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Microbial responses and nitrous oxide emissions during wetting and drying of organically and conventionally managed soil under tomatoes

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Abstract The types and amounts of carbon (C) and nitrogen (N) inputs, as well as irrigation management are likely to influence gaseous emissions and microbial ecology of agricultural soil. Carbon dioxide (CO₂) and nitrous oxide (N₂O) efflux, with and without acetylene inhibition, inorganic N, and microbial biomass C were measured after irrigation or simulated rainfall in two agricultural fields under tomatoes (*Lycopersicon esculentum*). The two fields, located in the California Central Valley, had either a history of high organic matter (OM) inputs (“organic” management) or one of low OM and inorganic fertilizer inputs (“conventional” management). In microcosms, where short-term microbial responses to wetting and drying were studied, the highest CO₂ efflux took place at about 60% water-filled pore space (WFPS). At this moisture level, phospholipid fatty acids (PLFA) indicative of microbial nutrient availability were elevated and a PLFA stress indicator was depressed, suggesting peak microbial activity. The highest N₂O efflux in the organically managed soil (0.94 mg N₂O-N m⁻² h⁻¹) occurred after manure and legume cover crop incorpo-

ration, and in the conventionally managed soil (2.12 mg N₂O-N m⁻² h⁻¹) after inorganic N fertilizer inputs. Elevated N₂O emissions occurred at a WFPS >60% and lasted <2 days after wetting, probably because the top layer (0–150 mm) of this silt loam soil dried quickly. Therefore, in these cropping systems, irrigation management might control the duration of elevated N₂O efflux, even when C and inorganic N availability are high, whereas inorganic N concentrations should be kept low during times when soil moisture cannot be controlled.

Keywords Microbial respiration · Carbon dioxide efflux · Denitrification · Acetylene block · Phospholipid fatty acids

Introduction

Wet–dry cycles affect microbial processes and soil physical properties, and thus, carbon (C) and nitrogen (N) gaseous emissions. Changes in soil water potential (Ψ_s) influence substrate availability and microbial cell physiology (Stark and Firestone 1995). Soil water content affects soil gas diffusivity, which regulates production and consumption of nitrogen oxides, movement of N oxides in and out of solution (Firestone and Davidson 1989), and soil–atmosphere gas influx and efflux. Soil type and the amount of water applied influence the duration of denitrification fluxes. About 60% of anthropogenic N₂O emissions are estimated to come from agriculture (Mosier et al. 1998). The types and amounts of C and N inputs are likely to influence gaseous emissions. High N₂O and total denitrification (N₂O plus N₂) losses have been reported from manured fields with high C availability (e.g., Mogge et al. 1999) and after inorganic N fertilizer inputs (Bouwman 1996; Eichner 1990). Both irrigation and fertility management may be modified to minimize N₂O emissions.

Changes in moisture and C and N availability affect microbially mediated C and N transformations as well as soil ecology. Patterns of phospholipid fatty acid (PLFA) profiles can be used to assess changes in microbial community composition in response to altered environmental

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conditions (Bossio and Scow 1998) or as indicators of the physiological status of microorganisms (Kieft et al. 1994, 1997). In previous studies, changes in microbial community composition occurred in the course of a growing season, but there were also indications that PLFA profiles change in as little as a few hours in response to wetting (Lundquist et al. 1999b). However, it was unclear if these changes were mainly due to differential survival of certain types of microorganisms or to stress-related modifications of existing fatty acids by microbes.

We compared gaseous C and N emissions, microbial responses, and changes in microbial community composition during wetting and subsequent drying in an organically and a conventionally managed soil. Specifically, our objectives were to (1) estimate N₂O, total denitrification, and CO₂ flux after irrigation or rainfall events, (2) examine the effect of environmental variables and C and N pools on these fluxes, and (3) assess microbial activity, biomass, and community composition in response to soil moisture changes.

Materials and methods

Field sampling: gas efflux and soil variables

The field site was located at the University of California Davis Long Term Research on Agricultural Systems (LTRAS; <http://www.ltras.ucdavis.edu/>) project (38°32' 30"N, 121°52'30"W; 28 m elevation). The organic system, a tomato–corn rotation, receives a winter legume cover crop (hairy vetch, *Vicia villosa* Roth, and Austrian winter pea, *Pisum sativum* L.), animal (composted poultry) manure, and harvest residue as inputs (Table 1). The

Table 1 Soil characteristics and average yearly inputs of organic and conventional systems at LTRAS

Management type and rotation	Conventional (tomato/wheat)	Organic (tomato/corn)
pH (H ₂ O 1:1)	6.8	6.5
CEC (meq 100 g ⁻¹ soil)	30.8	33.9
Sand (%)	23	19
Silt (%)	55	58
Clay (%)	22	23
Bulk density 0–50 mm (Mg m ⁻³)	1.15	1.15
Bulk density 50–150 mm (Mg m ⁻³)	1.29	1.23
Organic C (g kg ⁻¹)	10.3	12.8
Organic N (g kg ⁻¹)	1.0	1.4
Dry matter inputs (Mg ha ⁻¹ yr ⁻¹)	6	23
N inputs (kg N ha ⁻¹ yr ⁻¹)	165	350

Values are means. $n=3$. Bulk density was calculated from measurements taken on the cores retrieved from the field in June ($n=21$)

conventional system receives only inorganic fertilizer (granular, banded) and harvest residue and employs a tomato–wheat rotation. Both systems are furrow irrigated. The soils were sampled during a tomato (*Lycopersicon esculentum*) growing season, when the cropping systems were in their third year. The soil type was Yolo silt loam, a fine silty, mixed, nonacid, thermic Typic Xerorthent.

