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Biodegradation of Clomazone in a California Rice Field Soil: Carbon Allocation and Community Effects

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ABSTRACT: Degradation pathways for the herbicide clomazone in a California rice field soil were characterized via pulse-labeling of anaerobic (flooded) and aerobic (moist) soil microcosms. Clomazone-derived ^{13}C in the major C pools of a rice ecosystem and soil phospholipid fatty acid (PLFA) profiles were analyzed over time to determine if (1) the compound accumulates in the microbial biomass, (2) it affects temporal microbial population dynamics, and (3) it is either preferentially metabolized or cometabolized. In anaerobic microcosms, the compound was rapidly biotransformed to ring-open clomazone, upon which it persisted in the aqueous phase, whereas aerobic microcosms degraded it slower but a greater percentage was mineralized. Anaerobic biomass decreased after clomazone was added, and aerobic actinomycete abundance differed between treatments and controls. Additionally, PLFA and ^{13}C PLFA were statistically similar between treatment and controls. Thus, microbial cometabolism is likely to be the dominant degrading mechanism governing clomazone fate in California rice fields.

KEYWORDS: *clomazone, ^{13}C herbicide, ^{13}C PLFA, FAME, stable isotope probing, biodegradation, contaminant degradation, ^{13}C pulse label*

INTRODUCTION

Clomazone (Figure 1, trade names Cerano, Command) is a popular isoxazolidinone herbicide used on California rice fields to provide control of flood-tolerant weeds. It is also used on many other crops across the world, and is steadily gaining in popularity due to its effectiveness and low occurrence of weed resistance. The compound was registered for use in California in 2002, and its application has increased every year to over 40800 kg (a.i.) applied to over 100 000 ha in 2010.[1] The physicochemical properties indicate that the compound will partition primarily into the water column, as evidenced by its strong water solubility (1100 mg/L), weak sorption characteristics (K_f = 0.47−5.3), weak volatility (vapor pressure = 1.44×10^{-4} Torr), resistance to hydrolysis, and slow photodegradation ($t_{1/2}$ = 100−200 days).[2−4] This makes the compound highly bioavailable to soil microorganisms under both moist and flooded soil conditions.

Recent efforts have been directed at quantifying rates of clomazone microbial degradation and identifying biotransformation products under simulated California rice field conditions.[5] Under highly anaerobic flooded conditions, clomazone was shown to be reduced with a first order half-life of 7.9 days and transformed primarily via reductive ring-opening, whereas in a moist aerobic soil it was found to degrade much slower, with $t_{1/2}$ = 47 days. Flooded rice field half-lives for clomazone are reported elsewhere at 7.2 and 14.2 days for water and soil, respectively.[6] Mervosh et al. [3] noted several factors governing aerobic clomazone degradation in an Illinois soil, namely organic carbon content, temperature and moisture.

In California, rice growers must wait until applied herbicides dissipate to acceptable levels prior to field water discharge, in some cases leaving fields fallow for an extended time period if persistent. Thus, if a compound is biodegraded quickly, it will help the grower maximize the growing season. Soil microorganisms can expedite overall field dissipation by directly metabolizing and accumulating herbicides in the environment and can be a major factor in determining a compound's overall environmental fate. Microbes utilize organic substrates as terminal electron acceptors to generate energy for metabolic tasks and incorporate substrates into biomass where the compound can be metabolized, partitioned into cellular components, and eventually mineralized. This may occur through exogenous or endogenous enzyme activity.[7] In contrast to preferential metabolism, herbicides may be cometabolized incidentally in the presence of a primary growth substrate. It is unknown which of these pathways clomazone follows in California fields, and how this may differ between aerobic (moist) and anaerobic (flooded) conditions. In this study, we attempt to trace clomazone-C into microbial and microbial-derived

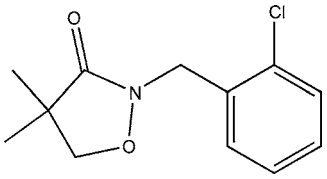

Figure 1. Structure of clomazone (2-[(2-chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone).

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pools in a typical California rice field soil to determine the timing and magnitude of metabolism and/or cometabolism

The effects of clomazone on community structure are widely unknown and may impact biodegradation efficiency. California rice field soils are typically a clay loam deficient in organic carbon;[8] thus, clomazone could serve as a potentially labile C source. If so, this would result in increased biomass of clomazone degraders. Conversely, clomazone may be toxic to microbes, as has been shown toward photosynthesizers,[9−11] and antimicrobial effects have been widely shown in other isoxazolidinone derivatives such as cycloserine.[12] If toxic, clomazone application would result in deceased biomass. In either case, it is unknown if the compound will affect temporal microbial community dynamics and what these effects will be. Phospholipid fatty acids (PLFAs) have been widely utilized to depict such changes in soil microbiota. The abundance of branched, monounsaturated, polyunsaturated, 10-methyl, and cyclic PLFAs are commonly used as biomarkers for gram positive, gram negative, fungal, actinomycete and slow growth biomass, respectively.[13−15] In this investigation, we examined PLFA and PLFA component response to clomazone addition. To determine if treatment effects are significant, PLFA data is commonly combined with nonmetric multidimensional scaling (NMS) techniques to reduce dimensionality of PLFA data set for nonlinear relationships.[16−18]

METHODS

Soil Collection. The soil was an Esquon-Neerdobe thermic clay loam from the California Rice Research Station in Biggs, CA,[8] and was characterized by the UC Agriculture & Natural Resources (ANR) Analytical Laboratory at UCD. The soil contains 0.71% organic matter and 0.41% organic carbon. Soil was sampled from 0 to 10 cm, sieved through 2-mm mesh, and stored in plastic bags at 4 °C until experiments were conducted.

