and N in the extracts, which from previous tests were known to be near the detection limits for non-fumigated samples of the vegetable soil. The $CHCl_3$ was removed, and the soils were extracted as above. The amount of organic C in the extracts was determined by oxidation with dichromate in concentrated sulfuric acid and phosphoric acid, followed by titration of remaining dichromate with ferrous ammonium sulfate (Vance et al., 1987; Yeomans and Bremner, 1988). The Kjeldahl digestion of the extracts was carried out according to Wyland et al. (1994). The total ammonium in the digests was determined using a Lachat Quick Chem II Flow Injection Analyzer (Zellweger Analytical, Milwaukee, WI). The flush of C was converted to total MBC by multiplying by 2.64 (Vance et al., 1987), and the flush of N was converted to total MBN by multiplying by 1.86 (Brookes et al., 1985). We used the amount of organic N in the non-fumigated extracts as a measure of the DON, since the 0.5 M K_2SO_4 extract of non-fumigated soils should contain a variety of non-biomass N compounds (Badalucco et al., 1992).

Soil respiration was measured by placing 20 g of soil from each core into 30 ml serum bottles. The bottles were then sealed with crimp caps suitable for gas sampling and incubated at 25°C for 60 min. After the incubation, the headspace of the bottles was sampled with a 1 ml syringe. The CO_2 concentration in the samples was determined using an infrared gas analyzer (Horiba PIR-200, Horiba Instruments, Riverside, CA).

Two 10 g samples of moist soil from each core were analyzed for DOC with the extraction procedure for cold water soluble C (Davidson et al., 1987). Extracts were frozen until analysis with a Shimadzu Total Organic Carbon Analyzer (Shimadzu, Columbia, MD).

For inorganic N, triplicate samples (10 g of soil) were taken from each core. The samples were placed in centrifuge tubes with 25 ml of 2M KCl, shaken horizontally in an orbital shaker (200 rpm for 20 min), and then centrifuged (2000 rpm for 5 min). The extracts were kept at −20°C until analysis. The concentration of ammonium and nitrate in the soil extracts was determined colorimetrically using a Lachat Quick Chem II Flow Injection Analyzer (Zellweger Analytical, Milwaukee, WI).

Gravimetric moisture was determined after drying about 50 g of soil at 105°C for at least 48 h. In a separate set of cores, a soil drying curve was produced by comparing tensiometer measurements of soil matric potential with gravimetric moisture taken from 0–15 cm deep samples. The negative limit of the readings was −70 kPa.

2.4. Denitrification

In addition to the cores in the main experimental blocks, a separate set of four cores per site was sampled for denitrification. We decided against doing denitrification measurements on the cores sampled for all other variables because of the inhibitive effect of acetylene on nitrification. The flux of N_2O was measured in situ on capped cores (Folorunso and Rolston, 1984) with a single acetylene supply probe inserted in each cylinder. This was a plastic tube (5 mm internal diameter) with holes drilled throughout the underground length, set to a depth of 20 cm. A soil gas probe of plastic tubing (2 mm diameter) with a septum at the top and a perforated 6 cm long plastic tube (3 mm diameter) glued to the bottom end was inserted into each core to a depth of 15 cm. Acetylene was dispensed to the eight cores at a rate of 1.06 l min^{-1} 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 (9 ml) were taken from the headspace as well as from the soil gas at 30 and 60 min and injected into blood collection tubes (16 × 10 mm Vacutainer, Becton Dickson, NJ) and stored less than 24 h until analysis. The N_2O concentration in the gas samples was determined with a Carle Series 400 gas chromatograph (Chandler Engineering Company, Tulsa, OK) fitted with an electron capture detector. Sampling times for denitrification were concurrent with the destructive samplings for the rest of the variables.

2.5. Statistical analysis

Data from the two sites were analyzed separately since the main goal was to evaluate the time course of soil variables following sieving for each soil type. Within each soil type, the differences between sampling times were tested by analysis of variance (ANOVA) using the General Linear Model function of SAS version 6.11 (SAS Institute, 1991). Mean separations were conducted using the Duncan test to determine differences between individual means.

