Identification of Microbes Responsible for Acetate Consumption in Soils Under Different Wetting Regimes

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Summary

Acetate is a critical compound in the soil carbon cycle, as it is a terminal product of complex organic matter fermentation under anaerobic conditions and is a major substrate for aerobic and anaerobic respirations. Our goal was to use modern molecular methods to identify microbes involved in soil acetate turnover under different oxygen tensions/soil saturation conditions. We showed that incubation of several soils (two from the Imperial Valley, California, and one from a Korean rice paddy) with water alone caused significant shifts in microbial community composition, but that the addition of acetate caused further detectable shifts. We developed a method, known as stable isotope probing (SIP), which allowed us to directly link the ability to utilize acetate as a carbon source to the identity of the organism. Applying this technique to soils in microcosm incubation experiments showed that the acetate-utilizing community is a subset of the greater overall community and that, under anaerobic conditions, iron (III)- and sulfate-reducing bacteria simultaneously utilize acetate, a finding that contradicts predictions based on bulk chemistry studies. We are currently pursuing the dynamics of acetate utilization, i.e., whether the acetate-utilizing community changes over incubation time and changes in O_2 tension. Over the longer term, these studies promise to provide insight into soil carbon loss and transformation under anaerobic conditions, methane production in water-logged soils, quantification of impacts of anthropogenic inputs of water on transformations and transport of carbon in soils, and the overall soil carbon cycle.

Keywords: acetate, microbes, stable isotope probing, carbon

Objectives

Objective 1: Validate the utility of a new molecular method—stable isotope probing (SIP)— for identification of acetate utilizers in defined and pure mixed cultures.

Objective 2: Demonstrate the utility of SIP for identification of acetate utilizers in sterilized, homogenized soil microcosms with defined culture inocula (NOTE: this objective was omitted as it was deemed redundant with objective 3 and was determined to be unnecessary for completion of the project. Time saved by this omission was used to further pursue Objective 3).

Objective 3: Use SIP to identify the acetate-utilizing community in natural, homogenized soil microcosms under varying wetting regimes.

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Approach and Procedures

Soil Parameters

Soil parameters (pH, water-holding capacity, water content, total organic content, soil texture) and soil and air temperatures were measured by standard methods (Bartels 1996). Soil acetate concentration was determined in an aqueous extract by ion chromatography.

Acetate Turnover

This experiment was designed to determine the optimal acetate concentration and incubation time to allow complete acetate utilization. Samples were incubated either "aerobically" (room air headspace) or "anaerobically" (sparged with N_2 gas prior to incubation). ^{14}C-labelled acetate in distilled water was used to make soil slurries. Respired acetate ($^{14}CO_2$) and acetate remaining in the liquid phase were determined by liquid scintillation chromatography.

Pure Culture SIP

Our initial studies focused on two organisms: *Paracoccus denitrificans*, an acetate-utilizing facultative denitrifyer, and *Shewanella oneidensis* MR-1, a facultative denitrifyer unable to utilize acetate (Balows et al. 1992). Cells were grown in minimal medium in the presence of ^{13}C-acetate. Negative controls were the same cells grown in the absence of ^{13}C-acetate. Cells were grown separately and together in the same culture. Labeled DNA was separated from unlabeled DNA by density gradient ultracentrifugation in a CsCl-EtBr gradient as previously described (Radajewski et al. 2000).

Soil Community Dynamics

Soil slurries containing unlabeled acetate or water only were incubated under aerobic or anaerobic conditions (see acetate turnover, above) for varying times to examine overall changes in the microbial community by 16S rRNA DGGE analysis (Muyzer et al. 1993).

Soil SIP

Soil slurries containing ^{13}C-acetate were incubated under conditions identical to those in the soil community dynamics section. Extracted DNA was subjected to SIP (Radajewski et al. 2000) and the ^{13}C-labeled and unlabeled components were examined by 16S rRNA PCR-DGGE analysis (Muyzer et al. 1993) to determine the component of the community that utilizes acetate at different times and under different oxygen tensions.

Results

SIP With Pure Cultures

Our first goal was to demonstrate that SIP is able to separate DNA from *P. denitrificans* cells grown with ^{13}C-labeled acetate from those grown with either unlabeled acetate or glucose. DNA from *P. denitrificans* grown in media containing 10 mM ^{13}C-acetate as the sole carbon source

migrated to a position approximately 1 cm below DNA from *P. denitrificans* cells grown with 10 mM unlabeled acetate (*fig. 1 a-c*). This distance is sufficient to allow efficient extraction of the labeled band away from the unlabeled band.

When *S. oneidensis* (non-acetate utilizing) and *P. denitrificans* (acetate utilizing) cells were grown together in media containing 5 mM ^{13}C-acetate and 0.5 mM glucose as carbon sources, two bands were observed in the CsCl-EtBr gradient (*fig 1d*). The upper band migrated to a position equivalent to that of unlabeled DNA, and the lower band migrated to a position slightly higher than the fully labeled band. It is likely that the decreased separation of labeled from unlabeled DNA is due to incomplete labeling of *P. denitrificans* DNA, as these cells can utilize both glucose and acetate, leading to partial labeling of genomic DNA based upon the amount of each substrate utilized. DGGE analysis showed only *P. denitrificans* DNA in the heavy, labeled band, whereas the light, unlabeled band showed both *P. denitrificans* and *S. oneidensis* DNA (data not shown), thereby demonstrating that only the cells that utilize acetate are labeled.