Microcosms. To 125-mL serum vials, 50 g soil was added to 75 and 16 mL autoclaved water to achieve anaerobic (flooded) and aerobic (50% moisture-holding capacity) conditions, respectively. Anaerobic conditions were confirmed by resazurin indicator color in the water layer of 6 identical microcosms that were not extracted, and a pilot study that revealed E_h < 200 mV after 14 days (data not shown). The headspace was purged with N_2 gas (anaerobic) or air (aerobic). Samples were crimped shut with butyl stoppers and incubated in the dark at 30 °C for 14 d (anaerobic) or 7 d (aerobic). After incubation, 200 μg [U- ^{13}C-phenyl] 4.74 atom percent clomazone (the regulatory application rate) was added in 100 μL water; controls received only water. Samples were incubated at 30 °C, typical of summertime high temperatures in the California Central Valley. Samples (N = 3 for each soil type and treament) were taken at regular intervals over 90 d (anaerobic) or 140 d (aerobic). Anaerobic extraction intervals were shorter due to an expectedly faster degradation rate.

Sample Extraction and Analysis. *Headspace Fraction: CO_2 and CH_4.* On extraction day, two 10-mL aliquots of headspace were withdrawn from each anaerobic sample, placed into two evacuated 12-mL exetainers (Labco Ltd., High Wycombe, UK), and analyzed for CO_2 and CH_4−δ^{13}C using a Sercon Cryoprep system interfaced to an isotope ratio mass spectrometer (IRMS; Sercon Ltd., Chesire, UK). Aerobic headspace samples had one 10-mL aliquot removed, and were analyzed for only CO_2. Samples not extracted that day were purged with N_2 gas (anaerobic) or air (aerobic) and returned to the incubator.

Aqueous Fraction: DOC, Clomazone, and Metabolites. For anaerobic samples, the water layer was extracted by directly filtering the microcosm's aqueous phase through a 0.2-μm acrodisk filter (Nalgene USA). For aerobic samples, pore water was extracted by adding 50 mL water to 10 g soil, shaking for 1 h, and filtering as above. A subsample of the filtered extracts (30 mL) was stored at 4 °C and analyzed for DOC δ^{13}C using an O.I. Analytical Model 1010 TOC Analyzer (OI Analytical, College Station, TX) interfaced to a PDZ Europa 20−20 IRMS (Sercon Ltd., Cheshire, UK); the remaining extracts were stored frozen (−20 °C). Upon thawing, a 1-mL aliquot was amended with internal standard (5-hydroxyclomazone), and analyzed for clomazone and metabolites via LC/MS using a HP1100 HPLC coupled to an Applied Biosystems API 2000 triple quadrupole tandem mass spectrometer (Applied Biosystems, Foster City, CA), as outlined in Tomco et al.[5]

Soil Fraction: Microbial Biomass, Whole Soil and PLFA. On extraction day, soil was removed from microcosms with a spatula then freeze-dried and stored at −80 °C prior to extraction/analysis. Subsamples were analyzed for soil δ^{13}C and soil PLFA δ^{13}C following the procedures of Bossio, et al.[14] Briefly, 8 g soil was extracted with two 23-mL additions of 2:1:0.8 methanol/chloroform/phosphate buffer. Samples were centrifuged for 15 min at 1500 rpm and placed into a 125-mL separatory funnel containing 50 mL of 1:1 chloroform/phosphate buffer. Funnels were shaken for 2 min and allowed to sit overnight. The organic layer was collected and evaporated to dryness under N_2 gas at 37 °C. Chloroform (1 mL) was added, and the phospholipids fractionated via solid-phase extraction. The column was conditioned with 3 mL chloroform, the sample loaded, then 3 mL chloroform followed by 6 mL acetone passed through the column prior to elution with 3 mL methanol. The eluate was evaporated to dryness, and samples transesterified via mild alkaline hydrolysis with 1:1 KOH (1 M, in methanol)/toluene. Samples were incubated at 37 °C for 15 min, quenched with an equal portion of acetic acid, and extracted with two 3-mL additions of hexane. The extract was evaporated to dryness, and samples were transferred to autosampler vials with 150 μL hexane amended with C19:0 internal standard. Samples were stored at −80 °C and analyzed for fatty acid methyl esters (FAME) δ^{13}C using a Thermo GC/C-IRMS system composed of a Trace GC Ultra gas chromatograph (Thermo Electron Corp., Milan, Italy) coupled to a Delta V Advantage isotope ratio mass spectrometer through a GC/C−III interface (Thermo Electron Corp., Bremen, Germany). We chose to analyze phospholipids instead of total soil FAME because PLFA is specific to viable (living) microbes.[19]

