Supplemental Table and Figures

Fractionation of stable carbon isotopes during acetate consumption by methanogenic and sulfidogenic microbial communities in rice paddy soils and lake sediments

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Legends of the supplemental figures

Fig.S1: Acetate conversion to CH_4 and CO_2 in unbuffered suspensions of paddy soil from Vercelli (Italy) without additions (water control), with gypsum, and with CH_3F. The panels show the temporal change of (A) concentrations of acetate; (B) $\delta^{13}C$ of acetate; (C) partial pressures of CH_4 (1 ppmv = 10^{-6} bar); (D) $\delta^{13}C$ of CH_4; (E) partial pressures of CO_2 (1 ppmv = 10^{-6} bar); and (F) $\delta^{13}C$ of CO_2. Means ± SE, n = 2.

Fig.S2: Mariotti plots of (A, B) acetate consumption and (C, D) CH_4 production in (A, C) the absence (control) and (B, D) the presence of gypsum (+ sulfate) in 4 replicates of unbuffered suspensions of paddy soil from Vercelli.

Fig.S3: Acetate conversion to CH_4 and CO_2 in phosphate-buffered (pH 7) suspensions of paddy soil from Vercelli (Italy) without (H_2O) and with addition of acetate (acetate) and with acetate and CH_3F. The soil was preincubated in the absence of sulfate, but sulfate (gypsum) was for the experimental incubation. The panels show the temporal change of (A) concentrations of acetate; (B) $\delta^{13}C$ of acetate; (C) partial pressures of CH_4 (1 ppmv = 10^{-6} bar); (D) $\delta^{13}C$ of CH_4; (E) partial pressures of CO_2 (1 ppmv = 10^{-6} bar); (D) $\delta^{13}C$ of CO_2. Means ± SE, n = 3.

Fig.S4: Balance of the produced CH_4 + TIC against acetate consumed in phosphate-buffered suspensions of paddy soil from Vercelli and IRRI, and of sediments from the NE and SW basin of Lake Fuchskuhle. The figures show individual replicates (n = 3) of the unamended control (methanogenic conditions); of the experiment plus gypsum

($CaSO_4$-1); of preincubation and experiment plus gypsum ($CaSO_4$-2). The diagonal line indicates stoichiometric conversion (disproportionation) of acetate to CH_4 + CO_2.

Fig.S5: Acetate conversion to CH_4 and CO_2 in phosphate-buffered (pH 7) suspensions of paddy soil from IRRI (the Philippines) without addition of acetate (H_2O), with addition of acetate (acetate) and with addition of acetate and CH_3F (Acet + CH_3F). The soil was preincubated in the absence of sulfate, and sulfate (gypsum) was also not added for the experimental incubation. The panels show the temporal change of (A) concentrations of acetate; (B) $\delta^{13}C$ of acetate; (C) partial pressures of CH_4 (1 ppmv = 10^{-6} bar); (D) $\delta^{13}C$ of CH_4; (E) partial pressures of CO_2 (1 ppmv = 10^{-6} bar); (D) $\delta^{13}C$ of CO_2. Means ± SE, n = 3.

Fig.S6: Acetate conversion to CH_4 and CO_2 in phosphate-buffered (pH 7) suspensions of paddy soil from IRRI (the Philippines) without addition of acetate (H_2O), with addition of acetate (acetate) and with addition of acetate and CH_3F (Acet + CH_3F). The soil was preincubated in the absence of sulfate, but sulfate (gypsum) was for the experimental incubation. The panels show the temporal change of (A) concentrations of acetate; (B) $\delta^{13}C$ of acetate; (C) partial pressures of CH_4 (1 ppmv = 10^{-6} bar); (D) $\delta^{13}C$ of CH_4; (E) partial pressures of CO_2 (1 ppmv = 10^{-6} bar); (D) $\delta^{13}C$ of CO_2. Means ± SE, n = 3.

Fig.S7: Acetate conversion to CH_4 and CO_2 in phosphate-buffered (pH 7) suspensions of paddy soil from IRRI (the Philippines) without addition of acetate (H_2O), with addition of acetate (acetate) and with addition of acetate and CH_3F (Acet + CH_3F). The soil was preincubated in the presence of sulfate, and sulfate (gypsum) was also added for the experimental incubation. The panels show the temporal change of (A) concentrations of acetate; (B) $\delta^{13}C$ of acetate; (C) partial pressures of CH_4 (1 ppmv = 10^{-6} bar); (D) $\delta^{13}C$ of CH_4; (E) partial pressures of CO_2 (1 ppmv = 10^{-6} bar); (D) $\delta^{13}C$ of CO_2. Means ± SE, n = 3.

Fig.S8: Balance of the produced CH_4 + TIC against acetate consumed in phosphate-buffered suspensions of paddy soil from Vercelli and IRRI, and of sediments from the NE and SW basin of Lake Fuchskuhle. The figures show individual replicates (n = 3) of the

unamended control (methanogenic conditions); of the experiment plus gypsum ($CaSO_4$-1); of preincubation and experiment plus gypsum ($CaSO_4$-2). The diagonal line indicates stoichiometric conversion (disproportionation) of acetate to CH_4 + CO_2. The figure is identical to Fig.S4, but background production of CH_4 and CO_2 in the water control without addition of acetate was substracted.

Fig.S9: Mariotti plots of (A, B, E) acetate consumption and (C, D, F) CH_4 production in (A, C) the absence (no sulfate); (B, D) the presence of gypsum (with sulfate); and (E, F) after preincubation with sulfate and in the presence of sulfate in 3 replicates of phosphate-buffered suspensions of paddy soil from Vercelli.

Fig.S10: Mariotti plots of (A, B, E) acetate consumption and (C, D, F) CH_4 production in (A, C) the absence (no sulfate); (B, D) the presence of gypsum (with sulfate); and (E, F) after preincubation with sulfate and in the presence of sulfate in 3 replicates of phosphate-buffered suspensions of paddy soil from the IRRI.

Fig.S11: Mariotti plots of acetate consumption in the presence of methyl fluoride (CH_3F) in (A, C) the presence of gypsum (with sulfate); and (B, D) after preincubation with sulfate and in the presence of sulfate, in 3 replicates of phosphate-buffered suspensions of paddy soil from (A, B) Vercelli and (C, D) the IRRI.

Fig.S12: Acetate conversion to CH_4 and CO_2 in phosphate-buffered (pH 7) suspensions of lake sediment from the SW basin of Fuchskuhle without addition of acetate (H_2O), with addition of acetate (acetate) and with addition of acetate and CH_3F (Acet + CH_3F). The sediment was incubated in the absence of sulfate. The panels show the temporal change of (A) concentrations of acetate; (B) $\delta^{13}C$ of acetate; (C) partial pressures of CH_4 (1 ppmv = 10^{-6} bar); (D) $\delta^{13}C$ of CH_4; (E) partial pressures of CO_2 (1 ppmv = 10^{-6} bar); (D) $\delta^{13}C$ of CO_2. Means ± SE, n = 3.

Fig.S13: Acetate conversion to CH_4 and CO_2 in phosphate-buffered (pH 7) suspensions of lake sediment from the SW basin of Fuchskuhle without addition of acetate (H_2O), with addition of acetate (acetate) and with addition of acetate and CH_3F (Acet + CH_3F). The sediment was incubated in the presence of sulfate, and sulfate (gypsum) was also not added for the experimental incubation. The panels show the temporal

change of (A) concentrations of acetate; (B) $\delta^{13}C$ of acetate; (C) partial pressures of CH_4 (1 ppmv = 10^{-6} bar); (D) $\delta^{13}C$ of CH_4; (E) partial pressures of CO_2 (1 ppmv = 10^{-6} bar); (D) $\delta^{13}C$ of CO_2. Means ± SE, n = 3.

Fig.S14: Mariotti plots of (A, B) acetate consumption and (C, D) CH_4 production in (A, C) the absence (no sulfate) and (B, D) the presence (both preincubation and experimental incubation) of gypsum (plus sulfate) in 3 replicates of buffered suspensions of lake sediment from the NE basin of Fuchskuhle.

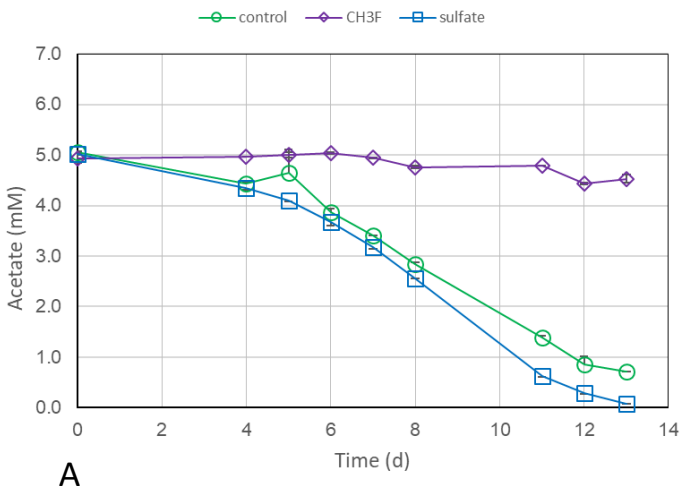
Fig.S15: Mariotti plots of (A, B) acetate consumption and (C, D) CH_4 production in (A, C) the absence (no sulfate) and (B, D) the presence (both preincubation and experimental incubation) of gypsum (plus sulfate) in 4 replicates of buffered suspensions of lake sediment from the SW basin of Fuchskuhle.

Fig.S16: Mariotti plots of acetate consumption in the presence of methyl fluoride (CH_3F) after preincubation and in the presence of gypsum (with sulfate) in 3 replicates of phosphate-buffered suspensions of lake sediment from the (A) NE basin and (B) SW basin of Fuchskuhle.

Table S1: Epsilon values (negative values) determined using Mariotti plots of ^{13}C-acetate or $^{13}CH_4$ in unbuffered or phosphate-buffer suspensions without and with addition of sulfate, also after preincubation with sulfate, and in the absence and presence of methyl fluoride

		ε									
						Preincubation				Preincubation	
Soil	δ^{13}C used	without sulfate		with sulfate		with sulfate		with sulfate + CH3F		with sulfate + CH3F	
		mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
Vercelli-soil	acetate	17.2	*0.93*	19.5	*0.34*						
	CH4	18.9	*2.03*	14.1	*1.96*						
IRRI-soil	acetate	20.2	*0.80*	20.6	*0.62*						
	CH4	21.6	*3.23*	19.4	*1.42*						
Vercelli-buffer	acetate	17.7	*0.53*	21.1	*1.27*	21.4	*0.20*	21.8	*2.47*	24.3	*0.54*
	CH4	29.7	*0.61*	22.9	*1.30*	24.9	*1.54*				
IRRI-buffer	acetate	20.0	*2.41*	19.1	*0.51*	21.2	*1.98*	23.8	*5.59*	10.1	*1.12*
	CH4	37.2	*6.86*	28.9	*3.68*	32.8	*6.82*				
NE-buffer	acetate	18.9	*1.01*			19.7	*1.41*			26.3	*3.28*
	CH4	27.9	*2.46*								
SW-buffer	acetate	11.4	*1.47*			14.3	*1.30*			17.8	*0.06*
	CH4	27.4	*3.20*								

Vercelli
Soil slurry



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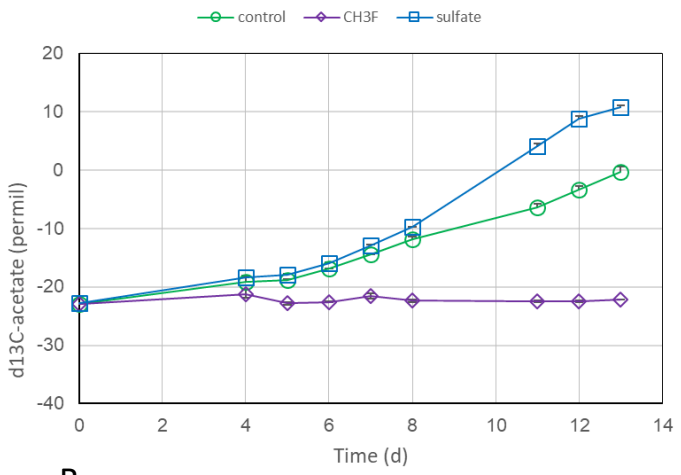