Soil Characteristics

Three soils with different physical and chemical characteristics were chosen for characterization of acetate utilization and determination of the identity of acetate utilizers. Field 1 and Field 2 are poorly drained, high clay content soils from the Imperial Valley Field Station and the rice paddy soil is from a Korean rice paddy. Fields 1 and 2 have similar pH, but other characteristics are quite different (Table 1). Soil samples were taken in May 2003, and soils were air dried, sieved through a 2-mm screen, and fully homogenized. Samples were stored at 4°C until used for various experiments.

Table 1. *Soil characteristics in rice paddy soil and irrigated soils (F1 and F2).*

	Rice paddy	Field 1	Field 2
Atm. Temp (°C)	21	36	37
Soil temp (°C)	18	26	26
pH	5.61	8.11	7.98
Water holding capacity (% volume)	26.2	30.2 ± 1.3	38.1 ± 1.4
Water content (%)	17.24 ± 0.40	10.82 ± 0.78	21.25 ± 2.01
Total organic carbon (%)	2.23 ± 0.19	1.85 ± 0.16	2.03 ± 0.15
Acetate (mM)	1.24	1.22	1.42
Texture	Sandy loam	Silty clay loam	Silty clay

Acetate Turnover

To determine that soils were incorporating acetate carbon into bacterial biomass, the optimal acetate concentration, and the optimal water content, soils were incubated aerobically (*fig. 2a*) or anaerobically (*fig. 2b*) at several different acetate concentrations.

Under aerobic conditions, we observed the same uptake rate in both Field 1 and Field 2 (*fig. 2a*). After 72 hours, approximately 8%, 25%, and 92% of the added ^{14}C remained in the aqueous phase in the 1 mM, 10 mM, and 100 mM acetate additions, respectively. Turnover was only observed in non-sterile soils. There are at least three places that the label could have gone: 1) microbial biomass, 2) CO_2 (product of respiration), or 3) adsorption on soil particles. Adsorption appears to be a minor component, as we were able to recover >98% of acetate added to sterile soils by our extraction method (data not shown). Therefore, we can determine how much acetate was incorporated into biomass:

Eq 1: [total added − (remaining + respired)] × % yield = incorporated

Based on these calculations, we determined that 1 mM acetate is likely to be insufficient to fully label cells, and 100 mM acetate is far in excess of what the microbes are capable of utilizing. Therefore, 10-20 mM acetate is the optimal concentration for acetate utilization by these soils.

Under anaerobic conditions, increasing water content led to earlier acetate turnover in all three soils (*fig. 2b*). Nevertheless, between 50% and >95% of acetate was consumed within three days in all soils, regardless of the water content. Therefore, water content does not appear to be a critical factor in acetate turnover.

After two weeks under anaerobic conditions, soil color changes were observed (*fig. 3*). Rice paddy soil was changed from a yellowish orange color to black, and field 1 and 2 soils also appeared to darken, although to a lesser extent than the rice paddy soil. This color change is likely the result of the reaction of H_2S (produced from sulfate by sulfate-reducing bacteria under anaerobic conditions) with iron and other metals in the soil, as a hydrogen sulfide odor was observed when the vials were opened. This color change also accompanied other chemical changes in the soils. Throughout the incubation, Field 1 and 2 soil samples maintained a slightly alkaline pH with only a gradual decrease over the course of the experiment to no lower than pH 7.4 (*table 2*). However, the rice paddy soil started at an acidic pH (5.3) which then increased to pH 7.2 for the first two weeks of incubation, after which time the pH began to decrease (*table 2*). Methane production was also observed in the rice paddy soil and, to a lesser extent, in Field 1 samples (*table 3*). No methane production was observed in Field 2 samples during the course of this incubation. Methane was detectable after 4 and 15 days incubation in acetate-added rice paddy and field 1 soil, respectively. No methane production was observed in soil samples incubated without acetate.

Table 2. pH variation during anaerobic incubation.

	Rice paddy		Field 1		Field 2	
	0 mM Acetate	20 mM acetate	0 mM Acetate	20 mM acetate	0 mM Acetate	20 mM acetate
Day 0	5.48	5.30	7.75	7.57	8.03	7.95
Day 2	5.49	5.31	7.77	7.58	7.95	7.95
Day 4	5.34	5.27	7.63	7.60	7.67	7.82
Day 8	5.38	5.39	7.33	7.26	7.51	7.39
Day 15	5.33	7.20	7.43	7.92	7.47	7.98
Day 20	5.31	6.85	7.43	7.40	7.48	7.50

Table 3. *Methane‡ production during anaerobic incubation.*

	Rice paddy		Field 1		Field 2	
	0 mM Acetate	20mM acetate	0 mM Acetate	20mM acetate	0 mM Acetate	20mM acetate
Day 0	-*	-	-	-	-	-
Day 2	-	-	-	-	-	-
Day 4	-	0.021%	-	-	-	-
Day 8	-	0.040%	-	-	-	-
Day 15	-	4.533%	-	0.017%	-	-
Day 20	-	5.158%	-	0.097%	-	-

‡ Methane concentration was determined by GC/TCD
* (-): below detection limit

DGGE of Whole Soil Communities

To determine the effect of water addition and acetate addition on the overall microbial community, we performed 16S rRNA PCR-DGGE on DNA extracted from soil samples incubated (either aerobically or anaerobically) either dry, with water added (1:1 vol:vol), or with varying amounts of acetate added (fig. 4).

Under aerobic conditions (*fig. 4a*), addition of water appeared to cause a significant shift in community composition in both Field 1 and Field 2 soils (compare F1 and F2 soil to F1 and F2 water, respectively). Addition of acetate, on the other hand, appeared to have less of an effect on the composition of the microbial community. Field 2 showed almost no detectable differences in DGGE pattern with the addition of either 1- mM or 100 mM acetate, although several new bands

do appear in Field 1 samples with the addition of the same acetate concentrations. It is important to note here that incubations were only for 48 h – probably insufficient times for the community to shift significantly. We are currently in the process of analyzing longer incubations under aerobic conditions.