The first sampling period (April), corresponding with the first irrigation of the year, was at the time of tomato transplanting. In the organic system, the winter legume cover crop (47 kg N ha⁻¹) and manure (133 kg N ha⁻¹) were incorporated 2 weeks earlier, and in the conventional system, N-P-K fertilizer (57 kg N ha⁻¹) was applied 1 week before transplanting. The second sampling (June) took place 2 weeks after the second of two (NH₄)₂SO₄ fertilizer side dressings (47 kg N ha⁻¹ application⁻¹) in the conventional system. A third sampling (August) was conducted after the final irrigation of the season. The last sampling (October) occurred after sprinkler irrigation to simulate a 90-mm rainfall. The soil had been fallow and dry for 2 months after the tomato harvest, and the plots under both management types had been disked in early September. In the conventional system, NH₄NO₃ (112 kg N ha⁻¹) for the subsequent winter wheat crop was applied 5 days before the sampling.

At each sampling period, efflux of CO₂, total denitrification, estimated as efflux of N₂O with acetylene inhibition (+C₂H₂) (Ryden et al. 1979), and N₂O (–C₂H₂) were measured 6 h, 30 h, and 54 h after the start of irrigation. There were three 12×12 m subplots in each of the 60×60 m plots per management type, with two +C₂H₂ and two –C₂H₂ sites per subplot. All sampling sites were on the shoulder of the tomato beds (350–500 mm from the centerline), where the most drastic changes in soil moisture were expected to occur.

A static chamber technique was used to measure gas efflux (Hutchinson and Livingston 1993). At the +C₂H₂ sites, a C₂H₂ concentration of 0.1–1.0% in the air of the soil pore space was achieved within 1 h of C₂H₂ flow through probes inserted next to the chambers, as verified at 150 mm depth. Thirty and 60 min after C₂H₂ flow shutoff, gas samples for analysis of N₂O were taken from the +C₂H₂ and –C₂H₂ chambers with nylon syringes (Sesi, Cournon D'Auvergne, France; VWR number 60350-066). At the –C₂H₂ sites, gas samples for analysis of CO₂ were removed from the chambers after 15 and 30 min. Ambient air was collected next to the chambers. The samples were stored in the syringes for 2–6 h. Concentrations of N₂O were determined by a gas chromatograph (GC) with a ⁶³Ni electron capture detector (HP 6890, Hewlett Packard, Palo Alto, CA) and a 2 m HayeSep Q column (Supelco, Bellefonte, PA, USA). Concentrations of CO₂ were determined using a GC with a thermal conductivity detector (HP 5890, Hewlett Packard, Palo Alto, CA).

Composite soil samples, consisting of three 150×20-mm-diameter cores, were collected within 150 mm of the chambers. Gravimetric soil water content (w) and inorganic N were determined daily, microbial biomass C (MBC) and dissolved organic C (DOC) were measured on the third day

of each sampling period. For inorganic N analysis, samples were extracted with 2 M KCl at a liquid to soil ratio of 5:1. The samples were shaken for 30 min, centrifuged, and the supernatant was collected. To measure MBC, the 1-d fumigation–extraction method (Vance et al. 1987) was used after removal of roots from the samples. Dissolved organic C was extracted with deionized H₂O (Lundquist et al. 1999a). All extracts were frozen until analysis. Water-filled pore space (WFPS) was calculated as

$$\% \text{WFPS} = (w \times \text{bulk density}) / [1 - (\text{bulk density}/2.65)] \times 100\% \quad (1)$$

where bulk density of the 50- to 150-mm layer, as determined in the intact cores (see below), was used. Soil water potential (Ψ_s) was estimated based on w by interpolating Ψ_s values from moisture retention curves generated for the organically and conventionally managed soils with a pressure plate apparatus.

Microcosms: wetting and drying time course

In July, 21 intact soil cores were collected in 300×130-mm-diameter PVC cylinders from random locations distributed over the entire length of a single bed in each of the plots described above. There was little soil compaction of the cores because the soils were relatively dry (Fig. 3) and because the PVC cylinders were sharply beveled. Three cores per management type were dismantled to measure soil variables (see below) before wetting. The rest of the cores were sealed off with perforated Parafilm to minimize gas losses through the bottom of the cores while allowing drainage of effluent, if necessary. The cores were then simultaneously rewet with deionized water to mimic furrow irrigation in the field. The water was fed into the cylinders 70 and 100 mm below the soil surface via plastic tubes inserted into the PVC cylinders. The head of the reservoir serving as water source was kept 20 or 50 mm, respectively, above the water entry points. To simulate soil drying in the field, 250-W heat lamps were placed about 0.5 m above the cores, which were also surrounded by fans.

The procedures for measuring CO₂ and N₂O efflux, measured 4, 10, 15, 23, 32, 51, and 99 h after wetting, were the same as in the field. After each gas sampling, three cores were dismantled. The 0- to 50-mm and 50- to 150-mm layer of soil was removed from the cylinders, weighed, and mixed. Soil respiration was measured in two 20-g subsamples per soil layer by the change in CO₂ concentration over 1 h in the headspace of a closed mason jar, as determined by a Horiba PIR-2000 (Horiba Instruments, Riverside, CA, USA) (Lundquist et al. 1999a). From each of the two upper layers, three subsamples for determination of soil moisture, inorganic N, MBC, and DOC were taken. Subsamples to measure soil moisture and inorganic N were also taken from the soil of the 150- to 300-mm layer. Soil extraction procedures were as above. For PLFA analysis,

one sample per core and layer was immediately frozen and stored at −20°C (Drenovsky et al. 2004).