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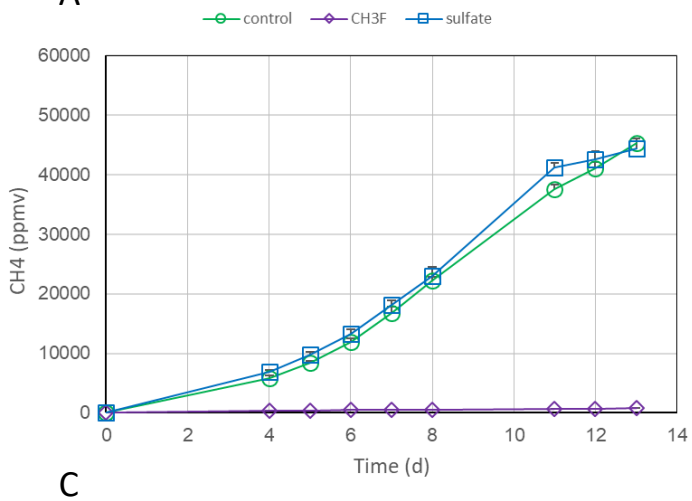

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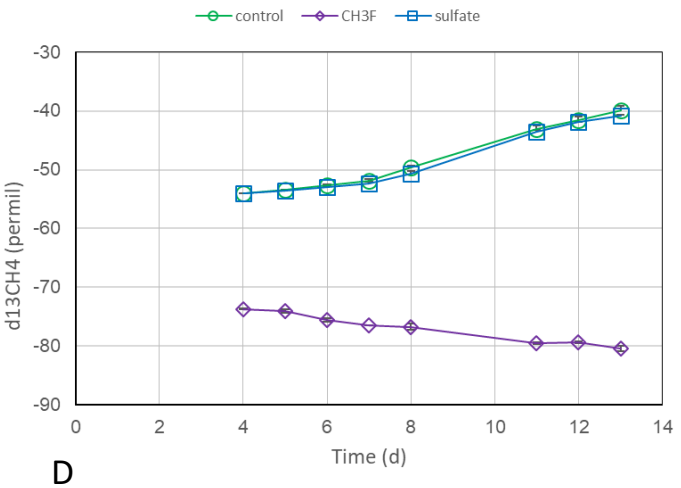

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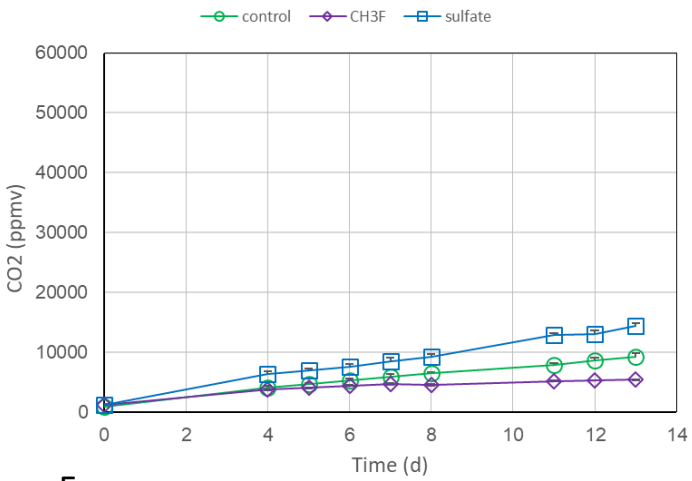

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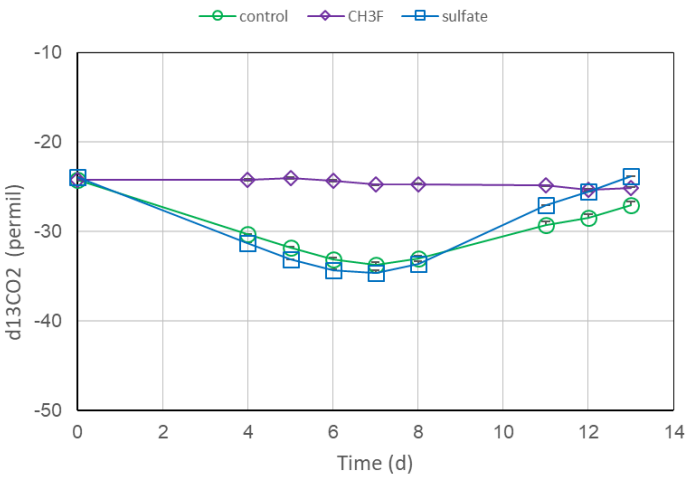
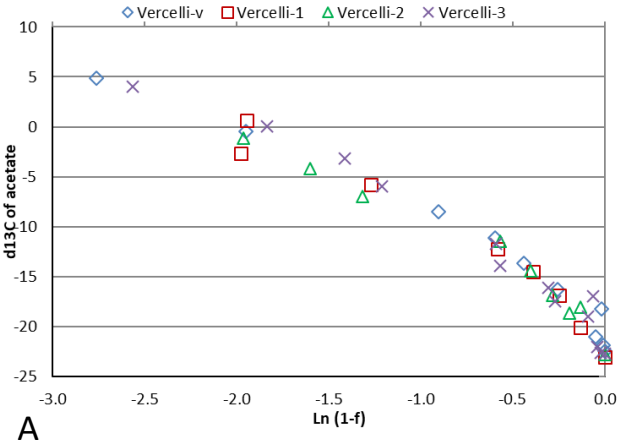


F

Fig. S1

Vercelli

control



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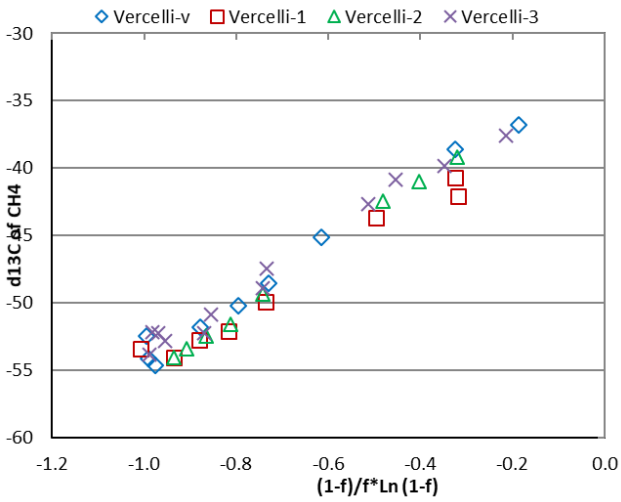
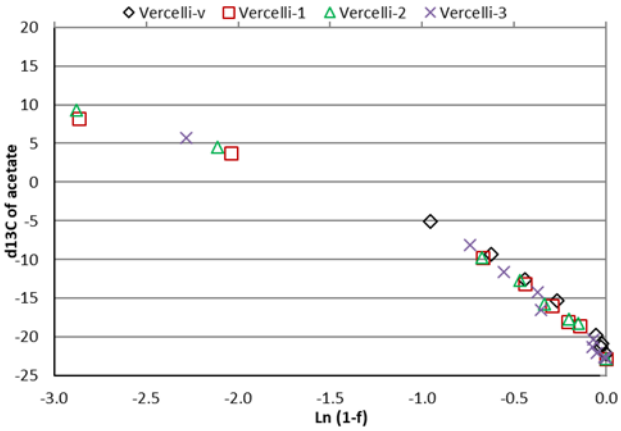


C

+ sulfate



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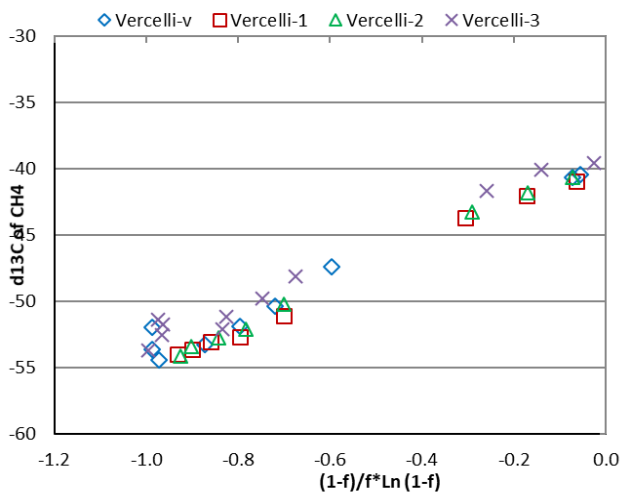
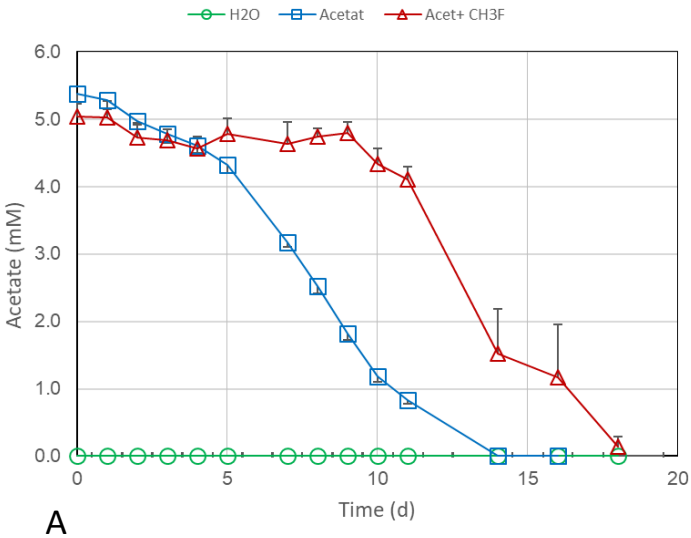


D

Fig. S2

Vercelli
with sulfate



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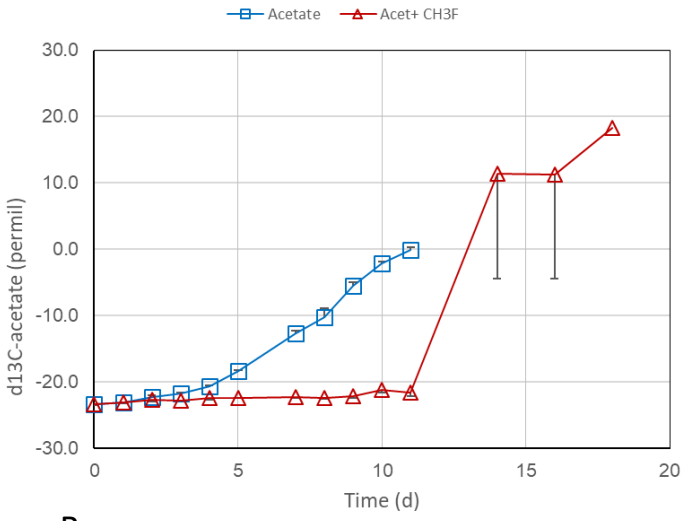