Calculations. *Mass Balance.* δ^{13}C (VPDB) was converted to atom percent, and the amount of clomazone-derived carbon calculated as the difference between enriched and control samples:

$$(\mu g\ \text{clomazone}^{13}C)_f = (\mu g\ C_f)\left(\frac{(\text{Atom}\%)_{f,\text{enriched}} - (\text{Atom}\%)_{f,\text{control}}}{100}\right) \quad (1)$$

with C_f being the carbon abundance in fraction f. Clomazone incorporation into each fraction was expressed as a percent of initial application amount:

$$\%\text{clomazone C} = 100 \times \frac{(\mu g\ \text{clomazone}^{13}C)_f}{(\mu g\ ^{13}C\ \text{added})} \quad (2)$$

To minimize effects of measurement variation on calculations, the means of each control pool were used as estimates for background atom %.

Statistical Analysis. Statistical analysis of PLFA data was performed using JMP 8.0 (SAS Institute). Following normalization (natural log), PLFA concentration and ^{13}C enrichment differences between treatments and controls and temporal effects were analyzed via 2-way ANOVAs. PLFA component abundance is expressed as a percentage of total PLFA abundance, and ^{13}C abundance is expressed as a percentage of the initial ^{13}C added. Nonmetric multidimensional scaling (NMS) analysis was performed in R (version 2.11.1) using the cmdscale command. Significance is defined at α = 0.05

RESULTS

Temporal Dynamics of Clomazone-C in Flooded (anaerobic) Soil. Under anaerobic conditions (Figure 2A), clomazone-C was found predominantly in the dissolved phase, and degraded quickly to the metabolite ring-open clomazone (RO). Clomazone-C in DOC decreased slightly over 90 days, coinciding with the formation of RO. At day 28, 54% of applied clomazone was transformed to RO, which did not degrade further. The sum of the clomazone + RO concentrations are

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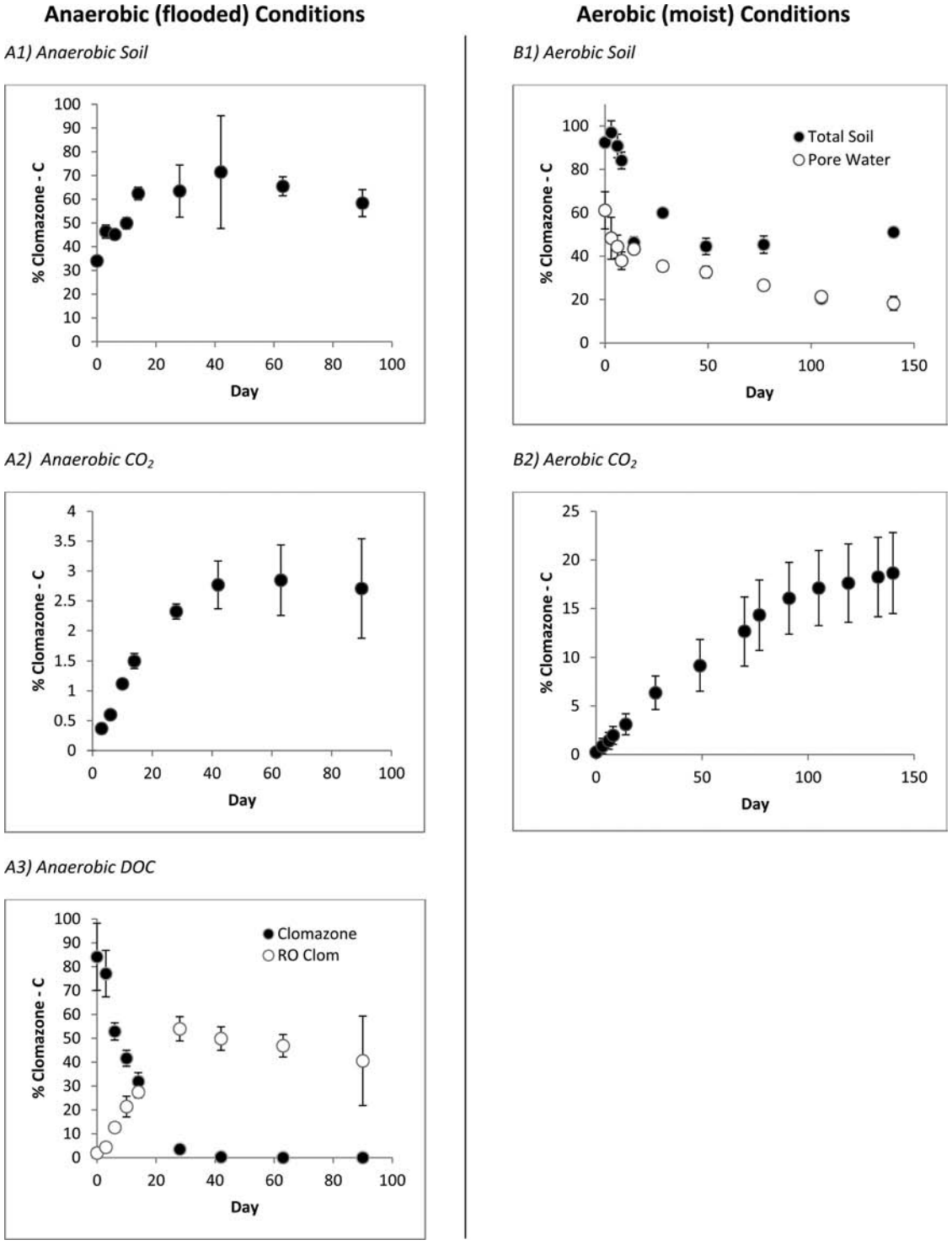