Canonical correspondence analysis (CCA) of

CANOCO version 3.11.5 (CANOCO, Microcomputer Power, Ithaca, NY) was used to compare the PLFA profiles both within and between sites. Patterns in multivariate data (concentrations of individual PLFA) and environmental variables (e.g., time since sieving, or grassland versus vegetable soil) are illustrated by CCA (ter Braak, 1987). PLFA with a high score of high absolute value on a CCA axis are highly related to the axis and to the environmental variable with high correlation to the axis. In all of the CCA in this study, axis 1 was created by the program to maximize correlation with an environmental variable. For the CCA of the individual sites, axis 2 was created by CCA to be highly correlated to a theoretical variable that best fits the PLFA data. The creation of this theoretical axis intentionally has no environmental basis. In addition to the grassland and vegetable CCA, a single CCA with both grassland and vegetable data was carried out. In this case, both axes were specifically created to maximize correlation with environmental variables, i.e., axis 1 with management (grassland versus vegetable soil) and axis 2 with time since sieving. A Monte Carlo permutation test of CANOCO was used to test for significant effects of time since sieving and/or site on the PLFA profiles of the soils. A total of 57 PLFA were detected in the grassland soil, and 51 PLFA were detected in the vegetable soil. Only consistently quantified molecules were used for the CCA analyses, i.e., molecules that occurred in every soil sample from a given site. This amounted to 33 different PLFA in the grassland soil, and 26 different PLFA in the vegetable soil. For the combined grassland and vegetable CCA, a total of 26 PLFA common to both data sets was used. Eigenvalues for PLFA distribution on the CCA biplots are available from the first author by request.

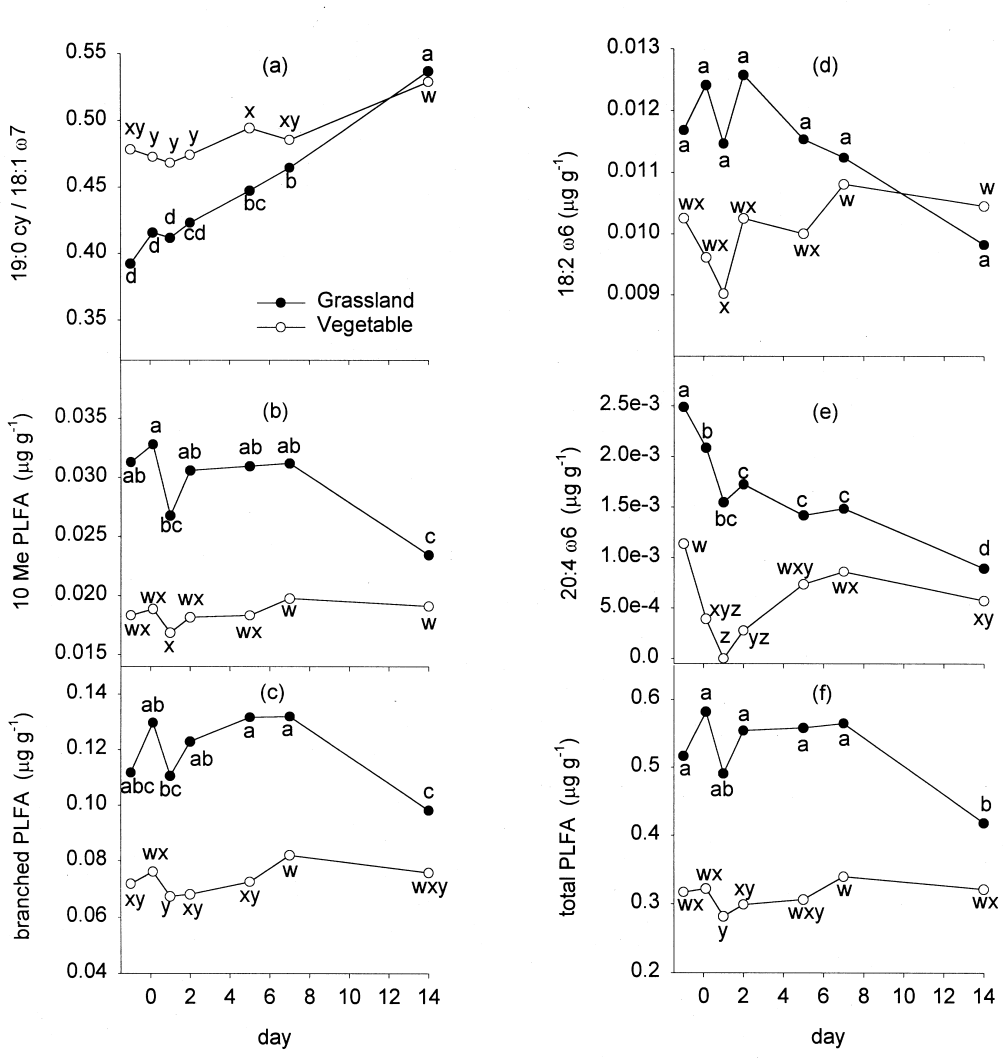

Fig. 2. Selected PLFA variables of the grassland and vegetable soils before and after sieving. Zero on the *x*-axis marks the time of sieving. Mean separations are shown separately for the two sites (*a*, *b*, *c*, and *d* for the grassland soil and *w*, *x*, *y*, and *z* for the vegetable soil). Note that the *y*-axis often does not begin at zero. $n = 4$ except for time 14, when $n = 3$ for the grassland soil, and time 5 when $n = 3$ for the vegetable soil.