Soil chemical analyses

Organic C in the MB extracts was measured by oxidation with dichromate in concentrated sulfuric and phosphoric acid, followed by titration of the unreacted dichromate with ferrous ammonium sulfate (Vance et al. 1987). For MBC, flush values are reported. Concentrations of NH₄⁺ and NO₃⁻+NO₂⁻ in the 2 M KCl extracts were determined by a flow injection analyzer (Lachat 8000, Zellweger, Milwaukee, WI, USA). The DOC extracts were analyzed using a Shimadzu TOC 5050 (Shimadzu, Columbia, MD, USA). For the PLFA study, duplicate 8-g dry weight soil samples were analyzed according to Bossio and Scow (1998). Briefly, the frozen samples were weighed and immediately extracted in a phosphate-buffered chloroform–methanol mixture. The PLFAs were separated from other lipids on solid-phase extraction columns, 0.50 g Si (Supelco, Inc., Bellefonte, PA, USA), and purified. The samples were analyzed, using a gas chromatograph (HP 6890, Hewlett Packard, Palo Alto, CA, USA) with a 25-m Ultra 2 (5% phenyl)-methylpolysiloxane column (J&W Scientific, Folsom, CA, USA), after addition of an internal standard to quantify individual fatty acids. The peaks were identified using MIDI software (MIDI, Inc., Newark, DE, USA) and bacterial fatty acid standards.

Statistical analysis

The data of both the seasonal field measurements and the wetting/drying time-course experiment were analyzed as split plots with soil management type as main plots and sampling period as subplots. To compare means within soil management types, Duncan's multiple range test was used. The least significant difference (LSD) is also shown in some of the figures. With respect to the field measurements that were taken several times during a sampling period, daily total denitrification and N₂O flux were compared. For the variables CO₂ flux, NH₄⁺, and NO₃⁻, the 3-day averages were compared because these parameters varied in inconsistent patterns within sampling periods.

The PLFA profiles were analyzed via canonical correspondence analysis (CCA) to assess the differences in PLFA composition in response to Ψ_s (ter Braak 1987, 1990). CANOCO 4 (Microcomputer Power, Ithaca, NY, USA) software was used to generate biplots. Only those 28 PLFAs that were found in every sample were used for the multivariate analysis. These 28 fatty acids, out of a total of 94, accounted for 94.5 (±0.2)% of the mass of all PLFA extracted. Axis 1 of the biplot was constrained to Ψ_s and the average of the three PLFA profiles per sampling time after wetting and soil type were the response variables. The eigenvalue of axis 1 is a measure of the variation of PLFA profiles explained by this axis, i.e., Ψ_s . Axis 2 of the biplot

is orthogonal to axis 1. The program used a Monte Carlo permutation to test the significance of Ψ_s in explaining variation among PLFA profiles.

Results

Field measurements: gas efflux and C and N pools

In general, the WFPS 30 h after the start of irrigation was $\geq 60\%$, and 54 h after irrigation, WFPS was usually $<60\%$ (Fig. 1). In the organic system, the 30-h N_2O ($-C_2H_2$) flux in April was higher than at any of the other sampling dates (Fig. 2). In the conventional system, the 30-h N_2O ($-C_2H_2$) flux in October was at least five times greater than at any other sampling date. The N_2O ($-C_2H_2$) flux 30 h after the start of irrigation was usually the highest of the three measurements per sampling period. Total denitrification, measured as N_2O ($+C_2H_2$) flux, showed a similar pattern as N_2O ($-C_2H_2$) emissions. Both, N_2O ($-C_2H_2$) and total denitrification did not differ between the two management types (main plot effect; $P>0.05$). In both soils, mean NH_4^+ concentrations were high in April, declined during the growing season, and were again elevated in October (Table 2). In the conventional system, the highest inorganic N concentrations coincided with the large fertilizer N inputs applied in October 5 days before sampling. Overall, inorganic N in the organic and conventional system did not differ between management types (main plot effect; $P>0.05$).

In the organic system, both CO_2 flux and DOC were greater than in the conventional system (Table 2). In both soils, mean DOC concentrations were higher after the 2-month fallow period after harvest compared to the growing season. In the organic system, mean DOC concentrations were also greater in April than in June and August.

During the growing season (April–August), MBC values were greater in the organically than in the conventionally

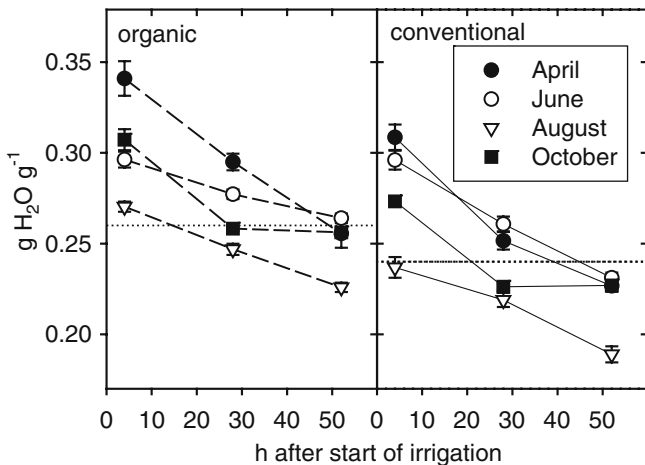


Fig. 1 Gravimetric water content (w) in organic and conventional soil in the field in the 0- to 150-mm layer. The dotted line indicates the corresponding w for 60% water-filled pore space in the 50- to 150-mm layer. Standard error of the mean shown as line bars. $n=12$