Figure 2. Clomazone-^{13}C recovery in the primary rice ecosystem C pools under (A) anaerobic (flooded) and (B) aerobic (moist) soil conditions. Data are expressed as means ± SD (N = 3).

assumed to account for all clomazone-C in DOC, since no other metabolite was found in significant quantities (>1%). Soil-bound residues increased from 34% at day 0 to 71% at day 42, then decreased to 58.4% recovered at day 90. Over 90 days, 2.8% of applied clomazone was mineralized to CO_2. Methane production increased exponentially throughout the experiment; however, no clomazone was recovered as $^{13}CH_4$. The combined DOC, CO_2 and soil-bound extracts accounted for 94−129% mass balance recovery over 90 days.

Temporal Aerobic (Moist Soil) Clomazone-C Dynamics. Figure 2B investigates the dynamics of aerobic clomazone-C degradation. The compound was found predominantly in the soil fraction, with 92.4% on day 0 and decreasing over time. Pore water degradation rate mirrored that of total soil. In contrast to anaerobic conditions, mineralization contributed significantly to dissipation, with 18.6% applied clomazone converted to CO_2 over 140 days. The combined soil and CO_2 fractions accounted for a mass balance of 86−98% through

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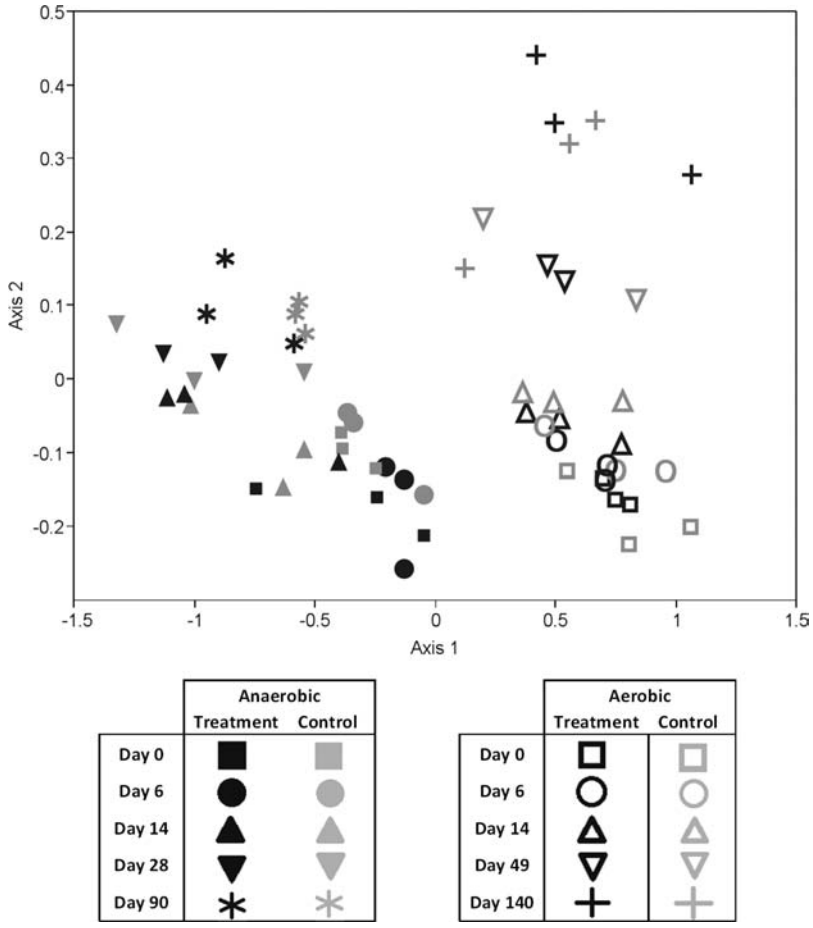

Figure 3. – NMS ordination plot of PLFA composition for both soil types (aerobic and anaerobic). Temporal and soil effects exhibit significance ($p < 0.0001$), and treatment effects are not significant at any time at $\alpha = 0.05$. PLFAs were grouped by biomarker (branched = gram+, monounsaturated = gram–, 18:2 ω6, 9c and 18:3 ω6c (6,9,12) = fungi, 10-methyl = actinomycetes, and cyclic = slow-growth) and natural-log transformed.

Table 1. Two-way ANOVA Tests of Significance ($\alpha = 0.05$) for Time, Treatment (Clomazone-added vs Water Control) and Interaction Effects of PLFA Biomarkers Detected in Anaerobic and Aerobic Soil[a]

	gram+	gram–	fungi	actinomycetes	slow growth
		Anaerobic			
Time	0.5494	<0.0001*	<0.0001*	<0.0001*	0.0678
Treatment	0.1229	0.5608	0.4676	0.6220	0.1597
Time × Treatment	0.9137	0.3262	0.7331	0.8666	0.7260
		Aerobic			
Time	0.1412	<0.0001*	0.1026	0.0005*	0.1385
Treatment	0.2580	0.6636	0.5923	0.4206	0.1588
Time × Treatment	0.0567	0.3454	0.8668	0.0191*	0.9057

[a]Significanct (*) effects are noted.

the first 8 days, and decreased to 70% at the end of the experiment.