Under anaerobic conditions, water addition had significant effects on microbial community structure (fig. 4b). Acetate addition also had significant effects on DGGE patterns in all three samples, although to different extents and requiring different times to observe the effect. In rice paddy soil, no clear effect was observable until day 8 of the incubation, whereas in Field 1 effects were detectable on day 1 and in Field 2 effects were detectable by day 4.

SIP of Intact Soil Communities

The changes in microbial community composition observed by DGGE of whole soil communities could be a direct result of increases in abundance of organisms that utilize acetate or could be due to indirect effects, such as changes in bulk geochemical parameters or downstream food web effects. To clarify whether the changes are primarily in acetate-utilizing components of the community or other community members, it is necessary to use a method that links function to identity, such as SIP. Therefore, we used SIP to determine the identity of the acetate-utilizing organisms in whole soil samples.

Isopycnic centrifugation of DNA extracted from soils incubated with ^{13}C-acetate allowed separation of ^{13}C-DNA, derived from acetate-utilizing organisms, from ^{12}C-DNA, derived from all other community members (*fig. 5*). 16S rRNA PCR-DGGE of ^{13}C-labeled DNA (*fig. 6*) showed significant differences compared to either the whole community DNA (*fig. 4*) or ^{12}C-labeled DNA (*fig. 6*) under both aerobic (*figs. 4a and 6a*) and anaerobic (*figs. 4b and 6b*) conditions, with enrichment in some bands and reduction in others. Aerobic samples were incubated for 8 days, while anaerobic samples were incubated for 15 days. These results indicate that the acetate-utilizing community is a component of the *in situ* community and is not due to the introduction of new community components.

One major advantage of the 16S rRNA PCR-DGGE method is that the bands can be cut from the gel and sequenced and the resulting sequence can be compared to public databases to determine the most closely related organism (i.e., the "identity") to the DNA. Table 4 reports the results of such sequencing of bands for aerobic conditions, and table 5 reports these results for anaerobic conditions (band numbers correspond to the numbers in *fig. 6*). Under aerobic conditions, *Bacillus* spp. and relatives are primarily detected by this approach. Cultured members of this group are obligately aerobic, heterotrophic, spore forming organisms (Balows et al. 1992). It may be this ability to form spores, which led to their enrichment in these samples, as the soils were stored (aerobically) dry for several months prior to this experiment; therefore, organisms capable of long-term survival without metabolic activity may have a competitive advantage in this situation.

Under anaerobic conditions, a more complex picture emerges. In all three soils, relatives of *Anaeromyxobacter* spp. are among the dominant members of the acetate-utilizing consortium (*table 5*). Previously cultivated members of this group have been found in rice soils and metal contaminated sediments, and are capable of iron (III) reduction (Petrie et al. 2003; Treude et al. 2003). Gene sequences related to this group have also been found in metal contaminated

sediments (Petrie et al. 2003). In all three soils, the other dominant members of the acetate-utilizing community are related to known sulfate-reducing bacteria. It is intriguing that both iron-and sulfate-reducing organisms apparently utilize acetate simultaneously, as a textbook thermodynamic approach would predict that sulfate reduction would not begin until iron reduction was complete, as more energy is available by oxidation of acetate with iron (III) than is available by oxidation with sulfate (Madigan et al. 1997). Based on our results, it is apparent that these two processes can and do occur simultaneously in anaerobic soils, and this process appears to be widespread (present in soils from a Korean rice paddy and two Southern California soils).

The presence of other gene sequences is more enigmatic. For example, despite the fact that acetate is a non-fermentable carbon source, a gene related to an obligately fermentative bacterium, *Clostridium quercicolum* (Balows et al. 1992), is present in the ^{13}C-labeled DNA of the rice paddy. It is currently unclear whether this sequence is a result of contamination from the ^{12}C band, is a result of cross feeding (i.e., excretion of ^{13}C labeled biomass by acetate-utilizing cells which is then utilized by non-acetate utilizing members of the soil community), or some other possibility. Other gene sequences, e.g., Bands F1-1, F1-2, F1-3, and F1-5 are not related to organisms that are known acetate utilizers, but are related to organisms found in other soils. It is again unclear whether these are true acetate utilizers or are present due to contamination or cross feeding.

Table 4. *Identity of DGGE bands from soil samples incubated with acetate under aerobic conditions (see figure 6).*

DGGE band	Nearest neighbor	Identity (%)	Assemblage
1	soil bacterium clone 107 (AY493974)	118/138 (85%)	
	Symbiobacterium sp. HN9 (AB052396)	105/124 (84%)	Actinobacteria
2	*Bacillus* sp. MB-11 (AF326360)	194/198 (97%)	Firmicutes
3	*Bacillus sphaericus* strain 205y (AF435435)	196/196 (100%)	Firmicutes
4	?		
5	Bacterium K2-24 (AY345429)	194/198 (97%)	
6	*Bacillus niacini* strain SAFN-019 (AY167811)	192/198 (96%)	Firmicutes
7	clone mb2430 (Z95735)	192/197 (97%)	
	Acidobacterium clone glen99_23 (AY150871)	187/197 (94%)	Acidobacteria
8	Actinobacterium clone uvi12 from soil aggregate (AY186841)	176/176 (100%)	Actinobacteria