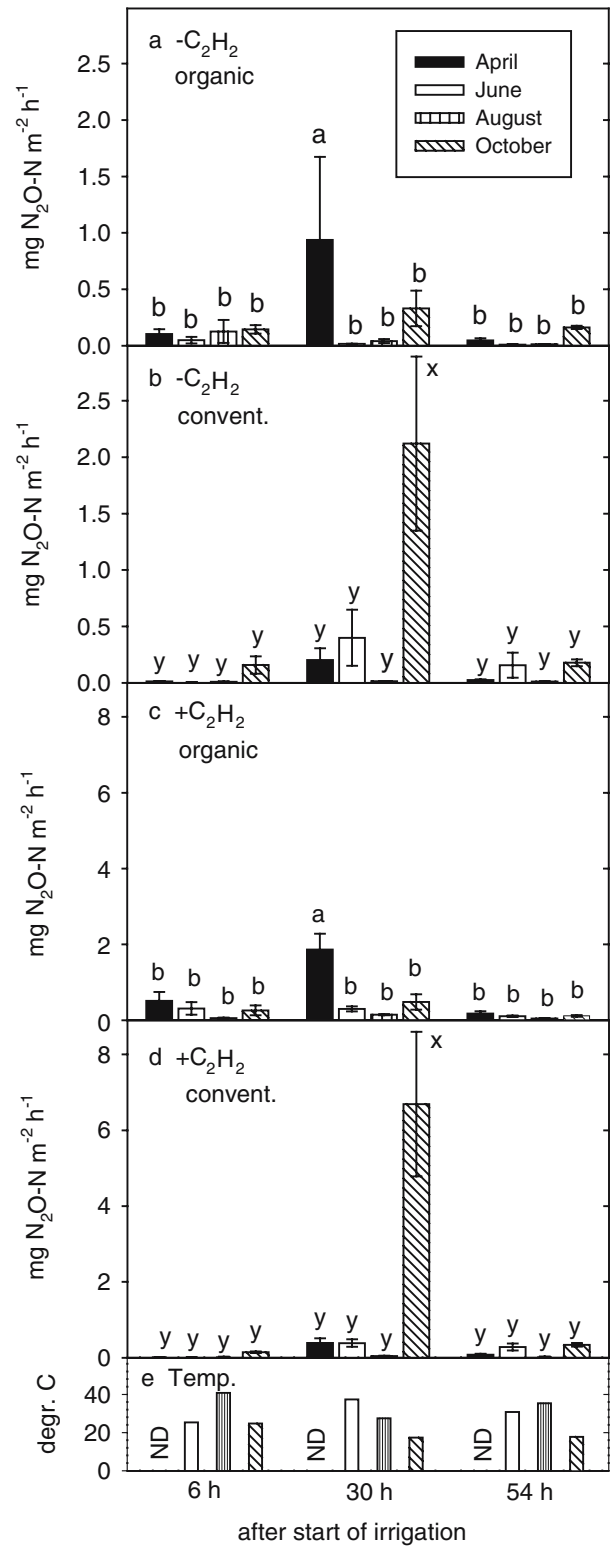


Fig. 2 a, b Nitrous oxide ($-C_2H_2$) flux and c, d total denitrification ($+C_2H_2$) after irrigation or simulated rainfall in the field. e Soil temperature (0–100 mm). Standard error of the mean shown as line bars. Means designated with the same letters are not significantly ($P>0.05$) different. $n=6$

Table 2 Inorganic N, microbial biomass C, and dissolved organic C in the 0- to 150-mm layer, CO₂ emission rates soil during irrigation events (April, June, August) and simulated rain (October), and results of ANOVA

Organic	NH ₄ ⁺ (mg N kg ⁻¹)	NO ₃ ⁻ (mg N kg ⁻¹)	MBC (mg C kg ⁻¹)	DOC (mg C kg ⁻¹)	CO ₂ (mg C m ⁻² h ⁻¹)
April	19.1 (2.2) a	7.1 (0.8) a	98.4 (20.6) a	36.0 (3.0) a	102.9 (18.1) a
June	0.6 (0.2) c	9.1 (1.8) a	112.0 (13.0) a	20.6 (0.6) b	107.1 (10.4) a
August	0.4 (0.1) c	8.2 (1.0) a	87.6 (12.7) ab	23.8 (1.2) b	94.3 (4.7) a
October	5.2 (0.4) b	3.2 (0.4) b	39.0 (12.8) c	31.6 (1.5) a	81.3 (10.7) ab
Conventional					
April	24.0 (6.1) x	4.7 (0.7) x	43.3 (16.3) c	9.2 (0.4) y	59.5 (9.7) b
June	7.5 (4.7) y	4.9 (1.2) x	58.6 (9.2) bc	12.4 (0.6) y	87.6 (9.3) ab
August	0.2 (0.1) y	7.4 (2.0) x	23.5 (9.0) c	12.7 (0.5) y	88.4 (6.0) ab
October	32.2 (4.1) x	9.0 (1.0) x	22.8 (7.3) c	20.0 (3.0) x	89.3 (13.6) ab
ANOVA					
Management	NS	NS	NS	***	**
Sampling period	***	**	***	***	**
Management × Sampling Period	***	***	NS	***	NS

Shown are means (SE). $n=6$ or $n=12$ (inorganic N). Means followed by the same letter are not significantly different

NS Nonsignificant

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

managed soil, although there was no consistent effect of management system on MBC. In both systems, the highest MBC concentrations were measured in June, and the lowest in October. In the organic system, mean MBC concentration in October was significantly lower than at any other sampling period.