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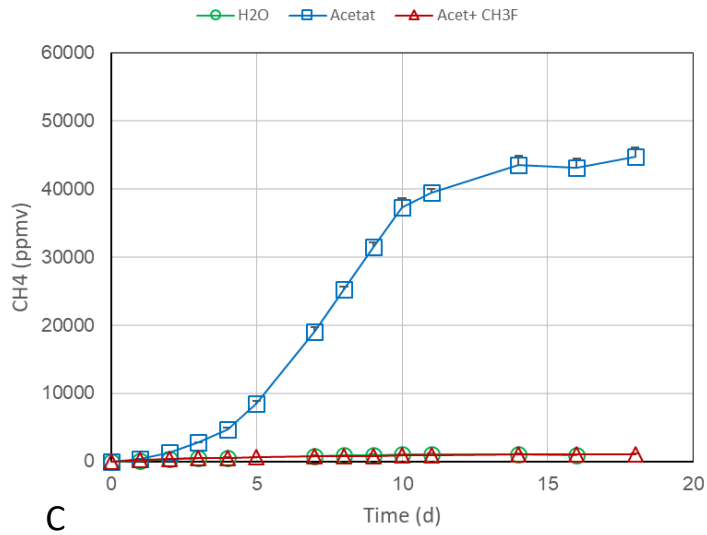

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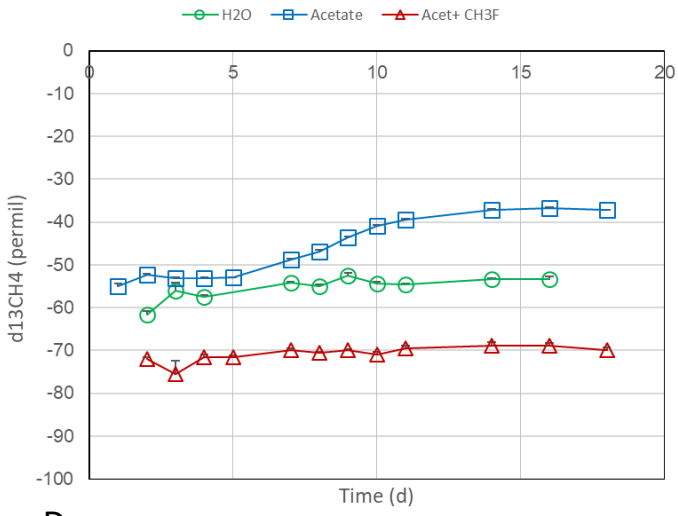

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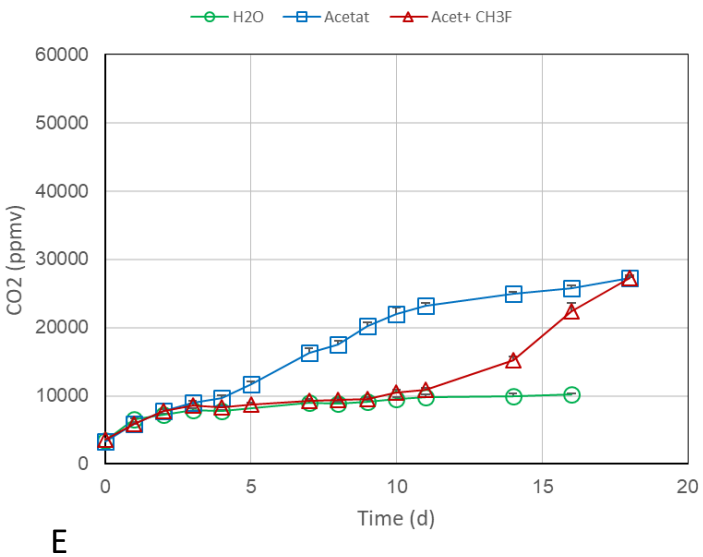

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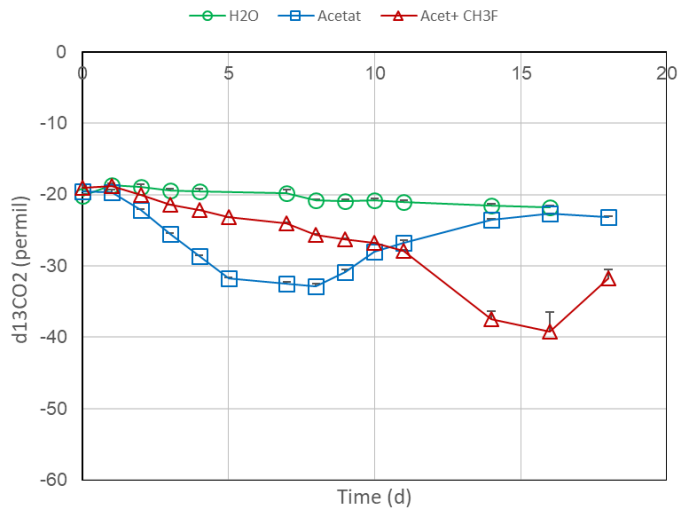

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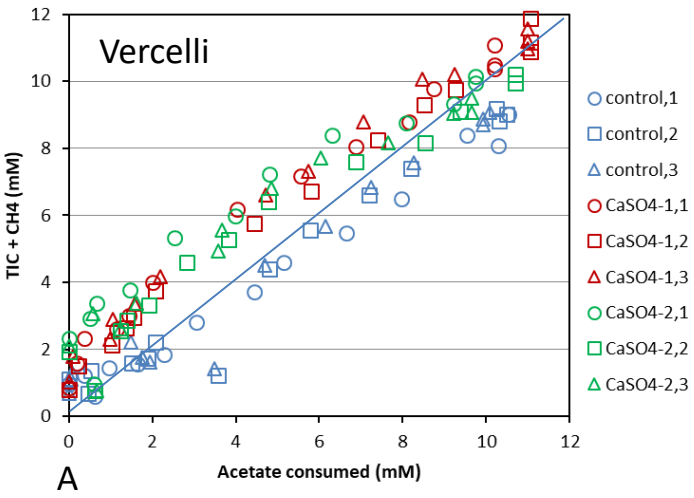
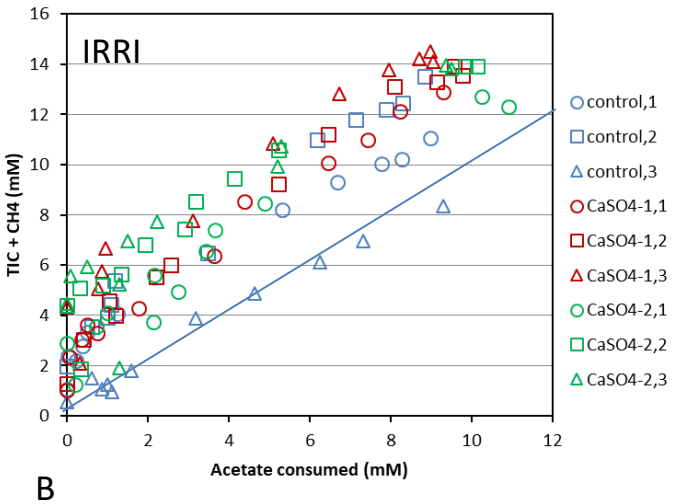
Fig. S3


Vercelli
TIC + CH4 (mM)
Acetate consumed (mM)
control,1
control,2
control,3
CaSO4-1,1
CaSO4-1,2
CaSO4-1,3
CaSO4-2,1
CaSO4-2,2
CaSO4-2,3

A


IRRI
TIC + CH4 (mM)
Acetate consumed (mM)
control,1
control,2
control,3
CaSO4-1,1
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CaSO4-2,1
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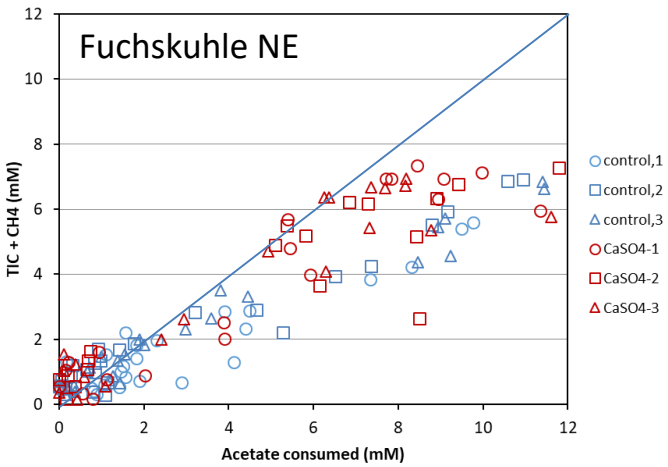
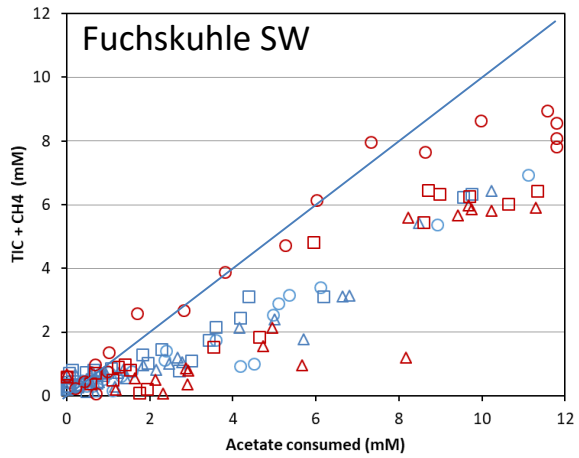
CaSO4-2,3

B


Fuchskuhle NE
TIC + CH4 (mM)
Acetate consumed (mM)
control,1
control,2
control,3
CaSO4-1
CaSO4-2
CaSO4-3

C


Fuchskuhle SW
TIC + CH4 (mM)
Acetate consumed (mM)
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control,2
control,3
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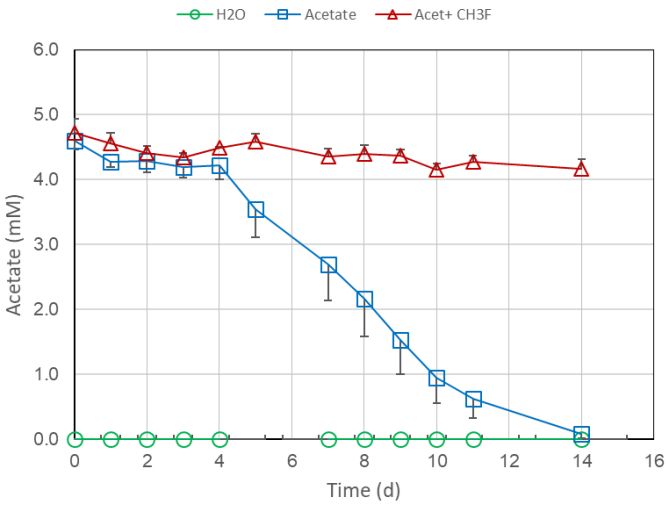
CaSO4-2
CaSO4-3

D

Fig. S4

IRRI
without sulfate



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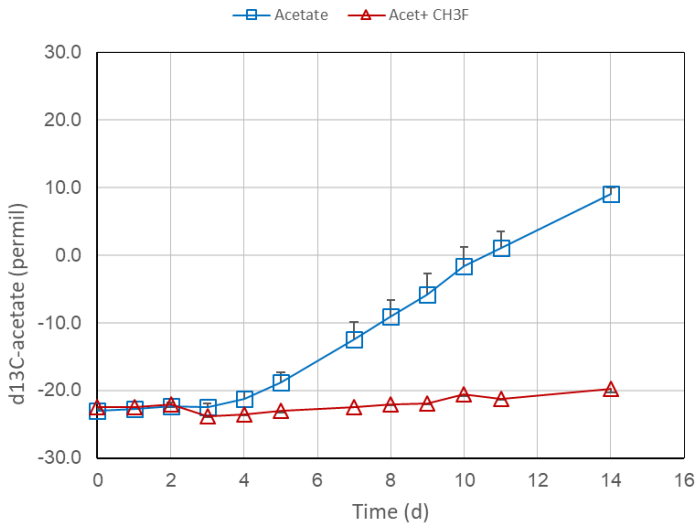