? = no sequence obtained

Table 5: *Identity of ^{13}C-labeled DGGE bands from anaerobic incubation (see fig. 6).*

DGGE band	Nearest neighbor	Identity (%)	Assemblage
Rice 1	*Clostridium quercicolum* 16S rRNA gene, strain DSM 1736(T) (AJ010962)	121/138 (87%)	Firmicutes
Rice 2	*Desulfitobacterium* sp. 16S rRNA gene (X81032)	129/138 (93%)	Firmicutes
Rice 3	*Anaeromyxobacter* sp. FAc12 (AJ504438)	133/138 (96%)	Delta proteobacteria
Rice 4	Uncultured cf. *Anaeromyxobacter* sp. (AJ504436)	144/157 (91%),	Delta proteobacteria
Rice 5	Uncultured cf. *Anaeromyxobacter* sp. (AJ504432)	134/139 (96%)	Delta proteobacteria
F1-1	clone PI_r173_c2 (AY374759)	46/49 (93%)	Delta proteobacteria
F1-2	clone R10 (AF407686)	46/48 (95%)	
F1-3	clone FW35 (AF523970)	124/153 (81%)	
F1-4	delta proteobacterium clone (AF503916)	46/47 (97%)	Delta-proteobacteria
F1-5	Perchlorate-reducing bacterium PMC 16S (AY265878)	73/81 (90%)	Beta-proteobacteria
F1-6	Uncultured cf. Anaeromyxobacter sp. (AJ504436)	108/131 (82%)	Delta-proteobacteria
F1-7	Uncultured cf. Anaeromyxobacter sp. (AJ504436)	128/138 (92%)	Delta-proteobacteria
F1-8	Uncultured cf. Anaeromyxobacter sp. (AJ504432)	139/141 (98%)	Delta-proteobacteria
F2-1	Desulfobacteraceae bacterium MSL53 (AB110538)	143/153 (93%)	Delta-proteobacteria
F2-2	Uncultured bacterium clone A144 (AY373403) Desulfobacteraceae	119/143 (83%)	Delta-proteobacteira
F2-3	Uncultured soil bacterium clone DS-33 (AY289481)	50/54 (92%)	Delta-proteobacteria
F2-4	Uncultured cf. Anaeromyxobacter sp. (AJ504436)	148/157 (94%)	Delta-proteobacteria
F2-5	Uncultured cf. Anaeromyxobacter sp. (AJ504432)	152/157 (96%)	Delta-proteobacteria

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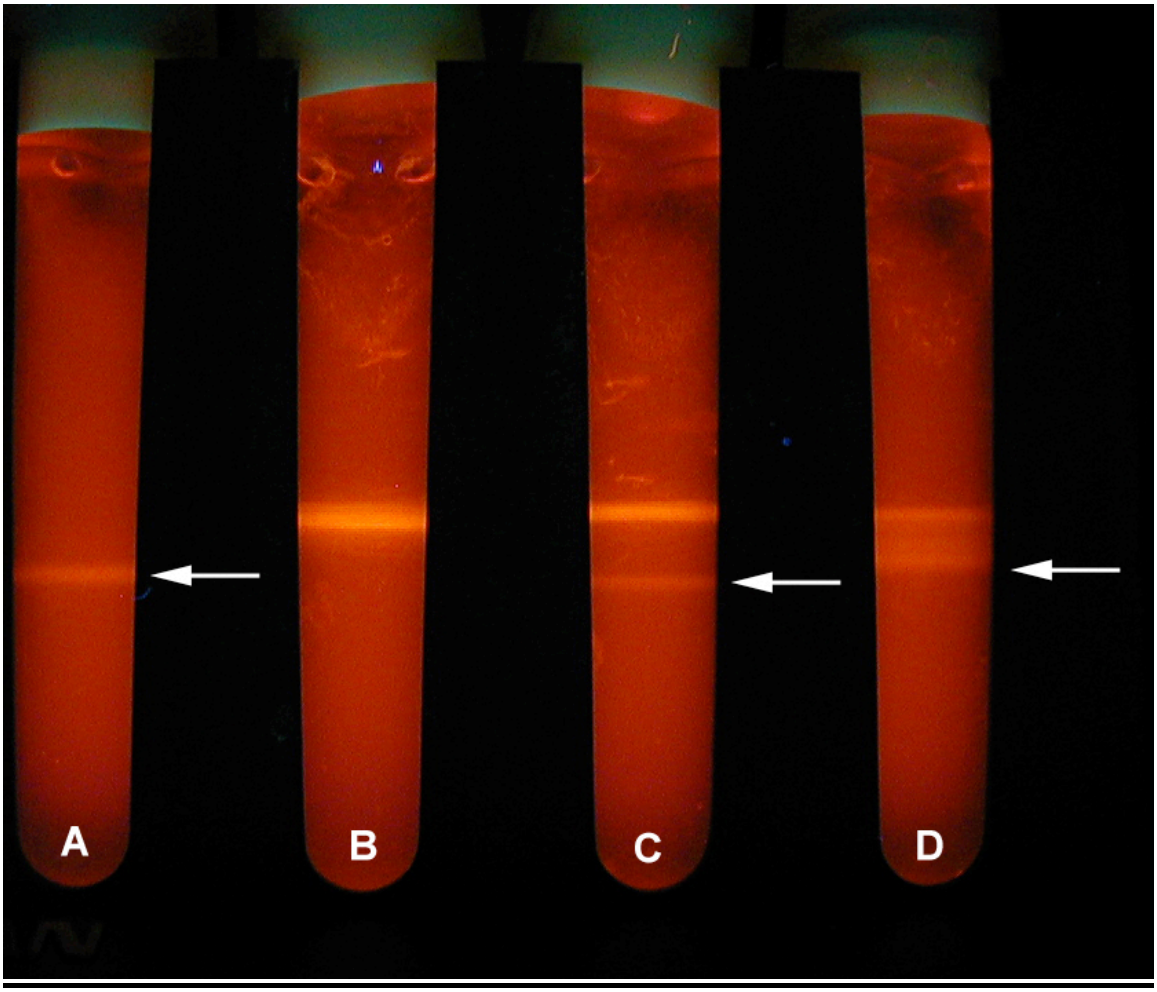