Microcosms: short-term effects of wetting and drying

In the microcosms, the changes in w were more pronounced in the surface layer (0–50 mm) compared to the 50- to 150-mm layer (Fig. 3). Mean N₂O (–C₂H₂) emissions were generally <0.02 mg N₂O-N m⁻² h⁻¹ and did not differ between soil management type or with time elapsed since wetting (data not shown). The C pools and C process rates and related measures, i.e., MBC, DOC, respiration, and CO₂ efflux, were all greater in the organically compared to the conventionally managed soil (Table 3). Respiration, as measured in the bioassay with 20 g of soil, responded rapidly to changes in soil moisture and increased significantly within 6 h of wetting in both soils (Fig. 3). However, the peak of CO₂ efflux from the intact soil cores occurred when the WFPS in the cores was approximately 60%. In the surface layer (0–50 mm) of both soils, MBC concentrations after about 3 days of soil drying were lower than maximum levels of MBC, reached shortly after the wetup. In the 50- to 150-mm layer of the organically managed soil, mean MBC concentrations decreased from their highest level after about 3 days of drying, whereas in the conventionally managed soil, there were no changes in MBC over time.

Mean total PLFA concentrations were greater in the organically compared to the conventionally managed soil but did not differ with time after wetting or between layers of either soil management type (Table 3). In the CCA ordi-

nation biplot of PLFA profiles in the 0- to 50-mm layer, axis 1, which was constrained to Ψ_s , explained 24% of the total variation (Fig. 4). The PLFA profiles separated along this axis. The Monte Carlo permutation test indicated that Ψ_s had a significant effect on microbial community composition ($P<0.05$). Axis 2, which explained 41% of total variation, separated PLFA compositions of the organic and the conventional treatment, with the exception of one sample (organic 12 h). In both soils, the PLFA profiles in the driest soil (<1.5 MPa; 0 and 100 h) and the soil at a Ψ_s of –0.08 (organic) to –0.1 MPa (conventional) were the farthest apart, and in both soils, the PLFA profiles showed a clockwise pattern with time. However, if the soil of the 0- to 50-mm layer of the organic and the conventional system was analyzed separately, changes in Ψ_s had a significant effect on microbial community composition only in the organic treatment ($P<0.01$; data not shown). In the 50- to 150-mm layer, Ψ_s had no effect on PLFA profiles, but axis 2, which explained 46% of total variation, separated organic from conventional PLFA profiles (not shown).

Table 4 lists the PLFAs that were most influential in separating the PLFA profiles before and after wetting. The fatty acids with a negative score were relatively more abundant at high Ψ_s , and those with positive scores were relatively more enriched at low Ψ_s . In fatty acid terminology, A:B ω C means A=number of C atoms, B=number of unsaturations, C=number of C atoms between closest unsaturation and aliphatic end of the molecule, i = iso (methyl branching), c=cis, and t=trans. The fatty acid 18:2 ω 6c, enriched in the organically compared to the conventionally managed soil in both layers ($P<0.01$), was particularly influential for the separation of the profiles on axis 1 and axis 2 of the biplot. The ratio of monounsaturated to saturated PLFAs (16:1 ω 5c+16:1 ω 7c+16:1 ω 11c+17:1 ω 9c+18:1 ω 7c+18:1 ω 9c)/(15:0+16:0+17:0+18:0+20:0) was higher in the organic than in the conventional treatment

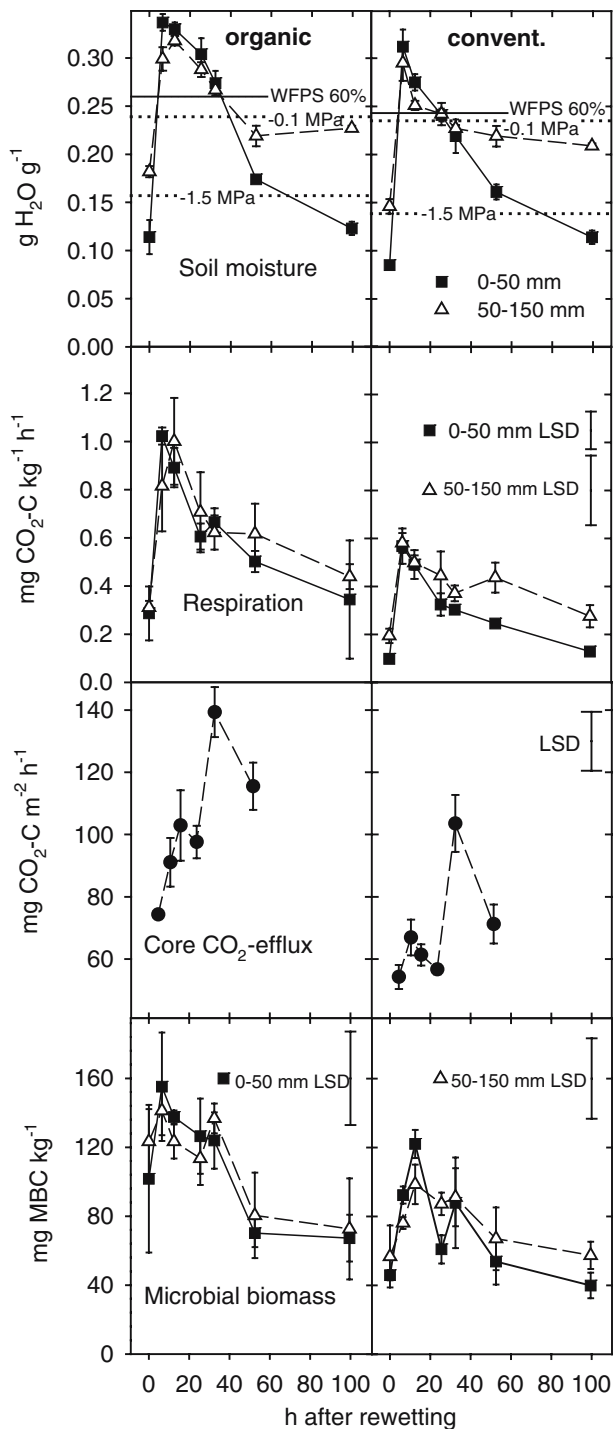


Fig. 3 Soil moisture (water-filled pore space shown for the 50- to 150-mm soil layer), respiration rates, CO_2 efflux, and microbial biomass C after wetting of intact soil cores. Values are means. Standard error of the mean shown as *line bars*. *LSD* least significant difference ($P < 0.05$). $n = 3$

($P < 0.001$). This ratio increased significantly after wetting in both soils in both layers and then decreased significantly with soil drying (Fig. 5). The ratio of the fatty acids, cyclo17:0/16:1 ω 7c, showed significant changes in the

opposite direction. The latter ratio was higher in the organic than the conventional treatment in the 0- to 50-mm layer ($P < 0.0001$).