3. Results

3.1. Moisture in grassland and vegetable soils

The average gravimetric moisture at the start of the experiment was 8.06% (−51 kPa) for the grassland soil, and 6.84% (−58 kPa) for the vegetable soil (Fig. 1). Both soils remained between −50 and −65 kPa for the first 2 days after sieving. Matric potential began to decrease slightly between 3 and 24 h after sieving in the grassland soil, but remained nearly unchanged for the first 2 days in the vegetable soil. Gravimetric moisture continued to decline for the rest of the sampling period. Fourteen days after sieving, mean gravimetric moisture was 5.45% for the grassland soil, and 4.59% for the vegetable soil.

3.2. Time sequence after sieving of the grassland soil

The total concentration of PLFA in the grassland soil remained statistically indistinguishable before sieving and up to 7 days afterwards (Fig. 2f). Between 7 and 14 days after sieving, however, total PLFA decreased significantly, and was also lower than in the initial sample of the intact cores (0.42 versus 0.52 μg g^{-1} soil).

Canonical correspondence analysis showed that sieving was followed by changes in PLFA composition, indicating changes in the microbiota of grassland soil through time. Axis 1, created by CCA to maximize correlation with the environmental variable, time since sieving, in Fig. 3 illustrates a sequential progression of changes in the overall composition of the PLFA profiles after the tillage simulation. Greater chronological separation occurred during the first week after sieving than during the subsequent week. Additionally, Fig. 3 shows that samples from before sieving and 3 h afterwards are already different in their PLFA profiles. The Monte Carlo test showed a significant change ($P < 0.01$) in the PLFA profiles of the grassland soils across different sampling times. Axis 2, a theoretical axis, is less informational than axis 1, but does show some clustering according to time since sieving.

Certain PLFA show changes with time since sieving. The PLFA with the highest absolute loading scores on axis 1 (Fig. 3) best explain the differences in microbial communities between early and late sampling times in the experiment. The individual PLFA varied in their response to sieving, and had different spatial arrangements along axis 1 (data not shown). The concentration of branched fatty acids, which mark the abundance of a broad group of bacterial taxa (Vestal and White, 1989; Zelles, 1997), declined between days 7 and 14, showing a pattern similar to the total PLFA (Fig. 2c and 2f). Fatty acids with a 10 Me branching (Fig. 2b), which are particularly abundant in actinomycetes (O'Leary and Wilkinson, 1988), declined 1 day after the tillage simulation and then again between days 7 and 14. The fatty acid 18:2 ω6 is a proposed fungal molecular marker (Vestal and White, 1989). Although concentrations before and after sieving were statistically indistinguishable, this PLFA tended to

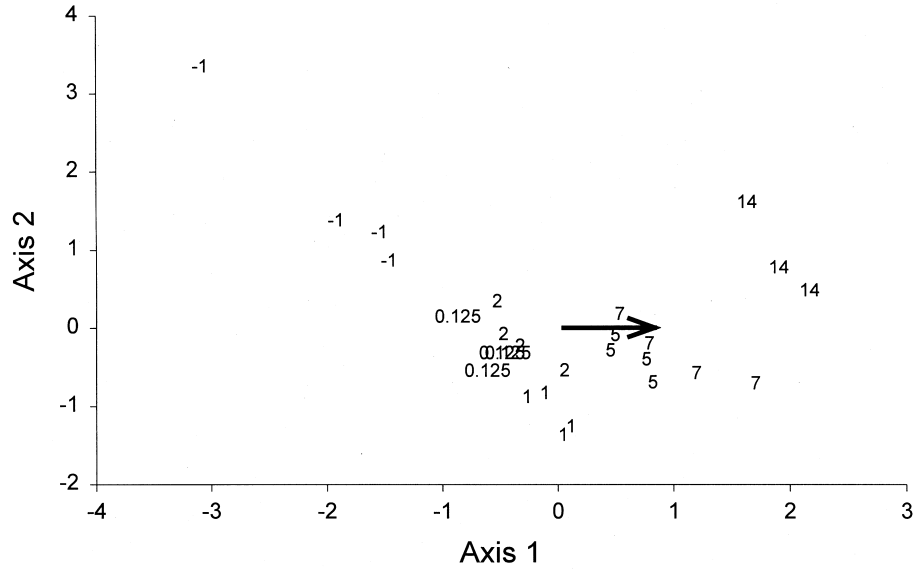

Fig. 3. CCA ordination biplot of the grassland soil. Shown are the number of days relative to the tillage simulation (−1, 0.125, 1, 2, 5, 7, 14). The time since sieving has a regression/canonical coefficient of 1.0 with axis 1, which represents 31% of the variation. Axis 2 represents 21% of the variation. The arrow shows the biplot score of the environmental variable (time relative to the tillage simulation). The eigenvalue distribution of the individual PLFA on the biplot is available from the first author. $n = 4$ except for time 14, when $n = 3$.

decrease in the second week of the experiment (Fig. 2d). The PLFA 20:4 ω6 had the highest biplot score on axis 1 of Fig. 3 (data not shown) and it declined after the disturbance, never recovering during the time frame of this experiment (Fig. 2e). This molecule, proposed to be an indicator of microeukaryotic biomass (Vestal and White, 1989; Zelles, 1997), 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).