Figure 1. Stable isotope probing of pure and mixed cultures with ^{13}C-acetate. (A) DNA from *Paracoccus denitrificans* grown in minimal medium containing ^{13}C-acetate as the sole carbon source. The approximately 1 cm arrow marks the labeled DNA band. (B) DNA from *P. denitrificans* grown in minimal medium containing unlabeled acetate as the sole carbon source. (C) DNA from ^{13}C-labeled and unlabeled *P. denitrificans* cells mixed after extraction. The approximately 1 cm arrow marks the labeled DNA band. (D) DNA from *P. denitrificans* and *S. oneidensis* grown in minimal medium containing ^{13}C-acetate and unlabeled glucose as carbon sources. The approximately 1 cm arrow marks the labeled DNA band.

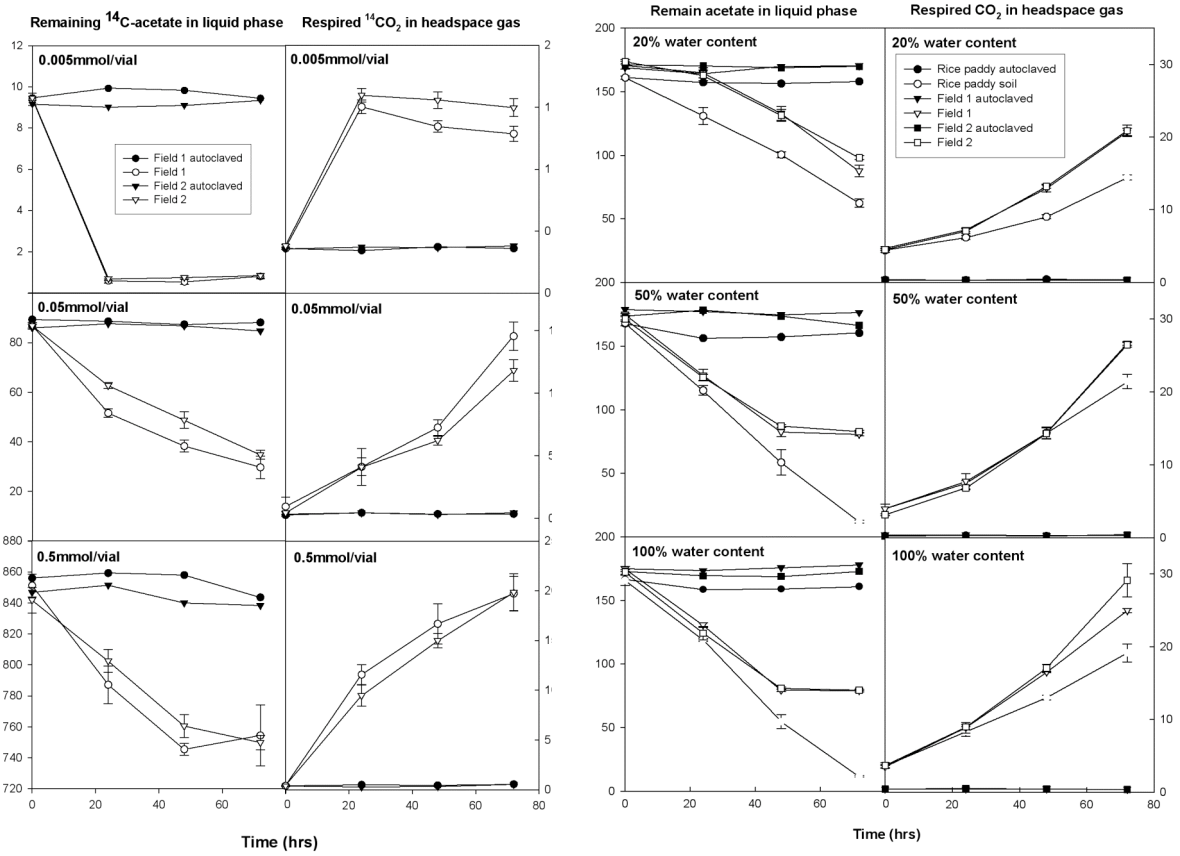

Figure 2 A&B. Loss of acetate and production of CO_2 in soil-acetate slurries incubated for the indicated times under (A) "aerobic" and (B) "anaerobic" conditions. Incubations were performed as described in the Approach and Procedures section of this report. 0.005 mmol vial^{-1} is equivalent to 1 mM. All anaerobic samples were incubated with 20 mM acetate.

Discussion

In order to understand the mechanisms and processes that govern the storage and flow of carbon in the soils that support California's diverse ecosystems, it is necessary to understand not only the processes themselves, but the communities which underlie those processes. Acetate is central to carbon transformations in anaerobic soils, as it is an end product of fermentation of more complex organic material, a product of acetogenesis from H_2 and CO_2, and a carbon and energy source for a number of aerobic and anaerobic metabolisms (Stams 1994; Jeyaseelan 1997). The bacterial and archaeal species that are responsible for acetate catabolism are unknown.