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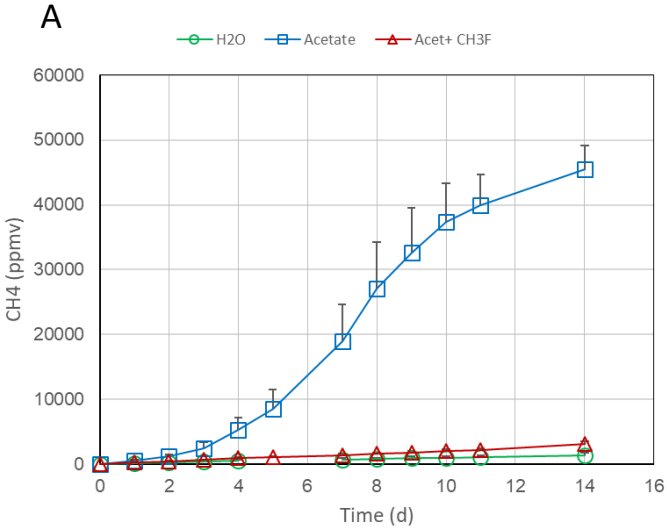

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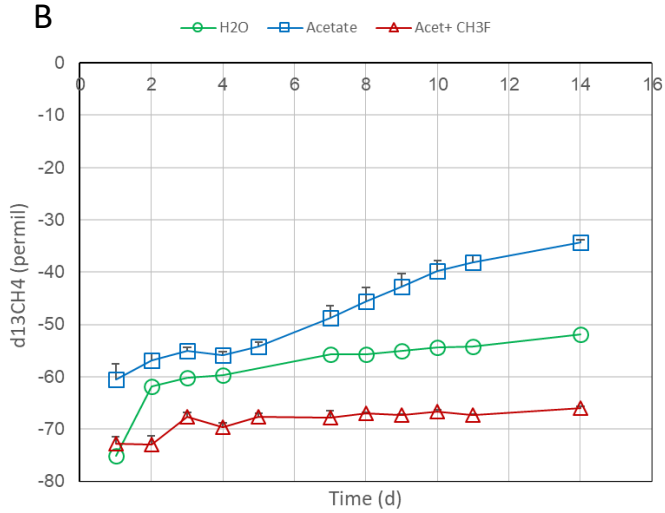

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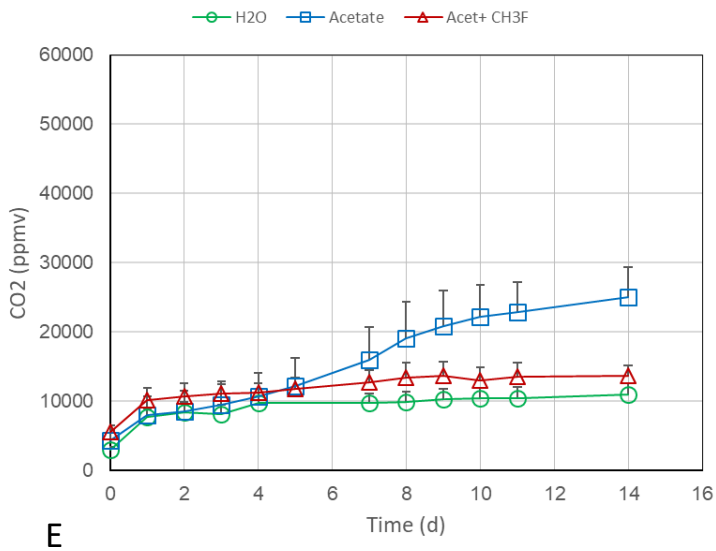

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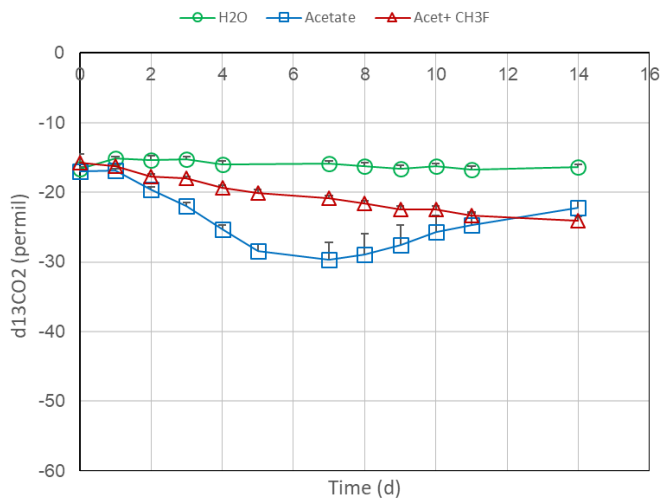

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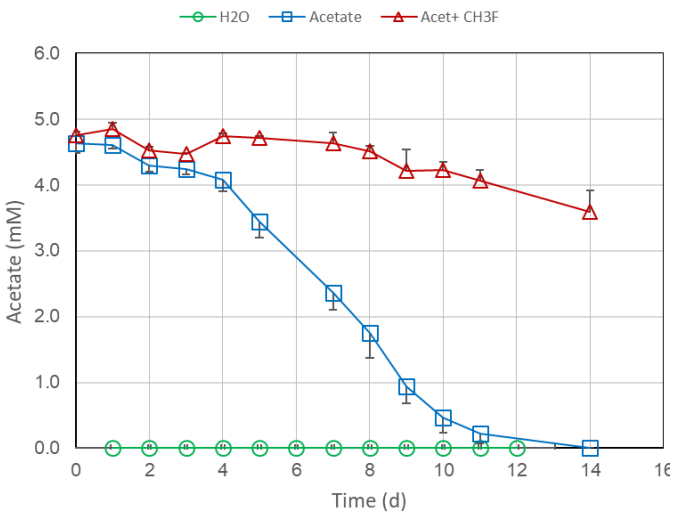
Fig. S5

IRRI
with sulfate



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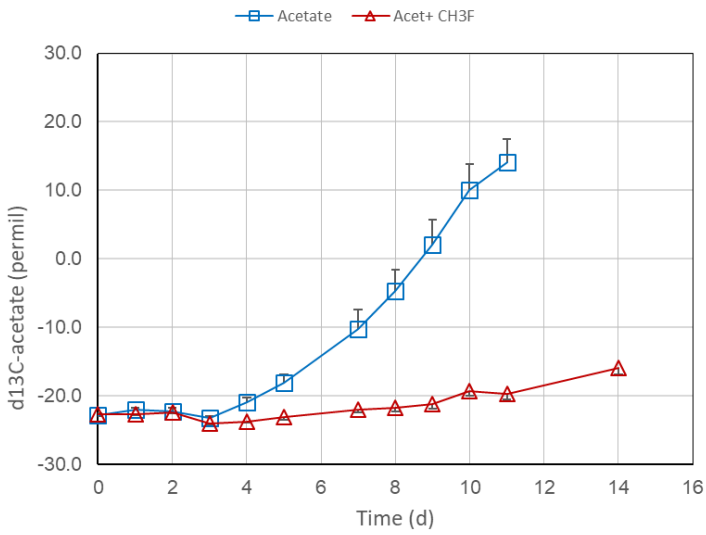

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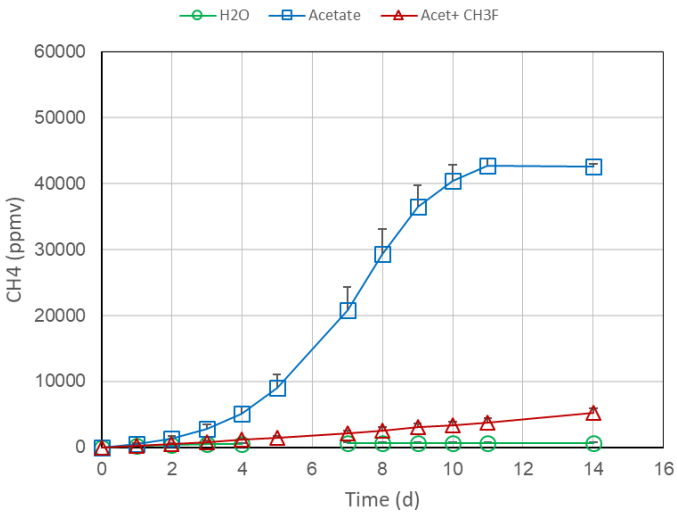

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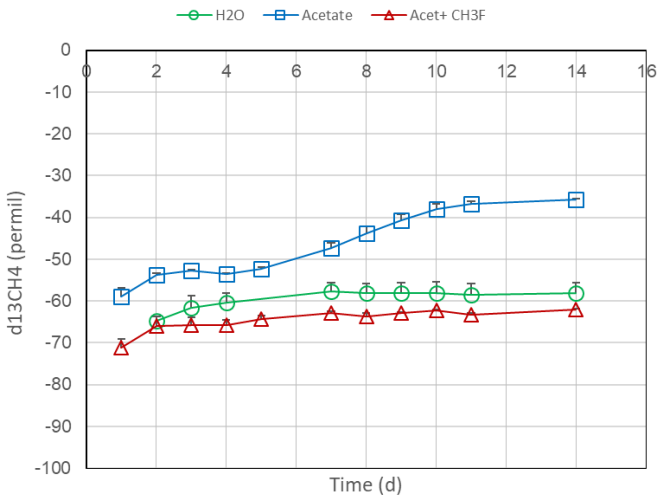

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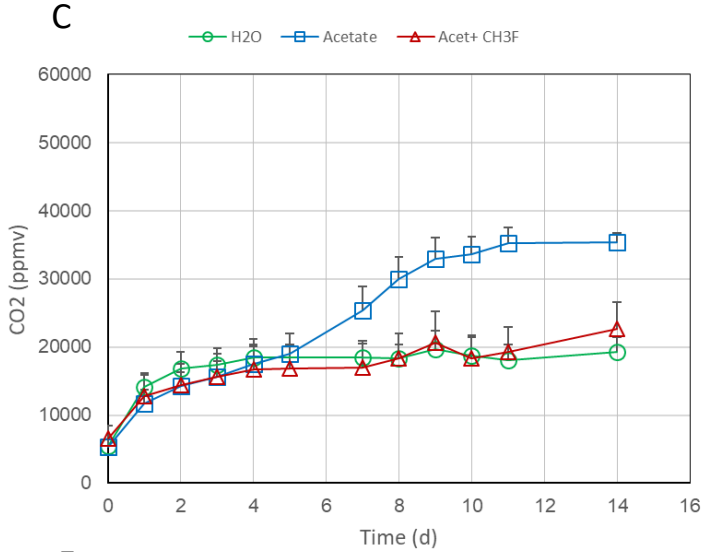

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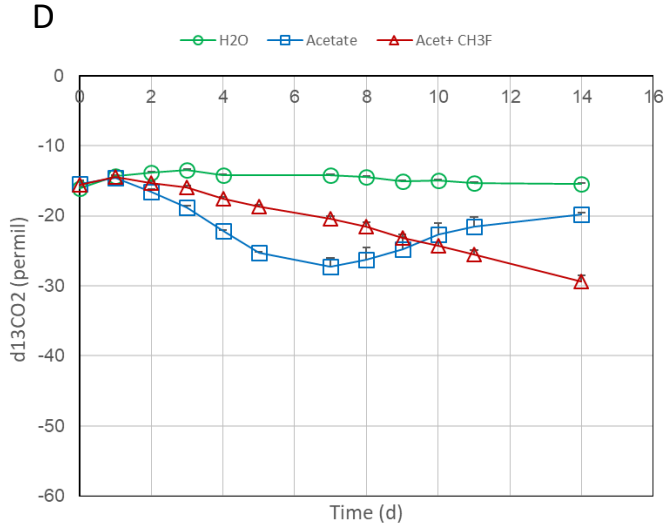
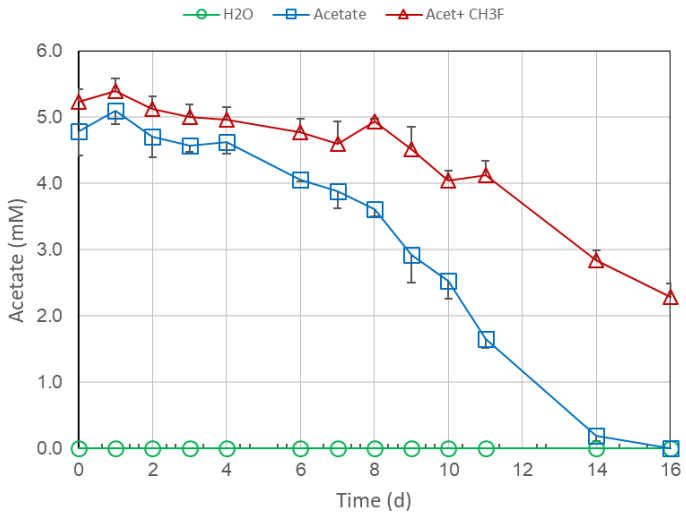