PLFA Biomarker Dynamics. PLFA concentration data were natural log-transformed and normalized to total PLFA, as in DeGrood, et al.[13] Each group was observed in detectable quantities, with gram+ and gram- bacteria accounting for the majority of the total biomass under both soil conditions. The effects of soil type, time and treatment on PLFA profile are presented in Figure 3 as a NMS ordination plot. Clear differences are apparent between anaerobic and aerobic soils ($p < 0.0001$), and temporal effects are significant ($p < 0.0001$). Treatment effects are not significant at $\alpha = 0.05$ for either soil type on all extraction days.

Under anaerobic conditions total PLFA biomass remained constant through day 14, then decreased through day 90, with treatment soil biomass significantly less abundant than control ($p < 0.0331$). Under aerobic conditions, total PLFA decreased significantly through day 140 ($p < 0.0001$) with no significant difference between treatment and control biomass. Results of two-way ANOVA tests for time, treatment and interaction (time x treatment) effects on PLFA functional groups are summarized in Table 1. Of the functional groups present, gram$^+$ and slow growth abundance did not vary with time or between treatment and control. Gram-, fungal and actinomycete abundance decreased significantly with time, and did not exhibit treatment or interaction effects. This indicates that

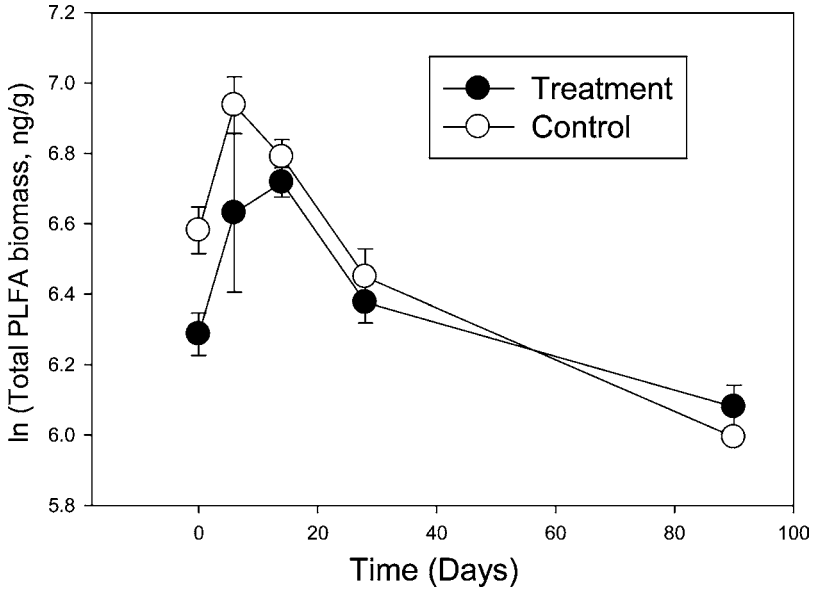

Figure 4. Temporal effects of anaerobic soil PLFA (total anaerobic biomass). Data are expressed as mean of ln Σ(all PLFAs), in ng/g. Error bars are ± SE. Treatment effect is significant ($p < 0.0331$).

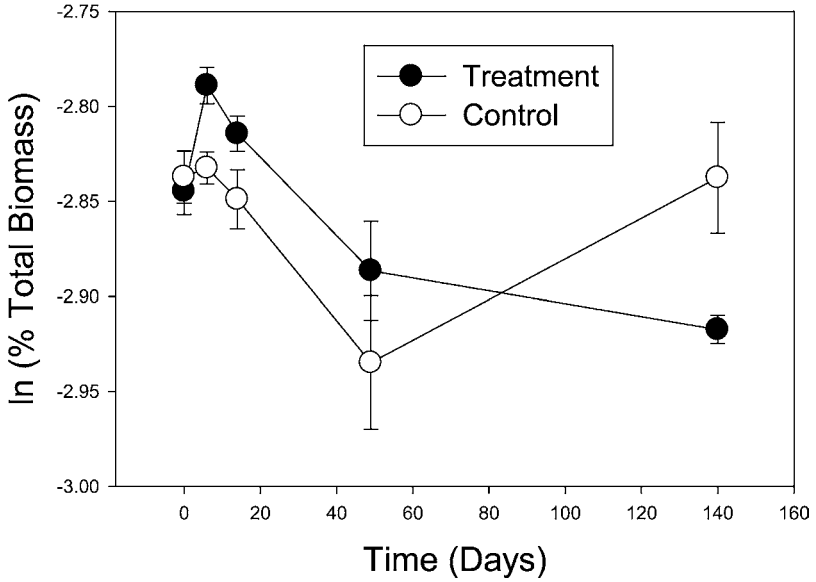

Figure 5. Temporal effects of actinomycete biomass in aerobic soil. Data are expressed as mean of Σ(10-methyl PLFAs)/ Σ(all PLFAs) and ln-transformed. Error bars are ± SE. Temporal and interaction (time × treatment) effects are significant ($p < 0.0005$ and 0.0191, respectively).

clomazone addition decreased the total anaerobic biomass (Figure 4), but did not preferentially decrease any microbial functional group. Under aerobic conditions, gram- bacterial abundance decreased with time, but did not exhibit treatment or interaction effects. Fungi and slow-growth bacterial abundance did not change with time, and did not show treatment or interaction effects. However, actinomycete abundance (Figure 5) varied with time, and interaction effects were significant ($p < 0.0005$ and 0.0191, respectively). This implies that clomazone addition altered the dynamics of actinomycete growth under aerobic conditions.