Part of the reason for this lack of understanding regarding who the main players are in acetate utilization, or indeed any process in environmental systems, is our lack of information about the diversity of microorganisms coupled with the lack of ability to link what is known about diversity to function. It has been estimated that we can culture less than 1% of bacteria and archaea from environmental samples (Staley and Konopka 1985), making it difficult to

determine the relevance of cultured organisms to the activities directly measured in the environment. Over the last 10 to 15 years, non-culture based techniques, especially those based on the small-subunit ribosomal RNA (16S rRNA) gene, have provided us with significant insights into the phylogenetic diversity and ecology of microorganisms (Amann et al. 1995; Head et al. 1998). However, it is not often possible to directly link phylogeny with function due to the complex and broad phylogenetic distribution of physiological traits within microbes (Pace 1997).

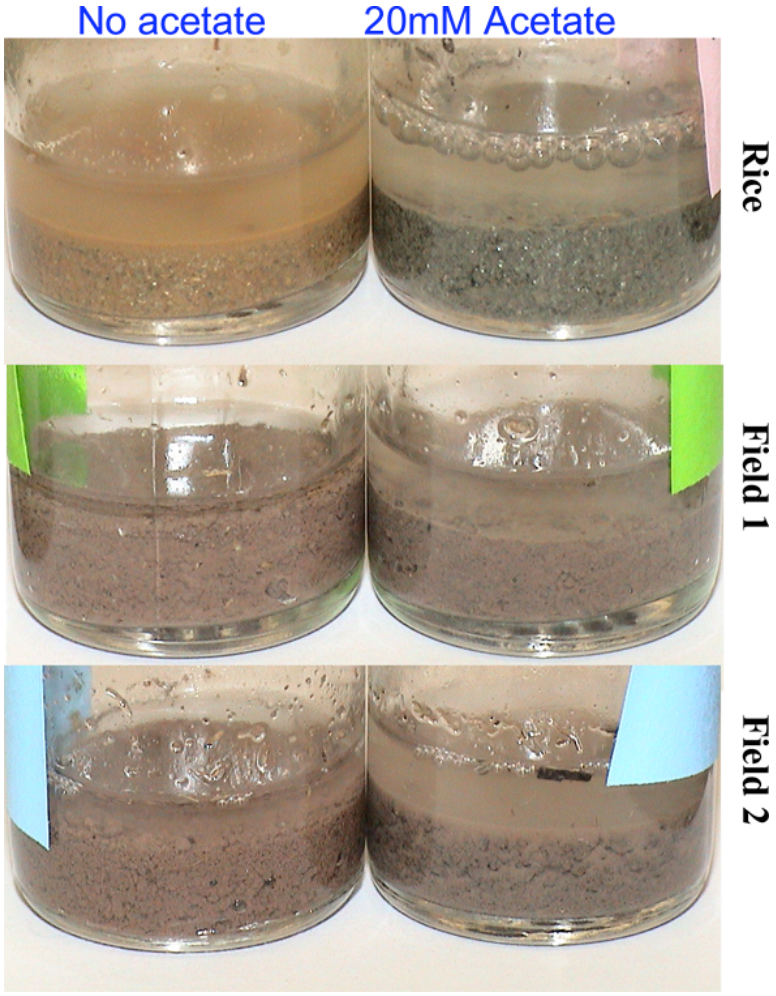

Figure 3. Soil color changes after two weeks "anaerobic" incubation.

Several methods have attempted to compensate for this deficiency by combining a molecular fingerprinting approach (such as 16S rRNA methods or phospholipid analysis) with isotope labeling methods (Hinrichs et al. 1999; Lee et al. 1999; Ouverney and Fuhrman 1999; Roslev and Iversen 1999; Cottrell and Kirchman 2000; Murrell and Radajewski 2000; Radajewski et al. 2000). Isotopes (either stable or radioactive) of carbon, nitrogen, sulfur, phosphorous, and other elements can be incorporated into compounds that are then consumed by the segment of the microbial population involved in turnover of that substrate, thereby labeling components of those cells (such as DNA or fatty acids). Labeled cells or their components are then separated and identified using one of a variety of approaches. The combination of molecular fingerprinting and

isotope labeling methodologies allows for the phylogenetic characterization of organisms that consume a particular substrate and are therefore involved in a process of interest.