In bacteria, the ratio of 19:0 cy to 18:1 ω7 has been proposed as an indicator of stress conditions, as it has been shown to increase under situations such as stationary growth, acidic conditions, low oxygen, and

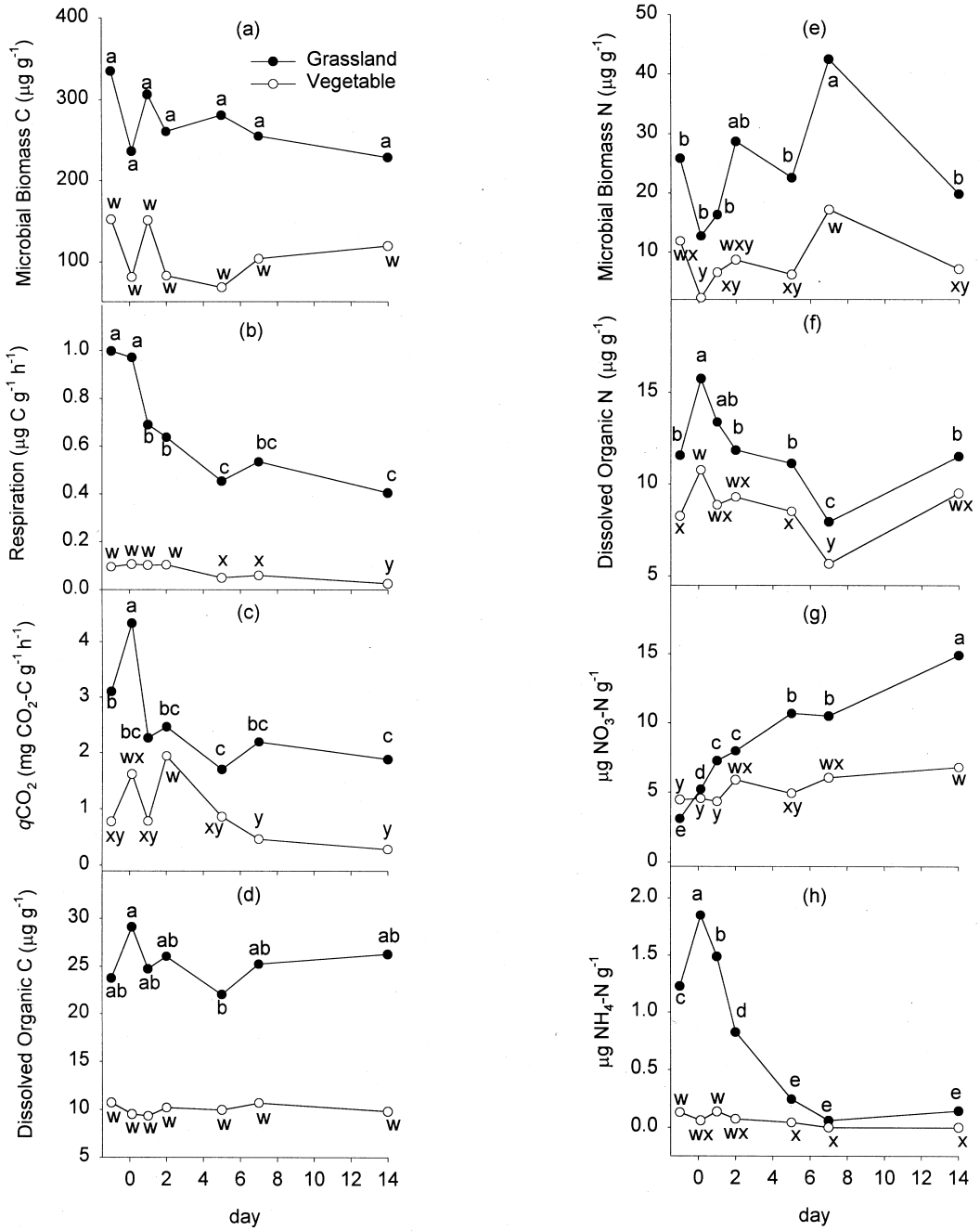

Fig. 4. Carbon and nitrogen pools and activity measurements of the grassland and vegetable soil before and after sieving. Zero on the *x*-axis marks the time of sieving. Note that *y*-axis often does not begin at zero. Mean separations are shown separately for the two sites (*a*, *b*, *c*, and *d* for the grassland soil and *w*, *x*, *y*, and *z* for the vegetable soil). $n = 4$ except for time 14, when $n = 3$ for the grassland soil, and time 5 when $n = 3$ for the vegetable soil.