Discussion

Controlling factors of N_2O flux

The differences in N_2O and total denitrification efflux appeared to be in part the result of short-term N and C availability and, thus, showed large seasonal variation. In the organic system, the greatest N_2O flux followed the cover crop and manure incorporations. At the time, inorganic N, particularly NH_4^+ , concentrations were relatively high. Mineralizable C was probably higher than at any other time of the year, and DOC was the highest of the four measurements. Thus, substrate for respiration may have been enhanced, as evidenced by the higher CO_2 flux in the organic than in the conventional system. By far the highest N_2O flux was recorded in the conventional system in October. Rates of N_2O emission in excess of $2 \text{ mg m}^{-2} \text{ h}^{-1}$ are among the highest ever measured in agricultural soil (Matson et al. 1998). In addition to the NH_4NO_3 fertilizer application, there had also been C inputs in the form of harvest residue ($1.8 \text{ Mg dry matter ha}^{-1}$), and DOC concentrations were at their highest level. This may have enhanced C availability, and thus respiration, after the simulated rainfall in October. High N_2O fluxes after N fertilizer applications and irrigation or rainfall have been reported in many different agroecosystems (Bronson and Mosier 1993; Matson et al. 1996). Nitrification may have contributed to N_2O flux at either of these two occurrences of high N_2O emissions since NH_4^+ pools were high in the organic system in April and in the conventional system in October. In fertilized tropical sugar cane soils, the highest N_2O emissions occurred where NH_4^+ concentrations were highest (Davidson et al. 1996). A substantial part of the N_2O flux in both soils, however, was probably due to denitrification, since the highest total denitrification rates, as measured by the acetylene block method that inhibits nitrification, were observed on the same dates as the highest N_2O ($-\text{C}_2\text{H}_2$) fluxes.

In addition to N inputs and C availability, soil moisture controlled N_2O emissions through its influence on both microbial activity and on gas transport. The use of WFPS is a useful predictor of either aerobic and anaerobic microbial activity in soil (Linn and Doran 1984), and a WFPS of 60% typically results in maximum microbial activity and the lower threshold for anaerobic respiration and denitrification (Paul and Clark 1996). In our soils, the estimated WFPS of 60% was also an approximate benchmark above which substantial N_2O flux was observed. In this silt-loam soil, WFPS did usually not stay above 60% for more than 2 days, and thus the high N_2O fluxes were relatively short-lived.

Table 3 Results of ANOVA in the time-course study with intact cores

Variable	Depth (mm)	Management	Time	Management× Time	Organic	Conventional
CO ₂ efflux		*	*	NS	Fig. 3	
Respiration	0–50	***	***	NS	Fig. 3	
	50–150	***	***	NS		
MBC	0–50	***	*	NS	Fig. 3	
	50–150	***	NS	NS		
DOC (mg C kg ⁻¹)	0–50	***	NS	NS	43.0 (5.7)	21.4 (2.5)
	50–150	***	NS	NS	22.3 (1.8)	11.3 (0.7)
NH ₄ ⁺ (mg N kg ⁻¹)	0–50	*	**	NS	1.3 (0.2)	0.9 (0.2)
	50–150	NS	NS	NS	0.7 (0.2)	0.5 (0.2)
	150–300	NS	NS	NS	0.4 (0.1)	0.8 (0.2)
NO ₃ ⁻ (mg N kg ⁻¹)	0–50	NS	NS	NS	41.9 (8.4)	47.2 (8.3)
	50–150	NS	NS	NS	2.8 (0.5)	2.4 (0.3)
	150–300	NS	NS	NS	1.8 (0.2)	2.1 (0.2)
Total PLFA (g kg ⁻¹)	0–50	***	NS	NS	20.9 (1.7)	12.2 (0.8)
	50–150	***	NS	NS	19.8 (1.0)	11.2 (0.6)

Means (SE) of the seven time points are shown for the variables dissolved organic C, ammonium, nitrate, and phospholipid fatty acids. $n=7$
 NS Nonsignificant
 * $P<0.05$; ** $P<0.01$;
 *** $P<0.001$

Microbial biomass C and respiration

The magnitude of the microbial biomass and activity measures was strongly influenced by the inputs these soils received. Microbial biomass C, total PLFA, respiration, CO₂ flux, and DOC were all higher in the organic than the conventional system, and this was probably due to the greater OM inputs in the organic system. Another contributing factor is the rotation, which keeps the soil in the organic system almost continuously ($\geq 80\%$) under plant cover, in contrast to the conventionally managed soil, which is fallow for long periods ($\geq 50\%$). Year-round cropping results in greater microbial populations compared to crop–fallow rotations (Campbell et al. 1991; Collins et al. 1992). In the short-term, the decline in MBC between

harvest and simulated rainfall in October in the organic system probably occurred because of the lack of plants and moisture during the 2-month fallow, dry period. Collins et al. (1992) also reported higher MBC concentrations in cropped compared to fallow soils. In the conventionally managed soil, when analyzed separately, MBC declined significantly between June and August/October (separate analysis not shown). A seasonal pattern of increasing MBC concentrations from planting to flowering and subsequent decline to MBC levels at planting, similar to the MBC fluctuation in the conventional system, has been shown in a similarly managed system in the same soil type (Gunapala and Scow 1998) and in continuous wheat and wheat/soybean rotations (Franzluebbers et al. 1994). All these results suggest a dependence of MBC on C products from roots.