PLFA ^{13}C Incorporation. We used PLFA ^{13}C enrichment as a proxy for clomazone-C incorporation into microbial biomass. PLFA concentration was adjusted for ^{13}C content and normalized to percent ^{13}C-clomazone recovery as in eqs 1 and 2. PLFAs did not become ^{13}C enriched over 90 and 140 days for anaerobic and aerobic conditions, respectively. This implies clomazone-C did not accumulate in the microbial biomass.

DISCUSSION

Stable isotope probing (SIP) and compound-specific isotope analysis (CSIA) are useful tools that have been applied to studies aimed at determining uptake of organic substrates into C pools, microbes and determining community effects. PLFA-SIP in particular has been utilized to depict uptake of the contaminants toluene[20] and 2,4-D,[21] and carbonaceous substrates, that is, leaf litter[22] and lignin.[23] Community response to allelochemicals in agricultural systems have been noted as well.[24,25] In this investigation, we present findings of C partitioning and community effects of the herbicide clomazone in a California rice field soil.

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Anaerobic (Flooded Soil). Clomazone-C was found to persist in the dissolved phase under anaerobic conditions, with ring-open clomazone the major fermentation product. This anaerobic biotransformation process was previously observed by Tomco et al.,[5] where extractable residue recovery was calculated with acetonitrile as the solvent. This reveals that solvent extraction efficiency of clomazone decreased over time, and that the formation of nonextractable bound residues is a major fate process. This likely explains low mass balance results achieved in this former study, and was recently discussed by Lerch et al., who found 2,4-D amended soil aged over 90 days to be less likely to biodegrade than soil aged 15 days. In this study, we measured total soil ^{13}C via direct combustion, so solvent extraction efficiency was not an issue, and lead to higher clomazone recoveries. Our findings suggest that up to 71% of applied clomazone may partition into the soil fraction, suggesting if bioremediation of clomazone contaminated soil is desired, the primary focus should be on how to desorb the compound from soil surfaces after it has aged. Field E_h spatiotemporal variation is expected to strongly influence degradation kinetics, since rice fields exhibit simultaneous aerobic and anaerobic properties.[26] This may confound degradation kinetics of the biotransformation product RO in the field, and further research should address the timing and magnitude of RO degradation, since it is persistent under highly anaerobic conditions. A small amount of clomazone (2.8% of applied) was mineralized through anaerobic respiration processes, consistent with other studies that have shown anaerobic biodegradation of contaminants may occur by microbes capable of utilizing alternative electron acceptors, commonly fumarate, in respiration.[27,28]

Aerobic (Moist Soil). Aerobic soil will slowly degrade clomazone-C without forming measurable metabolites. Data from this study and a previous study [5] indicate that the formation ofsoil-bound residues may be a significant pathway, and the extent to which this occurs is governed by the compound's soil-water partition coefficient (K_d) and organic carbon normalized distribution coefficient (K_{oc}). For clomazone, this is 0.47–5.3 mL/g and 150 mL/g, respectively, depending on soil type.[2,29] As with anaerobic soil, nonextractable bound residues constitute a major pathway of aerobic dissipation, and regardless of the flooding regiment, the compound is expected to decrease in bioavailability with time, remaining persistent in the field. Other studies[29,30] have indicated clomazone to have a low sorption affinity, however, the soil aging process may confound this. Aerobic mineralization was a major pathway, with 18.6% of applied clomazone recovered as $^{13}CO_2$ over 140 days; the result of microbial respiration processes. That the compound did not partition into microbial phospholipids suggests clomazone is transformed via pathways where energy substrates are either transformed into other cellular components such as proteins or polysaccarides, or rapidly respired but not incorporated into biomass, as was found in Phillips, et al.[31]

PLFA Profile and Community Dynamics. Aerobic soil actinomycetes were found to exhibit significant time and time x treatment effects, indicating that clomazone addition had a priming effect on these microorganisms under this soil condition. Phillips et al. [31] found microbial functional group distribution to change in response to addition of ^{13}C cellobiose, while the total biomass did not change. This is very similar to what was found in our experiment, where actinomycetes changed in response to clomazone addition, but the total biomass did not. Aerobic soil actinomycete abundance over time is presented in Figure 5. It is not known whether actinomycetes are capable of degrading clomazone, or if clomazone inhibits other populations with similar niches. Future research on clomazone utilization by actinomycetes and relationship to soil ecology would strengthen the correlation found here.