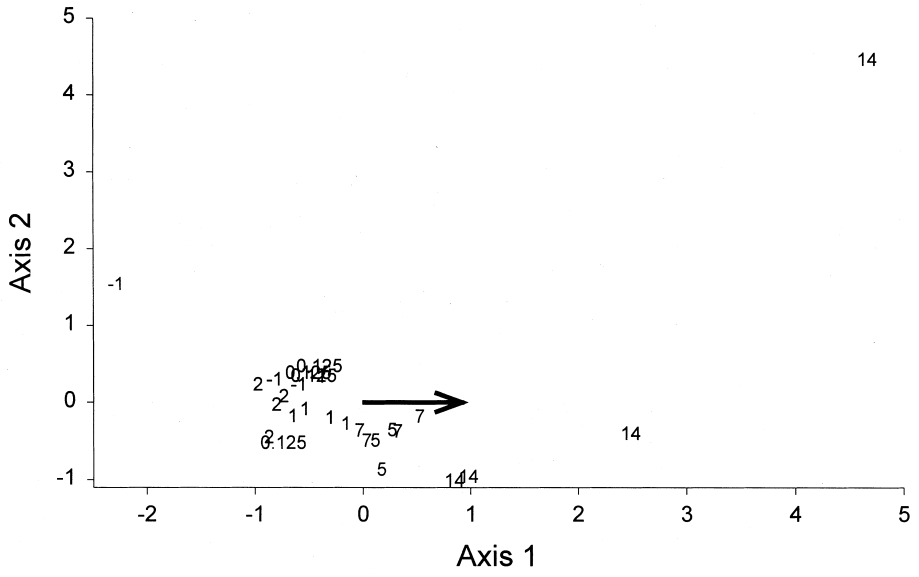

Fig. 5. CCA ordination biplot of the vegetable production soil. The arrow shows the biplot score of the environmental variable (time relative to the tillage simulation). The time since sieving has a regression/canonical coefficient of 1.0 with axis 1, which represents 14% of the variation. Axis 2 represents 29% of the variation. The eigenvalue distribution of the individual PLFA on the biplot is available from the first author. $n = 4$ except for time 5, when $n = 3$.

high temperature (Guckert et al., 1986). A significant increase in this ratio occurred between days 2 and 4, and again between days 7 and 14 (Fig. 2a), indicating that microbial stress increased through the experiment.

The MBC of the grassland soil remained statistically similar (mean value of 250 μg g^{-1} soil) throughout the entire post-sieving period (Fig. 4a). This generally corroborated the results from the total PLFA concentration, which also showed no significant change during the first week after sieving (Fig. 2f).

The initially high rate of respiration of the grassland soil (1.0 μg CO_2–C g^{-1} h^{-1}) significantly decreased between 3 and 24 h after sieving, and continued to decline gradually through the duration of the experiment (Fig. 4b). The metabolic quotient of the grassland soil (mg CO_2–C h^{-1} g^{-1} MBC) increased within 3 h after sieving but then declined less than 1 day later (Fig. 4c). There was no apparent relationship between respiratory activity and DOC. Dissolved organic C remained essentially unchanged throughout the entire post-sieving period, and the mean values for the different sampling times remained nearly statistically indistinguishable (Fig. 4d).

Large fluctuations in MBN occurred in the 5 days after sieving, but differences between times were not significant (Fig. 4e). After sieving, DON increased significantly then rapidly declined (Fig. 4f). Seven days after sieving, a brief increase in MBN occurred, followed by a decline a week later.

Simulated tillage was followed by immediate net N mineralization. Ammonium, which was always <2 μg N g^{-1} soil, increased rapidly immediately after sieving, then declined within 2 days and remained at levels less than the initial value present in intact cores (Fig. 4h). Nitrate, however, gradually increased five-fold during the experiment (Fig. 4g). Net nitrification was therefore stimulated during the entire 14-day period following disturbance. No measurable denitrification was observed in any of the grassland cores during the experiment (data not shown).

3.3. Time sequence after sieving of the vegetable soil

The total PLFA concentration in the vegetable soil underwent a brief decline between 3 and 24 h after sieving from 0.32 to 0.28 μg g^{-1} but afterwards returned to the initial level in intact cores (Fig. 2f). Initial total PLFA values were not statistically different from those at 14 days after sieving.

Sieving was followed by significant temporal changes in the microbial community structure of the vegetable soil. The Monte Carlo test indicated that the profiles of this soil at different sampling times were statistically different ($P < 0.01$). Axis 1 of Fig. 5, which represents the variability attributed to time relative to the tillage simulation, showed only slight separation between the early sampling times (−1, 0.125, 1, and 2 days after sieving) and later samplings. Axis 2 added very little information.