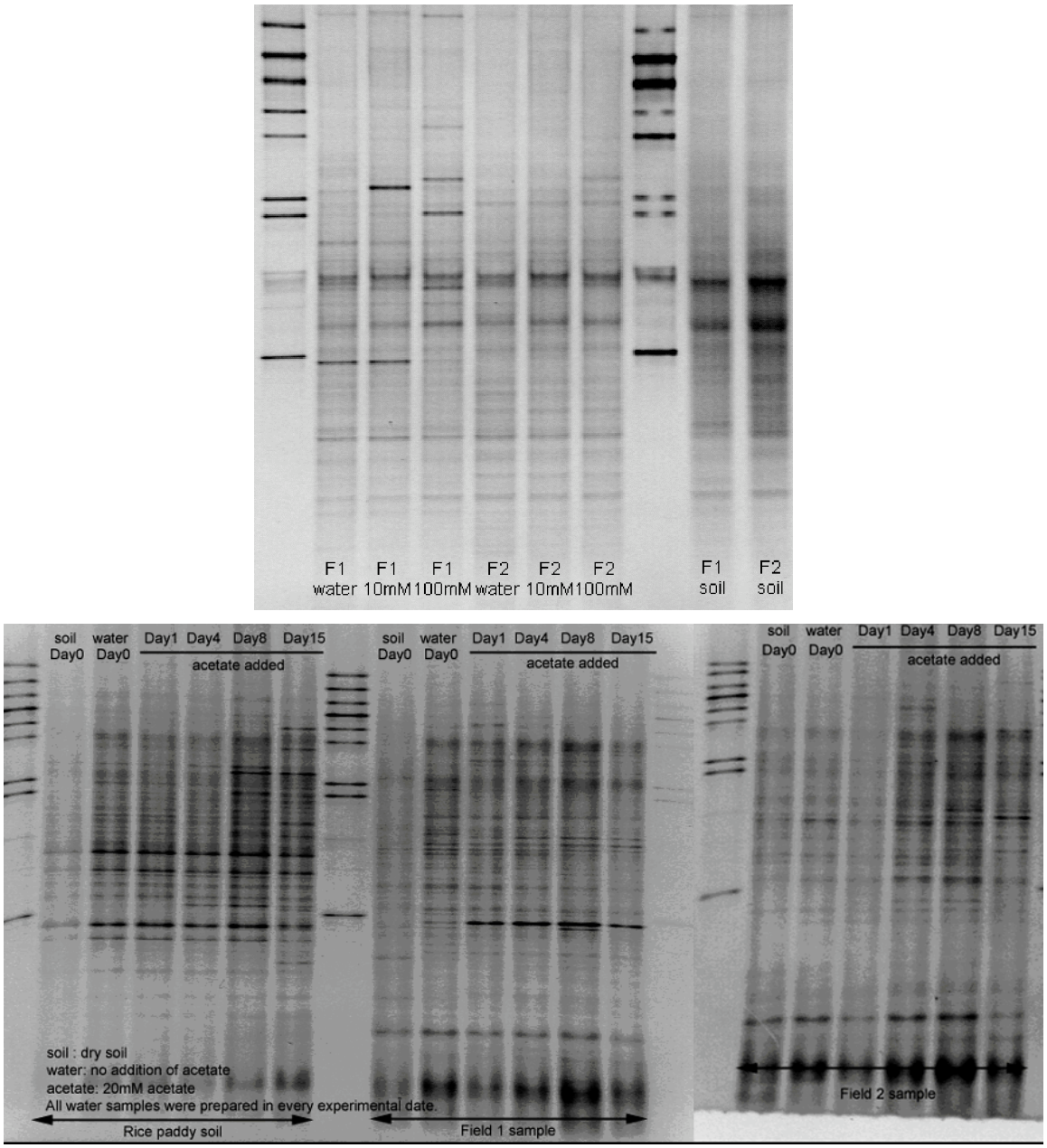

Figure 4. Affect of water only or water + acetate on the microbial communities in soil samples. DGGE was carried out as described in Approach and Procedures. A (top). Fields 1 (F1) and 2 (F2) incubated dry (soil), with water addition only (water), or with the indicated concentration of acetate under "aerobic" conditions. B (bottom). Rice paddy soil, field 1, and field 2 samples, samples incubated dry (soil), with water addition only (water) or with acetate (acetate added) under "anaerobic" conditions for the indicated times.

We have been utilizing a SIP, an approach that combines molecular microbiology and stable isotope labeling techniques (Radajewski et al. 2000; Whitby et al. 2001; Manefield et al. 2002; Morris et al. 2002). To date, SIP has only been used for substrates that are utilized by a small fraction of the microbial community, e.g., methane, carbon dioxide, or phenol. Because acetate is a compound that is central to the functioning of soil microbial communities, it most likely has a more complex cycling and to be utilized by a larger fraction of the total community than previously studied compounds.

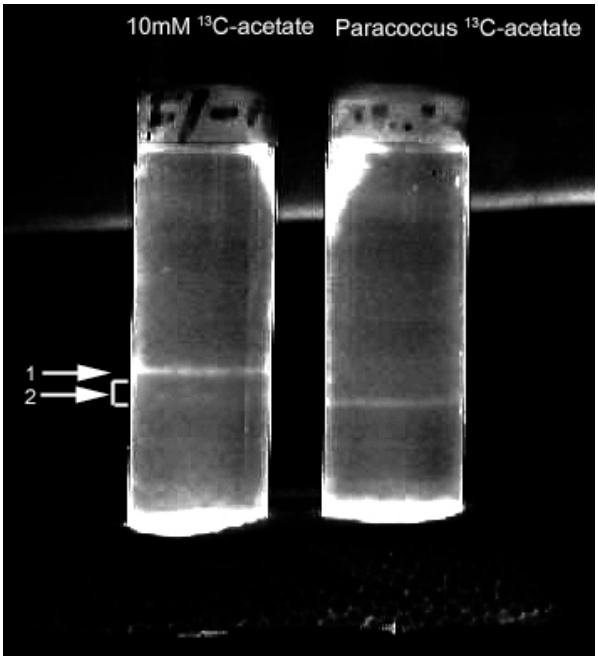

Figure 5: Isopycnic centrifugation of DNA extracted from soil incubated with ^{13}C-acetate and Paracoccus ^{13}C-labeled DNA (*see fig. 1*).