The total anaerobic biomass (Figure 4) decreased in response to clomazone addition. However, when the total biomass is partitioned into functional groups, none was found to vary in response to clomazone addition at $\alpha = 0.05$ significance. This implies that the entire anaerobic community was uniformly affected by the herbicide at early time points, and rebounded to baseline abundance over time. Under aerobic conditions, no effect on total biomass was significant. An NMS ordination plot (Figure 3) suggests PLFAs exhibited soil type and temporal effects, but not treatment effects. This illustrates that clomazone, which represents a very small proportion of total soil carbon, does not perturb the dynamics of the microbial community as a whole when applied in regulatory abundance. This observation is different from previous studies examining microbial community changes in response to pesticide addition,[32−35] which show community effects vary according to compound structure, dose, and frequency of use. Since most clomazone-C under both soil conditions was recovered in DOC or respired CO_2, and not in PLFA, this suggests the compound is degraded by cometabolic processes. As with thiobencarb, another popular chloroaromatic herbicide, amendment with organic carbon,[36] phosphate, and copper [37] are expected to alter the dynamics of clomazone biodegradation.

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Notes

The authors declare no competing financial interests.

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REFERENCES

(1) CDPR, Summary Report; California Environmental Protection Agency: Sacramento, CA. http://www.cdpr.ca.gov/docs/pur/purmain.htm, 2010; Last accessed October 12, 2012.

(2) Senseman, S.; Armbrust, K. *Herbicide Handbook*; Weed Science Society Of America: Norfolk, VA, 2007.

(3) Mervosh, T. L.; Sims, G. K.; Stoller, E. W. Clomazone fate in soil as affected by microbial activity, temperature, and soil-moisture. *J. Agric. Food Chem.* **1995**, *43*, 537−543.

(4) Tomco, P. L.; Tjeerdema, R. S. Photolytic versus microbial degradation of clomazone in a flooded California rice field soil. *Pest Manage. Sci.* **2012**, *68* (8), 1141−7.

(5) Tomco, P. L.; Holstege, D. M.; Zou, W.; Tjeerdema, R. S. Microbial Degradation of Clomazone under Simulated California Rice Field Conditions. *J. Agric. Food Chem.* **2010**, *58*, 3674−3680.

(6) Quayle, W. C.; Oliver, D. P.; Zrna, S. Field dissipation and environmental hazard assessment of clomazone, molinate, and thiobencarb in Australian rice culture. *J. Agric. Food Chem.* **2006**, *54*, 7213−7220.

(7) Burns, R. G.; Dick, R. P. *Enzymes in the environment: activity, ecology, and applications*; CRC: Boca Raton, FL, 2002; Vol. *86*.