Branched PLFA, which are bacterial markers, decreased between 3 and 24 h after sieving, then gradually again by 7 days after sieving (Fig. 2c). The fungal molecular marker, 18:2 ω6, and the actinomycete marker, PLFA with 10 Me branching, remained

essentially statistically similar throughout the experiment (Fig. 2b and d). Fatty acid 20:4 ω6, an indicator of microeukaryotes, decreased very rapidly following sieving, then increased to pre-sieving levels within 4 more days (Fig. 2e), with a slight decline during the last week of the experiment. This indicates that sieving was an intense but temporary disturbance for microeukaryotes in the vegetable soil. The stress-indicator ratio of 19:0 cy to its precursor, 18:1 ω7, was relatively stable during the first week after sieving (Fig. 2a). It increased slightly in the vegetable soil near the end of the experiment.

In the vegetable soil, MBC and respiration remained low during the entire experiment (Fig. 4a and b). The MBC ranged from 80 to 150 μg C g^{-1} soil. Respiration remained unchanged for the first 2 days after sieving, then decreased between 2 and 5 days after sieving. DOC remained statistically unchanged (Fig. 4d). The metabolic quotient of the vegetable soil showed a significant increase 2 days after the tillage simulation (Fig. 4c).

The MBN in the vegetable soil declined significantly within hours after sieving (Fig. 4e). However, within 2 days, it recovered to pre-disturbance levels. Thereafter, MBN showed an increase at 1 week after sieving. The DON increased immediately after sieving, and fluctuated afterwards (Fig. 4f).

Nitrate increased between 1 and 2 days after sieving, then showed little change (Fig. 4g). The initial ammonium concentration was low (<0.2 μg N g^{-1} soil), and levels declined to below the detection limit after 5 days following sieving (Fig. 4h). Denitrification was not important in this soil (data not shown) as only two instances were recorded when N_2O concentrations were slightly above ambient levels.

3.4. Comparison of the PLFA from grassland and vegetable soils

We carried out a CCA to determine if PLFA composition was significantly related to site management regime and to time, using only molecules common to both soils (Fig. 6). A Monte Carlo test confirmed that the time since sieving had a significant effect on the soil PLFA profiles ($P < 0.01$).

There was a clear distinction on both axes between samples from grassland and vegetable production sites (Fig. 6). The cluster of samples from the vegetable production site was confined to a smaller linear distance on axis 1 than were the samples from the grassland site, indicating greater variability in the grassland soil. On axis 2, which is correlated with time since sieving, greater linear distance occupied by the grassland soil indicates greater variability through time than in the vegetable soil. The area occupied by the samples in Fig. 6 indicates that greater relative differences occurred in the grassland soil than in the vegetable soil, especially during the first week after sieving.

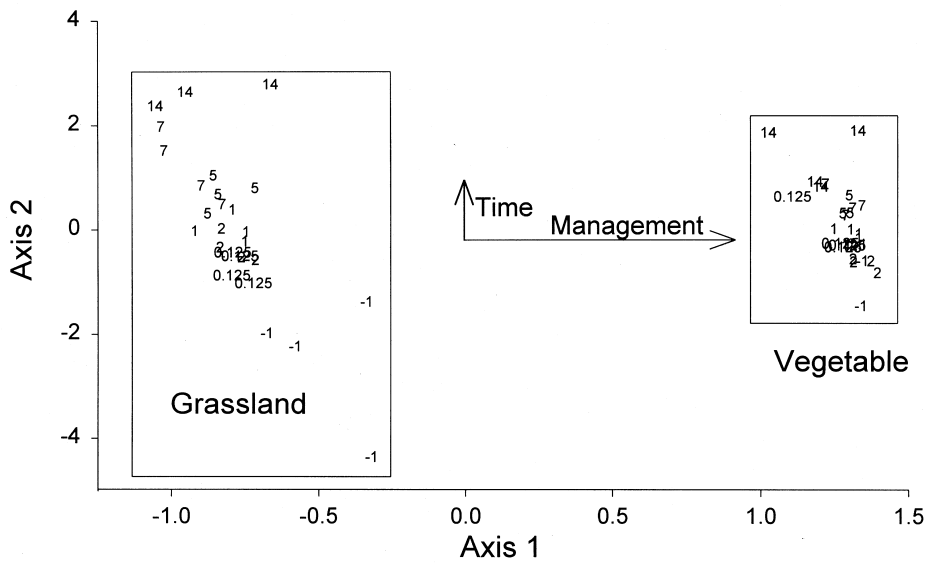

Fig. 6. CCA ordination biplot of the grassland and vegetable production soils. The arrows are the biplot scores of the environmental variables. Management (grassland or vegetable production) has a regression/canonical coefficient of 1.0 with axis 1, which represents 73% of the total variation. Time since sieving also has a regression/canonical coefficient of 1.0 with axis 2, which represents 5% of the variation. The grassland points cluster in the left rectangle, while the vegetable soil points cluster in the right rectangle. The eigenvalue distribution of the individual PLFA on the biplot is available from the first author. $n = 4$ except for time 14, when $n = 3$ for the grassland soil, and time 5, when $n = 3$ for the vegetable soil.