In the microcosms, the highest CO₂ efflux took place at 32 h after wetting, whereas the peak rate of respiration, as measured in the bioassay, was recorded at 6 h after wetting. Low soil aeration in the cores probably limited microbial activity in the early period of the experiment (0–32 h), and this may have resulted in lower CO₂ production. At WFPS $>60\%$, obligate aerobic processes and CO₂ production rates start to decline (Linn and Doran 1984). At 32 h, WFPS was 63 and 55% in organically and conventionally managed soil, respectively, near the optimal soil moisture/aeration value of 60% where microbial activity is expected to peak. Diffusional constraints due to the high w was less likely the cause of the delayed CO₂ flux peak since other workers (McCarty et al. 1999) did not find a marked difference between headspace and soil pore CO₂ partial pressures at WFPS of 73% in silt loam soil.

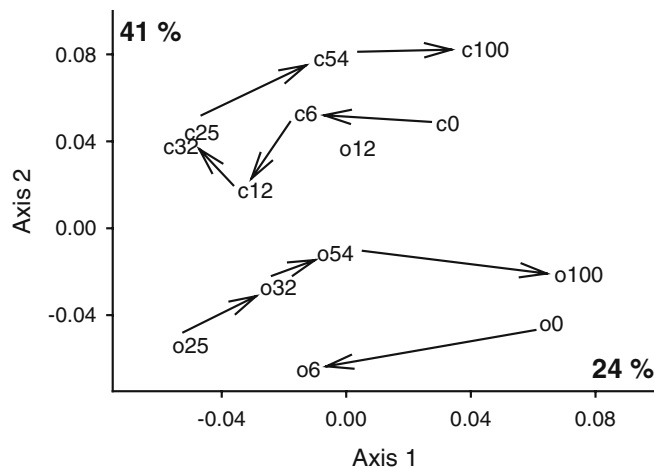


Fig. 4 Canonical correspondence analysis ordination biplot of the PLFA profiles for the 0- to 50-mm layer for organic and conventional soil before wetting (*o0*, *c0*) and during the 100-h drying period of the soil. *o* organic, *c* conventional, followed by number indicating hours after wetting. Axis 1 was constrained to soil water potential. Axis 1 and 2 have eigenvalues of 24 and 41%, respectively

Microbial community composition and soil moisture status

The changes in microbial activity in response to wetting and drying were accompanied by significant changes in microbial community composition, at least in the surface

Table 4 The most influential scores for axis 1 of the biplot (Fig. 4), and the most influential scores for the same analysis, using PLFA data of either the organic or the conventional soil

Molecule	Score			Specificity as Marker
	Biplot	Within organic	Within conventional	
i15:1	-1.61	-1.55	-1.09	Bacteria ^a
18:1 ω 7c; 18:1 ω 9t; 18:1 ω 12t	-1.55	-1.52	-1.54	Aerobic bacteria ^b
16:1 ω 7c	-1.48	-1.49	-1.70	Aerobic bacteria ^b
16:1 ω 7t	-1.45	-1.19	-1.87	Aerobic bacteria ^b
18:2 ω 6c	2.28	2.79	0.38	Eukaryotes, fungi ^c
16:1 ω 5c	1.34	1.18	1.30	Nonspecific
18:0	1.29	1.16	1.54	Nonspecific
i17:0	0.97	0.81	1.10	Eubacteria, actinomycetes, cyanobacteria, gram-positive bacteria ^a

Shown are the 8 greatest absolute values out of a total of 28

^aZelles 1997

^bVestal and White 1989

^cFederle 1986

layer of the organically managed soil. Although MBC did not increase significantly after wetting in the organic treatment, shifts for certain types of microbes occurred, based on PLFA profiles. The general patterns of the PLFA profiles were similar for the two systems (Fig. 4, biplot), which suggests that the abundances of microbes that respond to changes in Ψ_s shifted in about the same proportions and returned to similar community composition at the end of the dry-wet-dry cycle. The data of the 12-h samples of the organic system are unexplained exceptions that did not follow the general trends. The pattern of clockwise, gradual shifts in PLFA profiles suggested increases, followed by decreases of certain PLFAs during the time course, rather

than a direct correlation between concentrations of specific fatty acids and Ψ_s . For example, the 6- and 54-h samples were close to each other with respect to axis 1, although Ψ_s at those times was quite different.

The fatty acid 18:2 ω 6c, a biomarker for fungi (Federle 1986), was relatively enriched in the organically managed soil at low Ψ_s . In a previous study, this PLFA was consistently less abundant than in nonflooded rice fields (Bossio and Scow 1998) and was enriched in organically compared to conventionally managed systems in a similar soil (Bossio et al. 1998). At high Ψ_s in our soil cores, certain biomarkers of bacteria were relatively more abundant in the organic and conventional system (Table 4).