(8) USDA, Soil survey of Butte area, California, parts of Butte and Plumas Counties. http://soildatamart.nrcs.usda.gov/Manuscripts/CA612/0/Butte_CA.pdf, 2006; last accessed October 19, 2012.
(9) Mueller, C.; Schwender, J.; Zeidler, J.; Lichtenthaler, H. K. In *Properties and inhibition of the first two enzymes of the non-mevalonate pathway of isoprenoid biosynthesis*; Portland Press: Portland, ME, 2000; pp 792−793.
(10) Ferhatoglu, Y.; Barrett, M. Studies of clomazone mode of action. *Pestic. Biochem. Physiol.* **2006**, *85*, 7−14.
(11) TenBrook, P. L.; Tjeerdema, R. S. Biotransformation of clomazone in rice (Oryza sativa) and early watergrass (*Echinochloa oryzoides*). *Pestic. Biochem. Physiol.* **2006**, *85*, 38−45.
(12) David, S. Synergic activity of D-cycloserine and beta-chloro-D-alanine against *Mycobacterium tuberculosis*. *J. Antimicrob. Chemother.* **2001**, *47*, 203−206.
(13) DeGrood, S. H.; Claassen, V. P.; Scow, K. M. Microbial community composition on native and drastically disturbed serpentine soils. *Soil Biol. Biochem.* **2005**, *37*, 1427−1435.
(14) Bossio, D. A.; Scow, K. M.; Gunapala, N.; Graham, K. J. Determinants of soil microbial communities: Effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. *Microb. Ecol.* **1998**, *36*, 1−12.
(15) Findlay, R.; Dobbs, F. Quantitative description of microbial communities using lipid analysis. *Handb. Methods Aquat. Microb. Ecol.* **1993**, 271−284.
(16) Nkedi-Kizza, P.; Brown, K. D. Sorption, degradation, and mineralization of carbaryl in soils, for single-pesticide and multiple-pesticide systems. *J. Environ. Qual.* **1998**, *27*, 1318−1324.
(17) Santos, F.; Torn, M. S.; Bird, J. A. Biological degradation of pyrogenic organic matter in temperate forest soils. *Soil Biol. Biochem.* **2012**, *51*, 115−124.
(18) Bird, J. A.; Herman, D. J.; Firestone, M. K. Rhizosphere priming of soil organic matter by bacterial groups in a grassland soil. *Soil Biol. Biochem.* **2011**, *43*, 718−725.
(19) Drenovsky, R. E.; Elliott, G. N.; Graham, K. J.; Scow, K. M. Comparison of phospholipid fatty acid (PLFA) and total soil fatty acid methyl esters (TSFAME) for characterizing soil microbial communities. *Soil Biol. Biochem.* **2004**, *36*, 1793−1800.
(20) Hanson, J. R.; Macalady, J. L.; Harris, D.; Scow, K. M. Linking toluene degradation with specific microbial populations in soil. *Appl. Environ. Microbiol.* **1999**, *65*, 5403−5408.
(21) Lerch, T. Z.; Dignac, M. F.; Nunan, N.; Bardoux, G.; Barriuso, E.; Mariotti, A. Dynamics of soil microbial populations involved in 2,4-D biodegradation revealed by FAME-based Stable Isotope Probing. *Soil Biol. Biochem.* **2009**, *41*, 77−85.
(22) Moore-Kucera, J.; Dick, R. P. Application of C-13-labeled litter and root materials for in situ decomposition studies using phospholipid fatty acids. *Soil Biol. Biochem.* **2008**, *40*, 2485−2493.
(23) Bahri, H.; Rasse, D. P.; Rumpel, C.; Dignac, M. F.; Bardoux, G.; Mariotti, A. Lignin degradation during a laboratory incubation followed by C-13 isotope analysis. *Soil Biol. Biochem.* **2008**, *40*, 1916−1922.
(24) Kong, C. H.; Wang, P.; Gu, Y.; Xu, X. H.; Wang, M. L. Fate and impact on microorganisms of rice allelochemicals in paddy soil. *J. Agric. Food Chem.* **2008**, *56*, 5043−5049.
(25) Chen, K. J.; Zheng, Y. Q.; Kong, C. H.; Zhang, S. Z.; Li, J.; Liu, X. G. 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and 6-Methoxy-benzoxazolin-2-one (MBOA) levels in the wheat rhizosphere and their effect on the soil microbial community structure. *J. Agric. Food Chem.* **2010**, *58*, 12710−12716.
(26) Lerch, T. Z.; Dignac, M. F.; Nunan, N.; Barriuso, E.; Mariotti, A. Ageing processes and soil microbial community effects on the biodegradation of soil C-13−2,4-D nonextractable residues. *Environ. Pollut.* **2009**, *157*, 2985−2993.
(27) Lara-Martin, P. A.; Gomez-Parra, A.; Sanz, J. L.; Gonzalez-Mazo, E. Anaerobic degradation pathway of linear alkylbenzene sulfonates (LAS) in sulfate-reducing marine sediments. *Environ. Sci. Technol.* **2010**, *44*, 1670−1676.
(28) Garcia, J.; Rousseau, D. P. L.; Morato, J.; Lesage, E.; Matamoros, V.; Bayona, J. M. Contaminant removal processes in subsurface-flow constructed wetlands: A review. *Crit. Rev. Environ. Sci. Technol.* **2010**, *40*, 561−661.
(29) Mervosh, T. L.; Sims, G. K.; Stoller, E. W.; Ellsworth, T. R. Clomazone sorption in soil - incubation-time, temperature, and soil-moisture effects. *J. Agric. Food Chem.* **1995**, *43*, 2295−2300.
(30) Gunasekara, A. S.; dela Cruz, I. D. P.; Curtis, M. J.; Claassen, V. P.; Tjeerdema, R. S. The behavior of clomazone in the soil environment. *Pest Manage. Sci.* **2009**, *65*, 711−716.
(31) Phillips, R.; Zak, D.; Holmes, W.; White, D. Microbial community composition and function beneath temperate trees exposed to elevated atmospheric carbon dioxide and ozone. *Oecologia* **2002**, *131*, 236−244.
(32) Bending, G. D.; Rodriguez-Cruz, M. S.; Lincoln, S. D. Fungicide impacts on microbial communities in soils with contrasting management histories. *Chemosphere* **2007**, *69*, 82−88.
(33) Engelen, B.; Meinken, K.; von Wintzintgerode, F.; Heuer, H.; Malkomes, H. P.; Backhaus, H. Monitoring impact of a pesticide treatment on bacterial soil communities by metabolic and genetic fingerprinting in addition to conventional testing procedures. *Appl. Environ. Microbiol.* **1998**, *64*, 2814−2821.
(34) Saeki, M.; Toyota, K. Effect of bensulfuron-methyl (a sulfonylurea herbicide) on the soil bacterial community of a paddy soil microcosm. *Biol. Fertil. Soils* **2004**, *40*, 110−118.
(35) Seghers, D.; Verthe, K.; Reheul, D.; Bulcke, R.; Siciliano, S. D.; Verstraete, W.; Top, E. M. Effect of long-term herbicide applications on the bacterial community structure and function in an agricultural soil. *FEMS Microbiol. Ecol.* **2003**, *46*, 139−146.
(36) Schmelzer, K. R.; Johnson, C. S.; Viant, M. R.; Williams, J. F.; Tjeerdema, R. S. Influence of organic carbon on reductive dechlorination of thiobencarb in California rice field soils. *Pest Manage. Sci.* **2005**, *61*, 68−74.
(37) Gunasekara, A. S.; TenBrook, P. L.; Palumbo, A. J.; Johnson, C. S.; Tjeerdema, R. S. Influence of phosphate and copper on reductive dechlorination of thiobencarb in California rice field soils. *J. Agric. Food Chem.* **2005**, *53*, 10113−10119.