F

Fig. S6

IRRI
with sulfate, with preincubation



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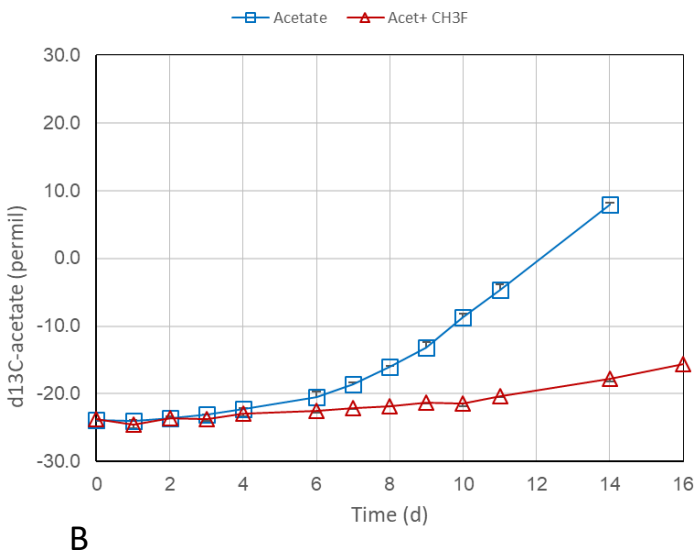

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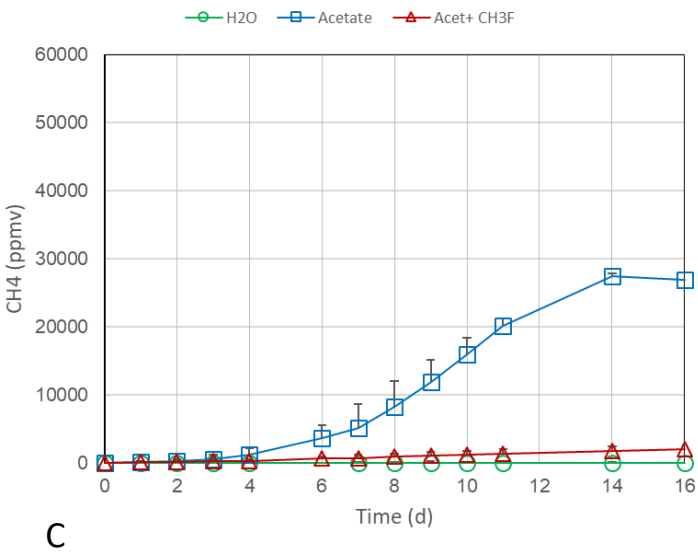

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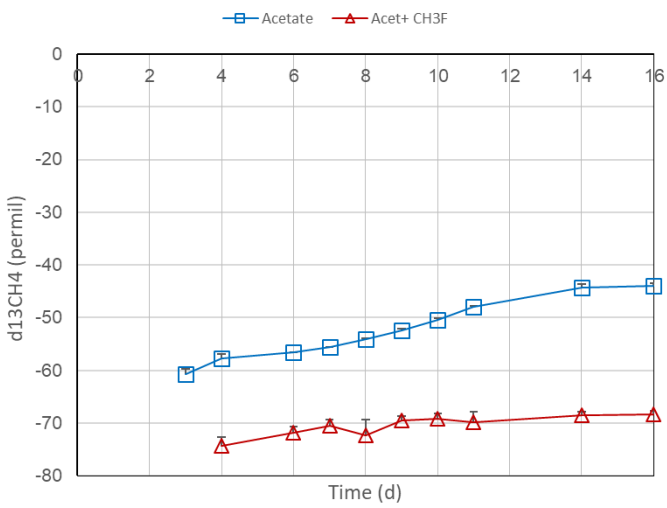

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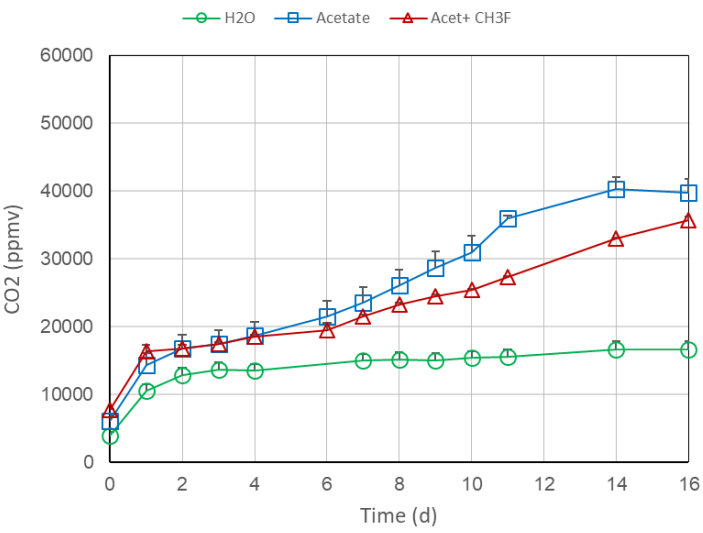

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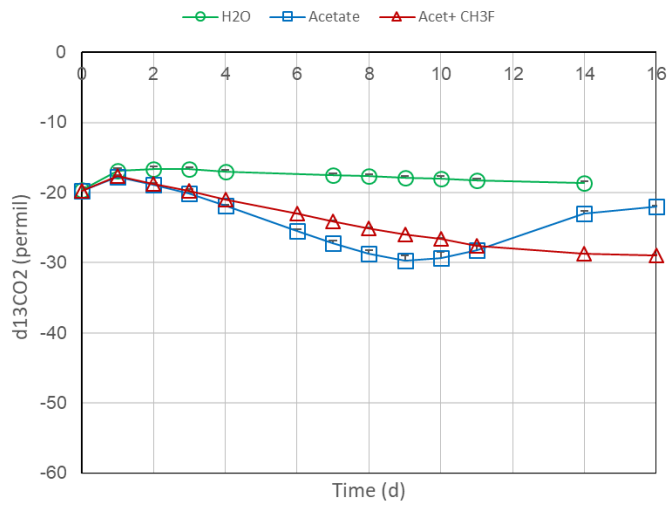


F

Fig. S7

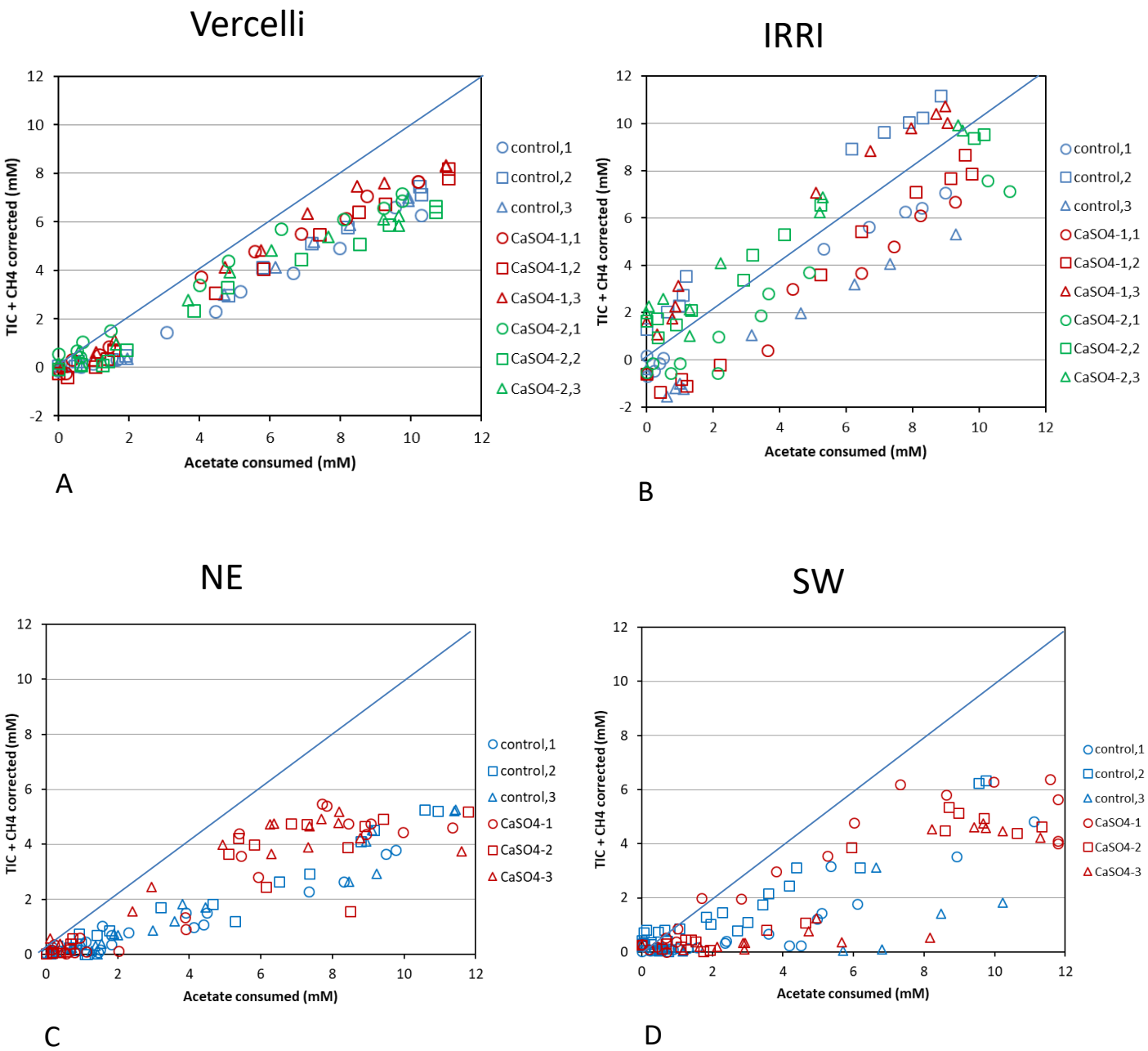
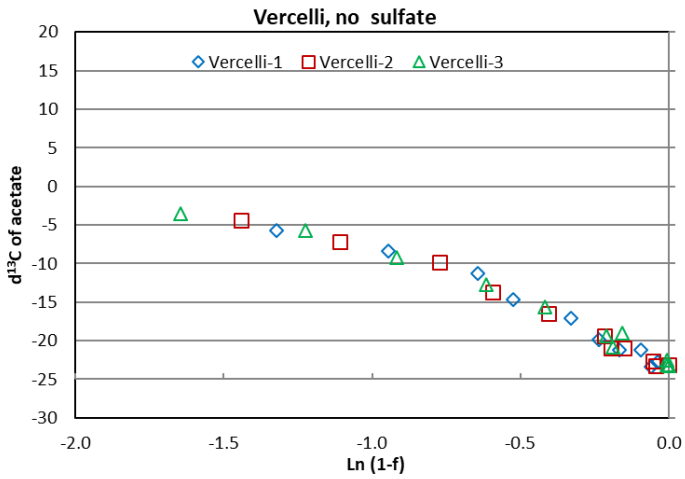