4. Discussion

In these sandy loam soils with different previous management history, sieving was followed by changes in microbial community structure, decreased respiration, and net production of nitrate, but relatively little change in soil microbial biomass. In the grassland soil, there were greater changes in PLFA composition, a higher increase in a stress marker, and a larger decline in respiration, and higher net nitrate production than in the vegetable soil. In the vegetable soil, initially low nutrient availability and microbial biomass may have limited the magnitude of the microbial response to disturbance.

4.1. C and N pools and microbial activity

Tillage initiates changes in many abiotic factors that are known to affect soil microbiology including physical disruption, aeration, and more rapid drying of soil. In this study, in which we simulated tillage by sieving, it is impossible to separate these complex effects on soil. Unfortunately, we were not able to maintain an unsieved control set of cores at the same moisture as the sieved treatment in the greenhouse. Soil from the two sites, however, was at very similar soil matric potential at the onset of the experiment, and soil drying rates were similar, yielding a useful time course for comparison. Microbial activity would be expected to decline when soil matric potential decreases < -50 kPa (Griffin, 1980). It would have been higher if soils had been water-saturated when sieved, yet moist rather than very wet soils were intentionally used in order to simulate actual tillage, which is only conducted when soil moisture is drier than field capacity.

At the range of soil moisture measured in this study, C and N mineralization rates, and MBC would be expected to be approximately 50–75% of the maximum levels present in very wet soils, based on an incubation study of a soil of similar texture (Franzluebbers, 1999). Thus, adequate moisture was present to test the hypotheses of reduced versus increased activity after simulated tillage. The decrease in soil moisture during the course of the experiment would have been expected to decrease rates of C and N mineralization and MBC by approximately 15%, based on the same study (Franzluebbers, 1999). In fact, no change in MBC or MBN occurred. The decrease in respiration was highest in the grassland soil, however, during the initial 2 days after sieving when little change (< 5 kPa) in matric potential had occurred (Fig. 2). Nitrate concentration also began to increase during this same period. Large changes in PLFA profiles also occurred at this time of little moisture change (Fig. 3). Thus, changes in moisture alone cannot account for the soil responses observed after sieving. The effect of soil disturbance versus drying on short-term biological changes after tillage merits further investigation.

We observed that respiration decreased in both soils after simulated tillage, but the decline occurred several days earlier in the grassland than the vegetable soil. Our results agree with those of Petersen and Klug (1994), who observed that physical disturbance of soil caused a decrease in the respiratory activity of the soil in a matter of days. In a subsequent study with a silt loam soil, we observed decreased respiration, as measured by soil incubation in sealed containers, in soil cores after rototillage compared to non-tilled control cores maintained at similar soil moisture in a growth chamber at $>90\%$ relative humidity (Calderón et al., in press). These data support the results of this study, showing decreased respiration in tilled soils. They also allay lessen methodological concerns that the respiration assay itself causes strong disturbance of soil that immediately overcomes the inherent level of soil metabolic activity.

Reicosky et al. (1995, 1997), Rochette and Angers (1999) and Ellert and Jantzen (1999) observed increased CO_2 emissions from the soil surface within hours after tillage in the field. In the silt loam soil described above, we also observed higher CO_2 emissions after rototillage than in non-tilled soils, despite lower respiration (Calderon et al., in press). Such bursts of CO_2 emissions from recently disturbed soil are best explained by a rapid release of dissolved CO_2 caused by a rapid equilibration of soil CO_2 with atmospheric CO_2 (Reicosky, personal communication). In addition, tillage can also increase the size of the voids in the soil, thus increasing oxygen flow into, and CO_2 release out of the soil (Reicosky and Lindstrom, 1993).

Our alternate hypothesis, that tillage exposes microsites rich in substrates which could lead to a burst in microbial activity and growth, is not supported by our results. The relative stability of the labile C pools such as DOC and MBC, together with the decreased respiration in both vegetable and grassland soils suggests that the tillage simulation did not expose substantial amounts of C from easily decomposable substrates. However, these results may only apply to coarse soils low in organic matter and microbial biomass such as the Chualar sandy loam. It has been hypothesized that soils rich in clay and high in surface area have high initial amounts of organic matter protected from microbial attack, and thus show the highest responses of organic N and C mineralization after a disturbance (Rovira and Greacen, 1957; Craswell and Waring, 1972). In the Chualar sandy loam, low clay content may result in low initial amounts of protected organic matter and relatively little change in availability of mineralizable C and N after soil disturbance, especially

in the frequently-tilled vegetable soil. Soils low in clay may create fewer barriers between the microbes and their necessary substrates (Crasswell and Waring, 1972), decreasing the likelihood of a response after tillage.