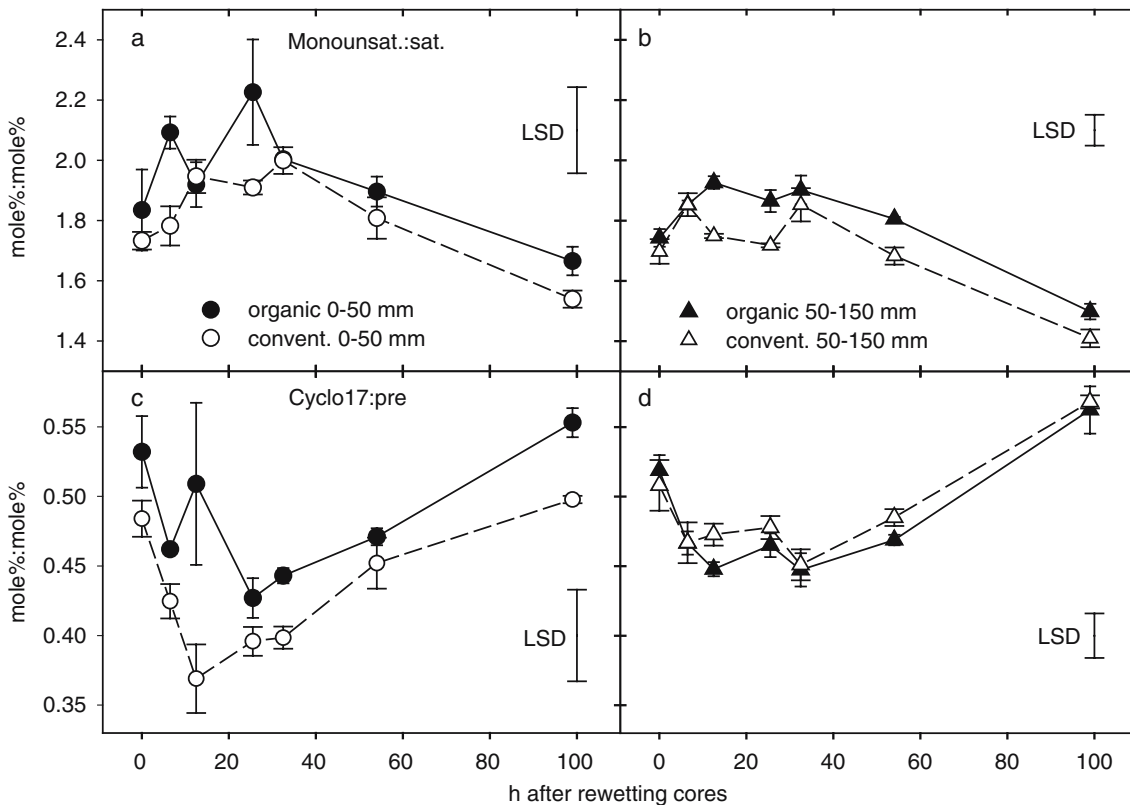


Fig. 5 Ratios of monounsaturated:saturated and cycloheptacosyl:preheptacosyl before wetting and during the 100-h drying period of the soil. Standard error of the mean shown as line bars. LSD least significant difference ($P < 0.05$). $n = 3$

The relative abundances of these biomarkers would be in agreement with the generally accepted notion that the abundance of active bacteria declines rapidly at Ψ_s below field capacity (Griffin 1981), whereas many fungi remain active at much lower Ψ_s (Beare et al. 1992).

The ratio of monounsaturated to saturated (monounsaturated: sat) PLFAs increased after wetting and then decreased to a lower than pre-wetup level, similar to MBC concentrations. There was a corresponding decrease in the cyclo17:0/16:1 ω 7c (17:0 cyclo:pre) ratio after wetting, followed by an increase. Monounsaturated fatty acids are associated with substrate availability (Zelles et al. 1992). They were higher after straw incorporation than after straw burning in rice cropping systems (Bossio and Scow 1998), and, as in the present study, the ratio of monounsaturated:saturated was greater in organically than in conventionally managed systems in similar soil (Bossio et al. 1998; Lundquist et al. 1999b) (Fig. 5). High saturated:monounsaturated ratios are indicators of desiccation stress (Kieft et al. 1994) or nutrient stress in pure cultures of *Pseudomonas* sp. (Kieft et al. 1997). Increases in the ratio of cyclopropyl fatty acids to their precursors, such as cyclo17:pre, also indicate stress. These ratios increased in pure culture studies of gram-negative bacteria with low nutrient availability and other stress factors (Guckert et al. 1986). The fatty acids that make up the two ratios account for about 50% of the total mass of PLFAs used in our CCA. The highest monounsaturated:saturated and lowest cyclo17:pre ratios occurred when PLFA profiles were separated most clearly from initial ($t=0$) and ending ($t=100$ h) profiles, at a WFPS of about 60%. This observation lends support to the hypothesis that peak microbial activity occurs at a WFPS of about 60%. Thus, the rapid changes in PLFA profiles may have been, in large part, due to rapid physiological adjustments by certain microorganisms and greater survival rates of fungi than bacteria in dry soil.

Implications for cropping systems management

The duration of N_2O flux during the cropping season was controlled by irrigation and evapotranspiration since this silt loam soil drains and dries quickly. The high N_2O flux after the fall N fertilizer application and simulated rainfall in the conventional soil demonstrated that inorganic N is a controlling factor of N_2O emissions. The choice of fertilizer (NH_4NO_3) may have influenced the magnitude of the fall emissions. Future research should involve testing of different fertilizer types within specific cropping systems. This study showed that multiple applications of small amounts of N fertilizer are preferable to large single doses. Spatially less concentrated inorganic N inputs are also recommended to reduce N_2O emissions (Beauchamp 1997). In the organic system, high carbon availability and high NH_4^+ concentration likely promoted the large N_2O efflux in the spring. The timing of the application of the organic inputs in this system cannot be changed. However, avoiding heavy irrigation might at least limit the duration of high N_2O efflux when both inorganic N and C availability are

high. Our data also suggest that high inorganic N availability should be prevented during times when extended or frequent rainfall are likely, as is the case in late fall and winter in this Mediterranean climate.

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