Vercelli
IRRI
NE
SW
TIC + CH4 corrected (mM)
Acetate consumed (mM)
control,1
control,2
control,3
CaSO4-1,1
CaSO4-1,2
CaSO4-1,3
CaSO4-2,1
CaSO4-2,2
CaSO4-2,3
CaSO4-1
CaSO4-2
CaSO4-3
A
B
C
D

Fig. S8

Vercelli



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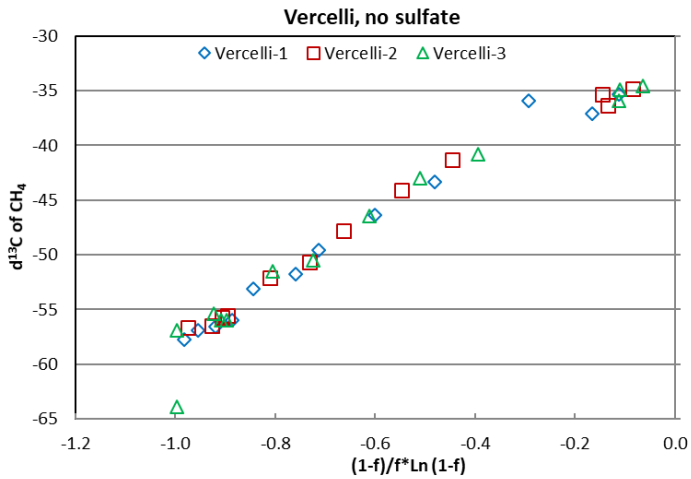

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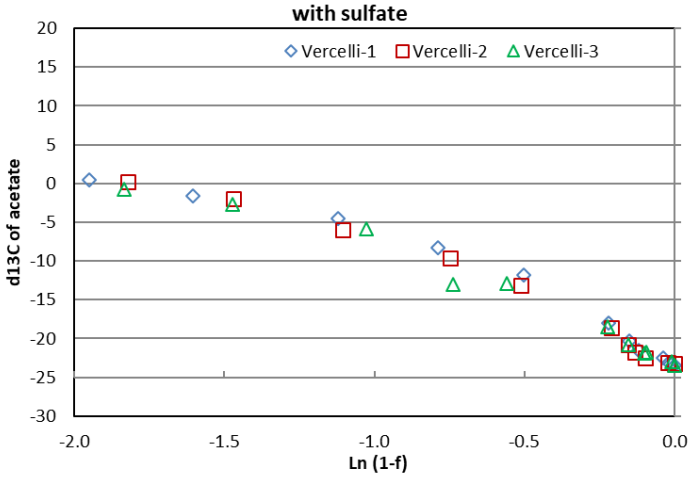

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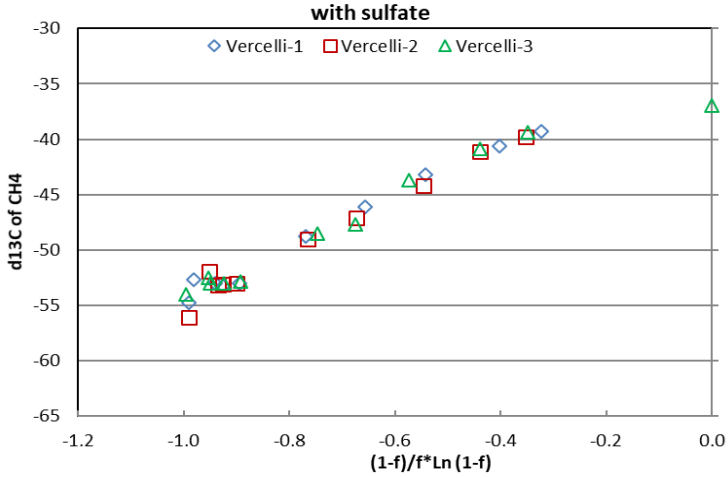

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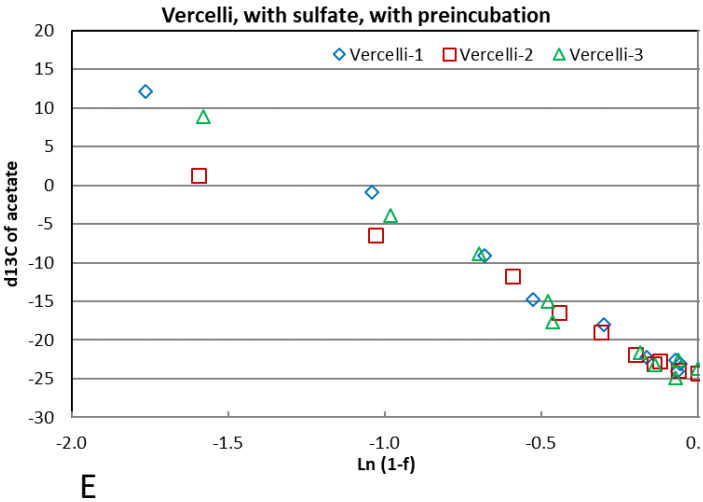

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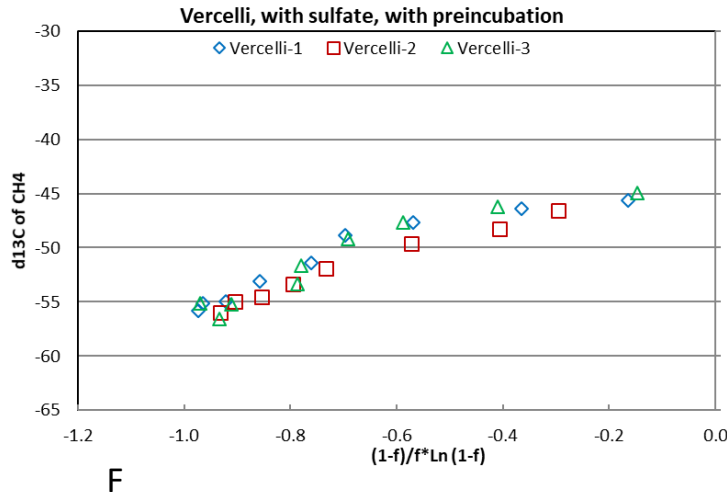
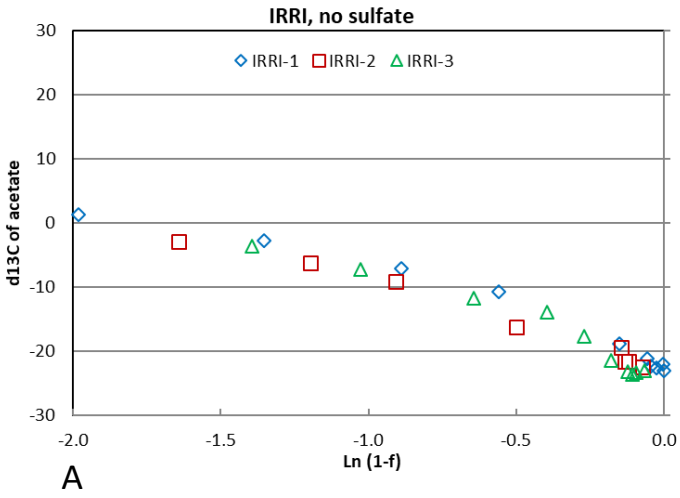


F

Fig. S9

IRRI



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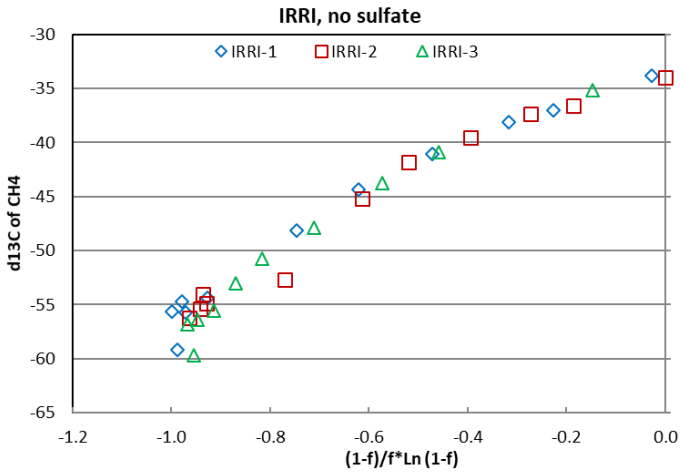

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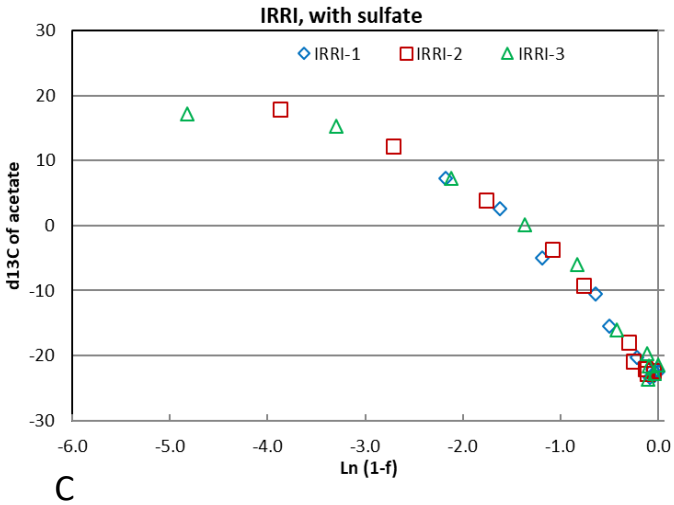

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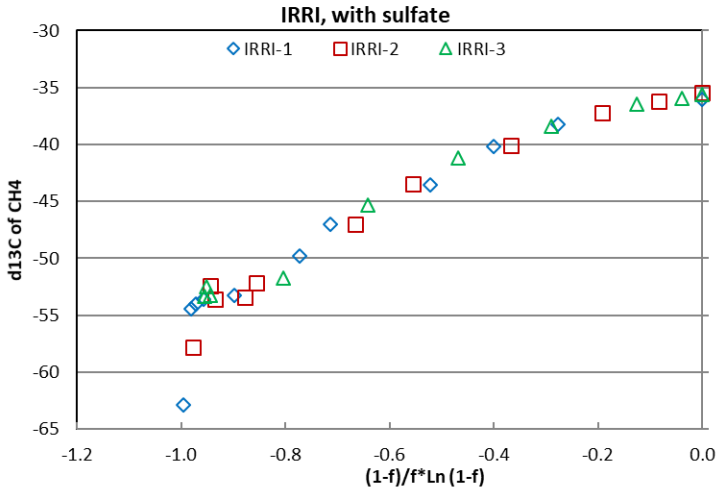

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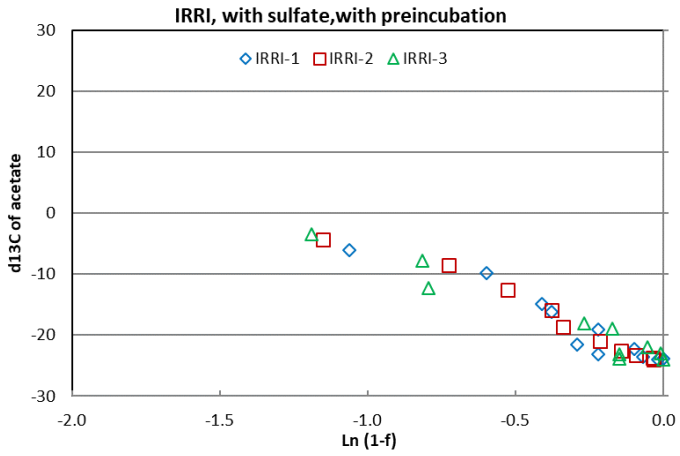