Methane is a potent greenhouse gas, and about 80-90% of atmospheric methane is a product of biological methanogenic processes (Cicerone and Oremland 1988; Heyer 1990), with about 20% of atmospheric methane arising from soils (Conrad 1996). Acetate is an important substrate for methanogenesis: about two-thirds of the methane produced in nature originates from acetate (i.e., acetoclastic methanogenesis), with the other one-third originating from the reduction of CO_2 at the expense of H_2 or formate (Ferry 1992). It is known that only methanogenic archaea (MA) are capable of producing methane biologically, and there is only one order of MA that are known to carry out methanogenesis from acetate: the Methanosarcinales (Ferry 1992). However, whether the Methanosarcinales are truly the only MA capable of acetoclastic methanogenesis is unclear, and the individual species responsible for this activity in a particular soil have never been determined. Because *Methanosaeta* spp. utilize a different pathway for acetoclastic methanogenesis than *Methanosarcina* spp., it is critical to determine which genus and species dominate in individual systems to understand the role of any particular system in methane production. We are currently completing studies of the Archaeal component of the acetate-utilizing community, and have preliminary indications that acetoclastic methanogens are utilizing acetate in direct competition with iron (III)- and sulfate-reducing bacteria under anaerobic conditions (data not shown). Therefore, this project has provided insight into the role of California soils in production of the greenhouse gas, methane.

We have also provided data that addresses another Kearney Foundation Mission Goal: quantification of impacts of anthropogenic inputs of water on transformations and transport of carbon in soils. Anaerobic processes in soils tend to primarily occur when the soil is flooded, as occurs during irrigation of agricultural soils. We obtained data on the impact of watering on the composition of the acetate-utilizing component of the soil microbial community. Since acetate is central to soil organic carbon transformation, information on how watering practices affect the

acetate-utilizers in the soil will, in turn, provide information on soil organic carbon transformation.

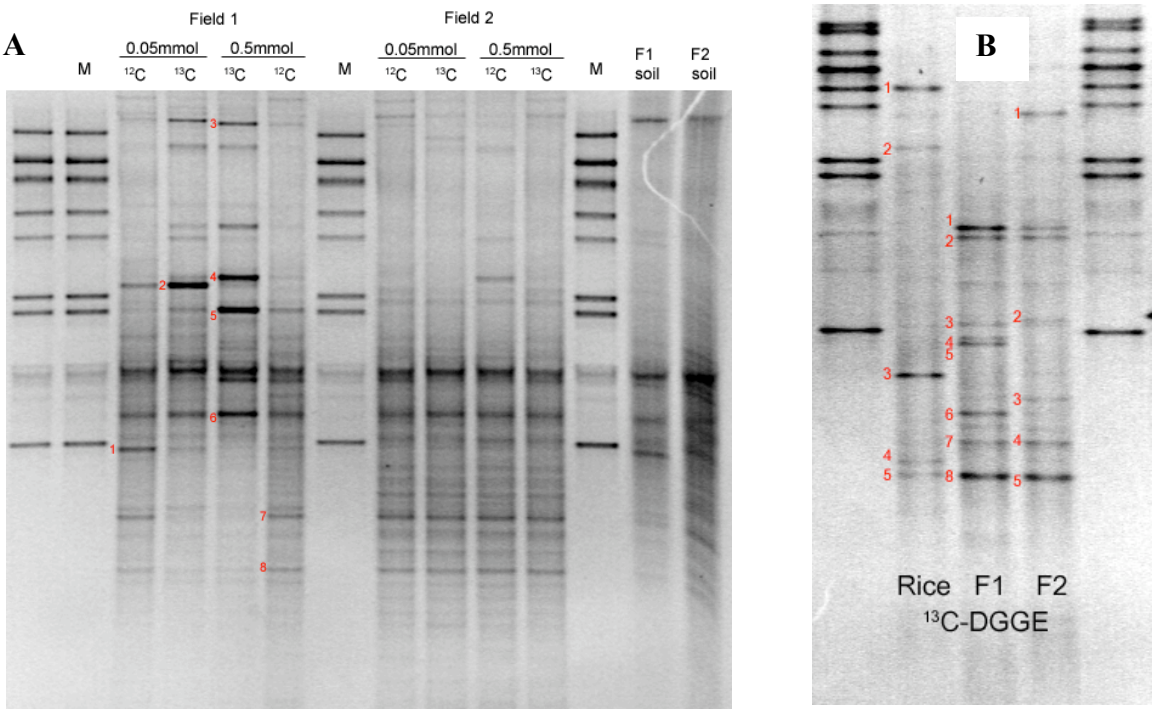

Figure 6. DGGE of light and heavy (A) or heavy (B) labeled bands from SIP of samples incubated with acetate under aerobic (A) or anaerobic (B) conditions. Numbered bands were excised and sequenced (*see tables 4 and 5*).

Our results have demonstrated that the SIP approach is capable of detecting the acetate-utilizing component of the microbial community and that a strict division between utilization of specific electron acceptors does not appear to occur at the microbial level—these organisms compete with each other for acetate even when one electron acceptor provides greater energy availability.

This project is not yet complete. We plan to continue our work by examining the dynamics of the acetate-utilizing community over time, i.e., does the acetate-utilizing community change with varying incubation lengths. This approach will allow us to eliminate contamination or cross feeding as explanations for the presence of particular bands as a) there is a low likelihood of continued contamination of the lower band by exactly the same gene sequences at all time points, thereby reducing the chance that a single gene sequence is due to contamination and b) if cross-feeding is occurring, it should be decreased by shorter incubation periods. Furthermore, we will be able to study food web dynamics using this approach to see how a substrate moves through the microbial food web (Lueders et al. 2004b). We also plan to improve the sensitivity of the SIP method by incorporating new developments in the field (Lueders et al. 2004a)

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This research was funded by the Kearney Foundation of Soil Science: Soil Carbon and California's Terrestrial Ecosystems, 2001-2006 Mission (http://kearney.ucdavis.edu). The Kearney Foundation is an endowed research program created to encourage and support research in the fields of soil, plant nutrition, and water science within the Division of Agriculture and Natural Resources of the University of California.