Net N mineralization and nitrification occurred in both grassland and vegetable soils, but at different rates. After the disturbance, nitrifiers rather than N immobilizers or denitrifiers appear to be a major users of mineralized organic N in the grassland soil. Exposure of small amounts of previously protected N sources may explain the increase in available DON and subsequent net mineralization and nitrification after sieving. Dead microbial cells may have been another source of mineralizable N (Marumoto et al., 1982). In cultivated soil, less organic matter is expected to be protected in macroaggregates due to repeated destruction of the binding agents that cause aggregation of microaggregates (Cambardella and Elliott, 1994), and thus, dead microbial cells may have been a proportionately larger source of the mineralizable N after sieving than in the grassland soil. Macroaggregates in the grassland soil would be expected to contain larger amounts of organic matter, and thus release a larger source of newly available substrate for microbes after simulated tillage (Tisdall and Oades, 1982; Elliott, 1986; Cambardella and Elliott, 1994).

Denitrification rates were low or undetectable in both soils and were not affected by sieving. Moisture conditions and mixing of the soil during the experiment were not favorable for the creation of the anaerobic microsites necessary for denitrification activity in this sandy loam.

4.2. PLFA and microbial community structure

Few differences were observed in total PLFA after sieving, and thus in the total microbial biomass. If sieving redistributed colonies and substrates, the biomass of some microbial groups might have been negatively affected by the new conditions while different groups of organisms thrived, resulting in relatively stable amounts of microbial biomass in the soil. During the first 2 days after sieving, however, large fluctuations in MBC, MBN, total PLFA and in some of the specific PLFA were observed (Fig. 2), despite lack of significant differences. High coefficient of variation (standard error divided by the mean) is typical of the microbial data that we have previously collected for this soil (Jackson, unpublished data). Complex processes may be occurring immediately after tillage, and further research is warranted to explore these dynamics.

In the vegetable soil, bacterial and actinomycete PLFA declined during the initial period after sieving, yet the bulk of the microbial community must have remained active since there was no reduction in respiration rate during the first two days. These results suggest that the microbes in the vegetable soil may be adapted to remain active after the tillage disturbance. The PLFA stress marker was unchanged during this initial period after the disturbance. In contrast, the grassland soil communities showed greater and more rapid changes after sieving at a moisture content typical of field tillage, while respiration rate decreased substantially. Petersen and Klug (1994) also observed a concomitant reduction of microbial activity with a shift in microbial community composition after physically disturbing the soil.

Unexpectedly, the fatty acid, 18:2 ω6, which is proposed as a fungal marker (Vestal and White, 1989), remained relatively constant in both soils after sieving, yet it was shown to be reduced by sieving in a previous study (Petersen and Klug, 1994). Fungal hyphae, because of their fibrous nature, would be expected to be damaged during the sieving process. The initial amounts of 18:2 ω6 in this study ranged from 2.0 to 3.3 mol% and were lower than those reported by Petersen and Klug (1994), which were >6 mol%. This may indicate that the Chualar soils analyzed in this study might have had initially low fungal biomass, which precluded the significant reduction in fungal marker after the disturbance.

With increased time after tillage, microbial community composition continued to change. For example, actinomycetes appeared to be sensitive to the slow decline in soil moisture. The increase in MBN, but not MBC, in both soils after 1 week suggests that a group of microorganisms with lower C/N ratio may have become more important after the impacts of the initial disturbance had subsided. By this time, microeukaryotes, as indicated by 20:4 ω6, had recovered to initial levels in the vegetable soil, but continued to decrease in the grassland soil. The different responses in the two soils suggests that different assemblages of microeukaryotes are present in the two soils, and/or, that food availability for microeukaryotes may have been disrupted by the more extensive changes in community composition that occurred in the grassland soil.

The stress-indicator ratio of 19:0 cy to its precursor, 18:1 ω7, increased throughout the experiment in both soils in response to simulated tillage. This is in agreement with results obtained by Petersen and Klug (1994). The relatively gradual increase in the stress indicator suggests that ongoing responses to disruption from simulated tillage, as well as soil drying, may partially explain the increase in microbial stress through time. Storage at low temperature before the experiment may have also contributed to microbial stress. Nevertheless, the grassland soil's stress indicator levels increased at a higher rate than those of the vegetable soil, further supporting the idea that the grassland soil

was more markedly impacted than the vegetable soil after tillage.

In the Chualar sandy loam, a large decrease in soil organic matter, microbial biomass, and community structure has occurred during the century-long transition between annual grassland and intensively managed vegetable production. Tillage events initiate different microbial responses in the grassland and vegetable soils. Moisture content before and after tillage may play an important role in determining microbial activity. In finer-textured soils with more protected organic matter, different responses to tillage might be expected to occur.

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