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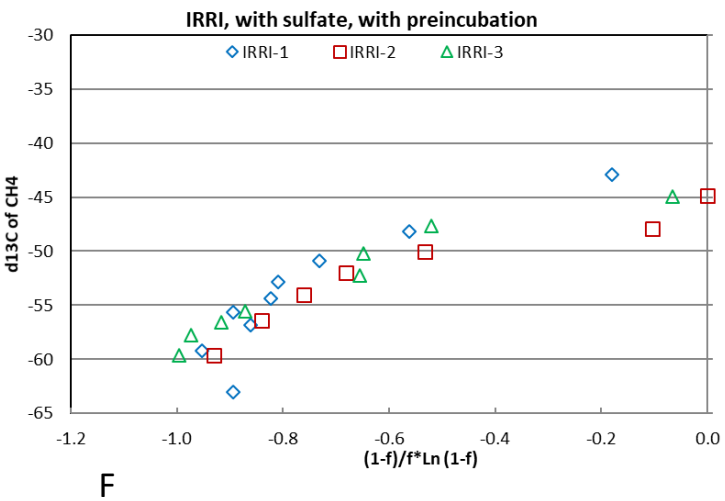
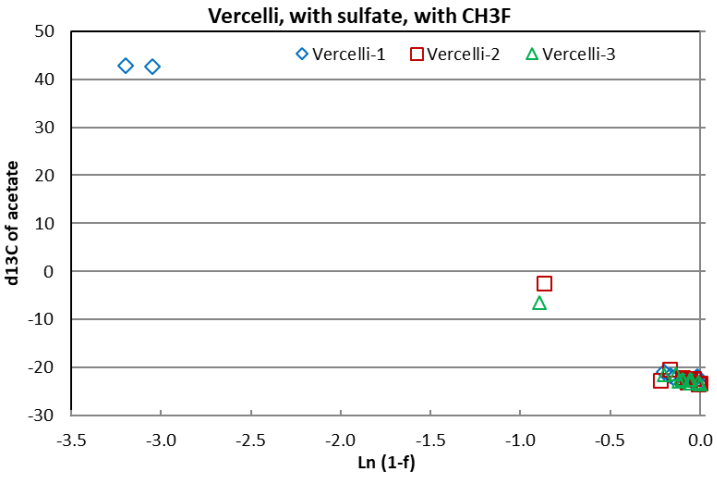


F

Fig. S10

Incubation with sulfate + CH3F



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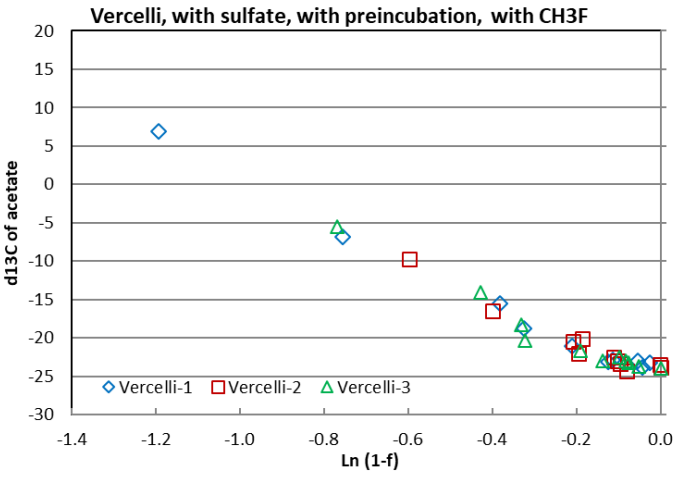

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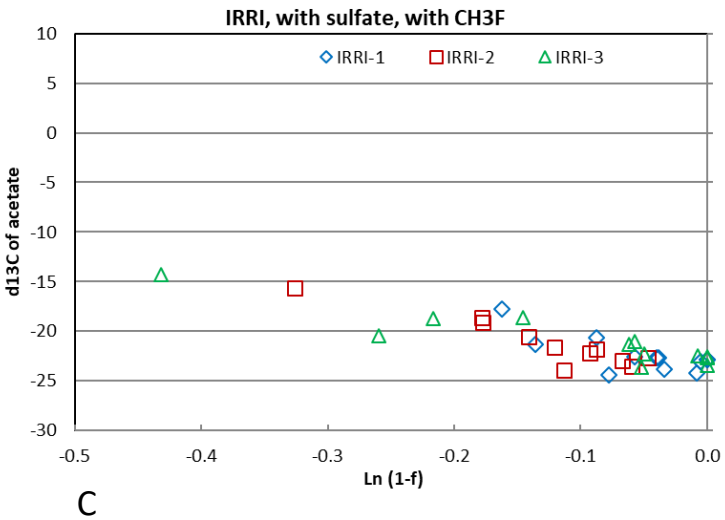

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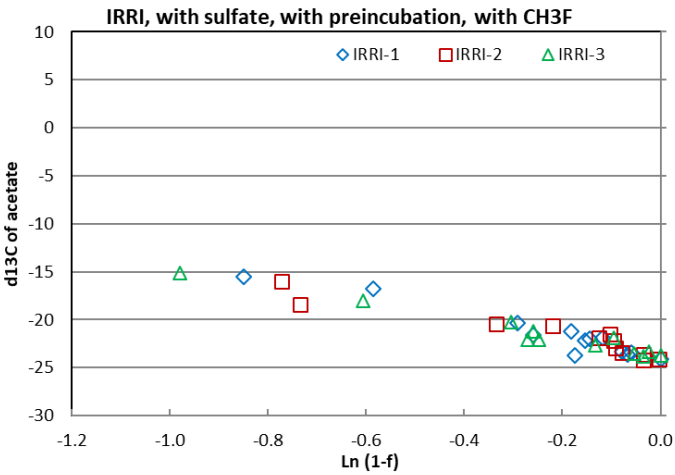
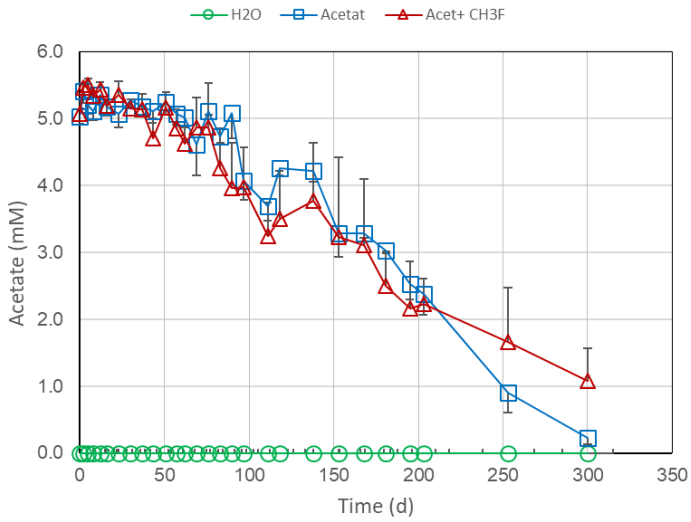


D

Fig. S11

Fuchskuhle – **SW**
without sulfate



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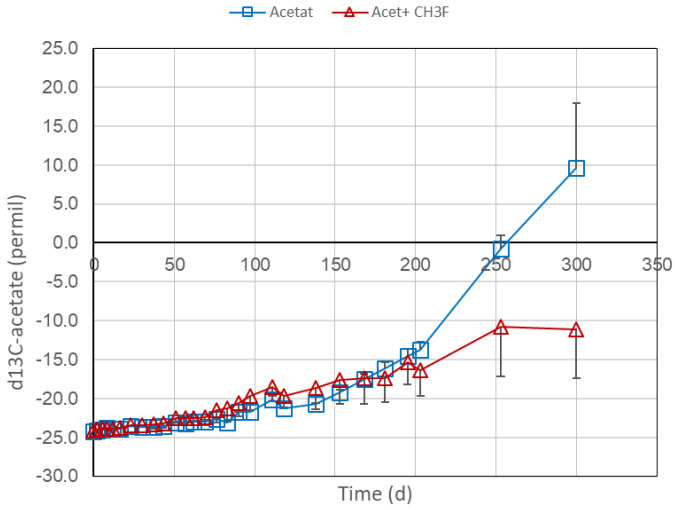

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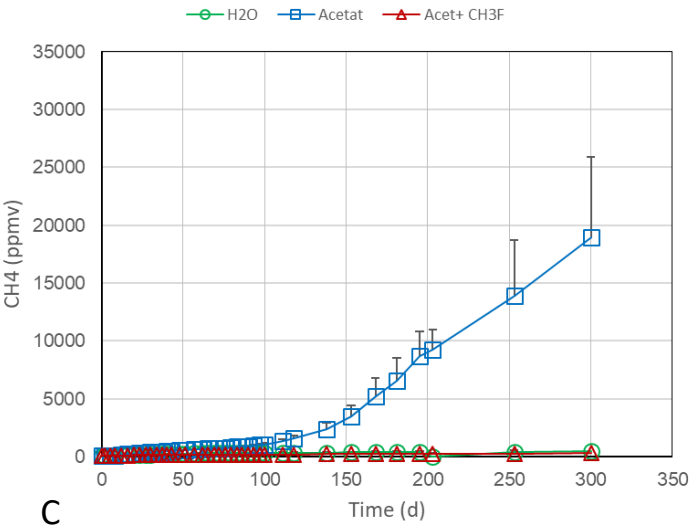

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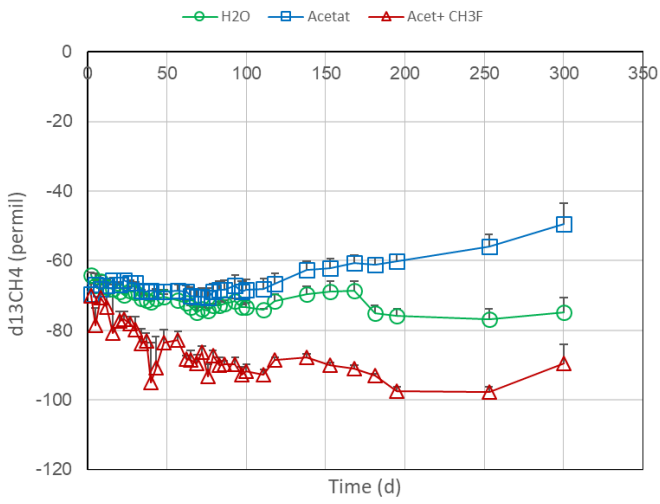
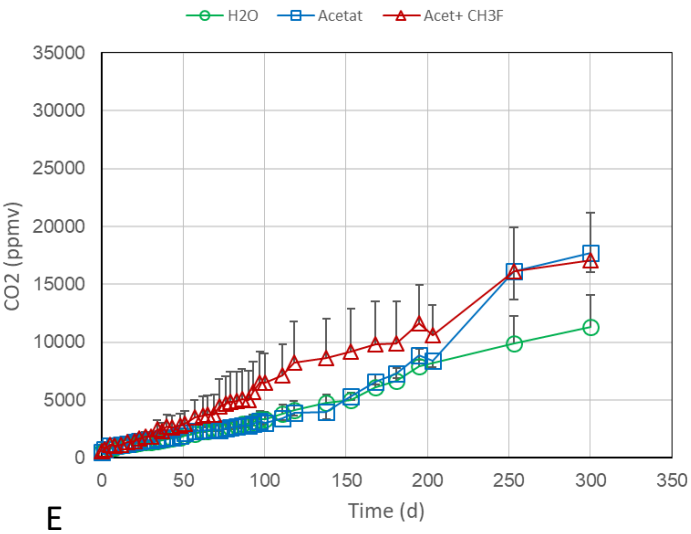


D



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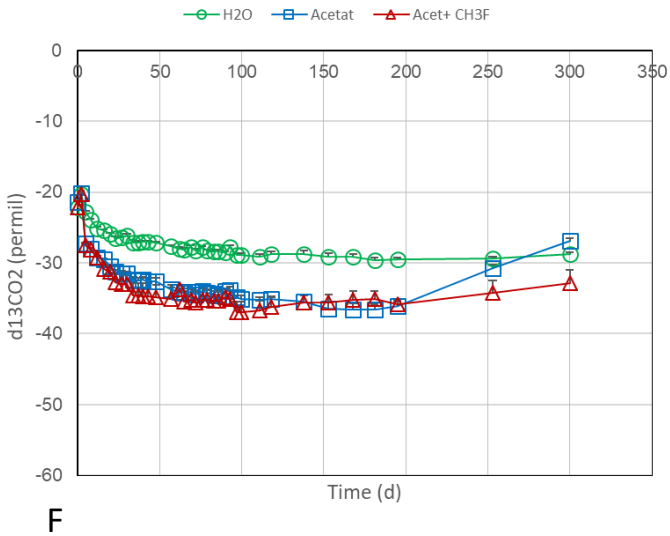
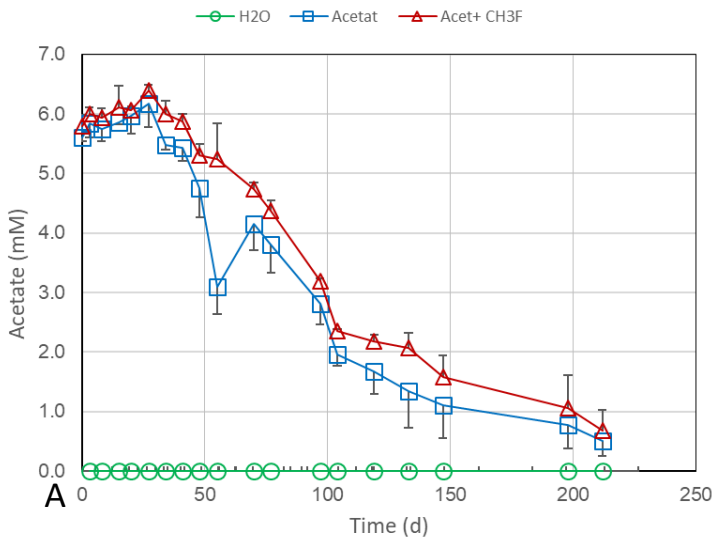


F

Fig. S12

Fuchskuhle – **SW**
with sulfate



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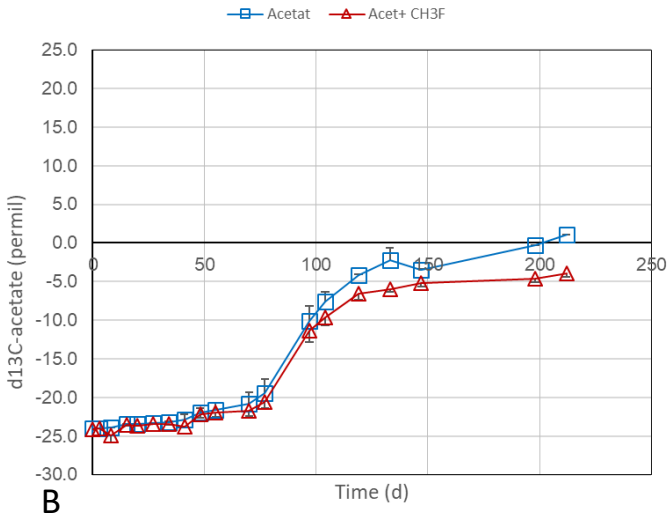

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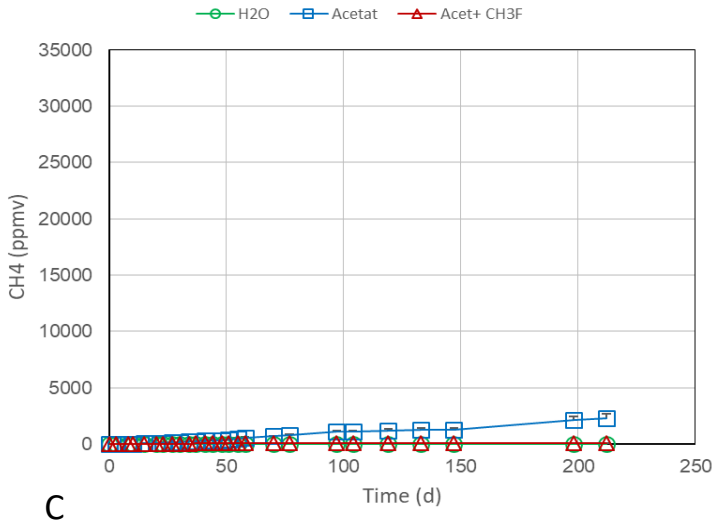

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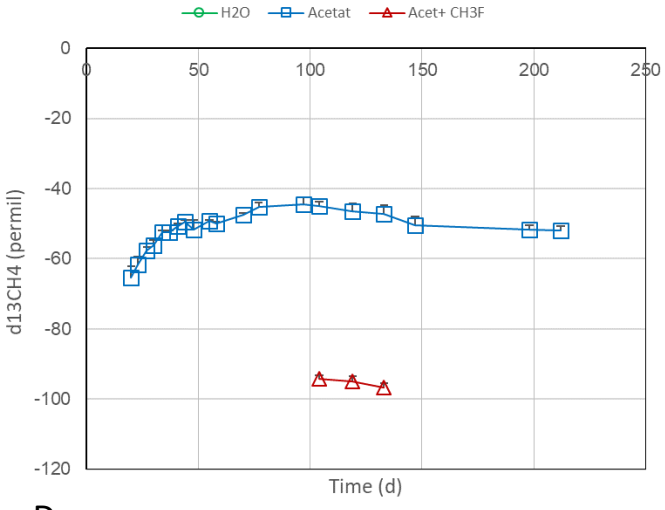

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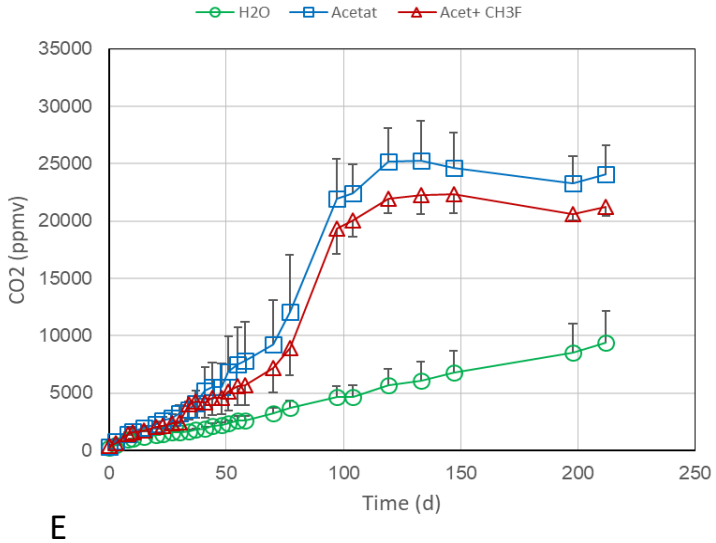

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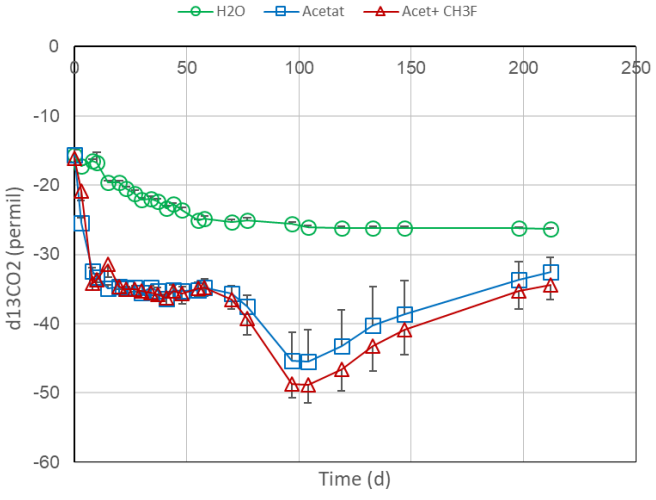
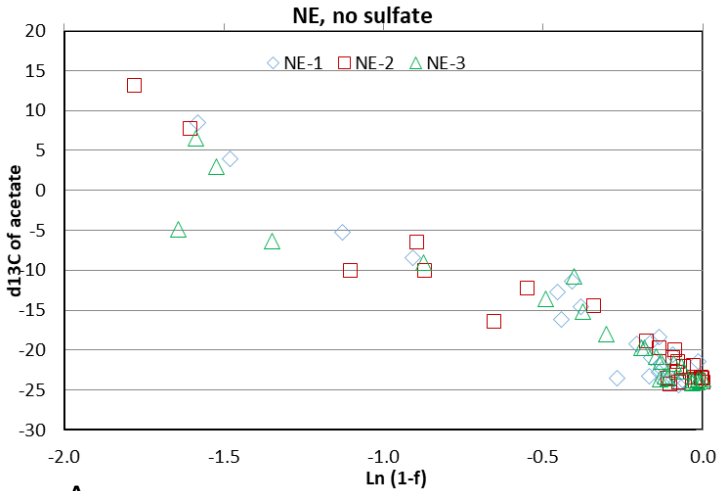


F

Fig. S13

Fuchskuhle – **NE**



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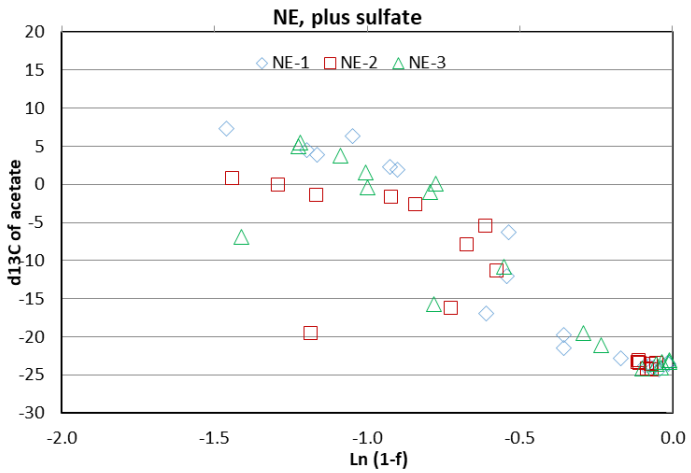

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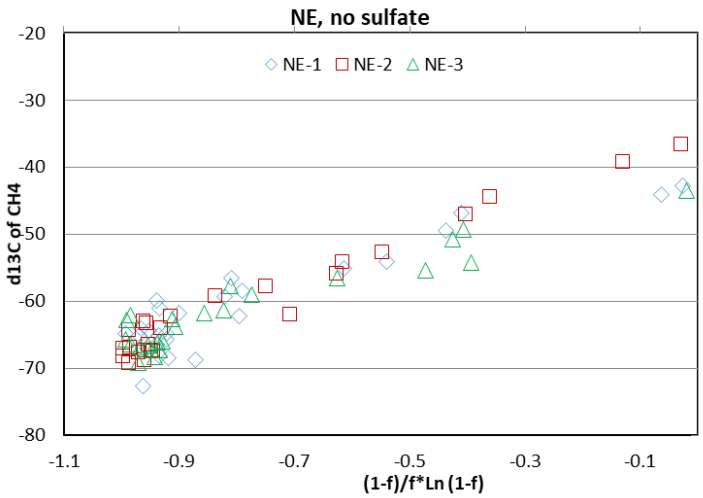

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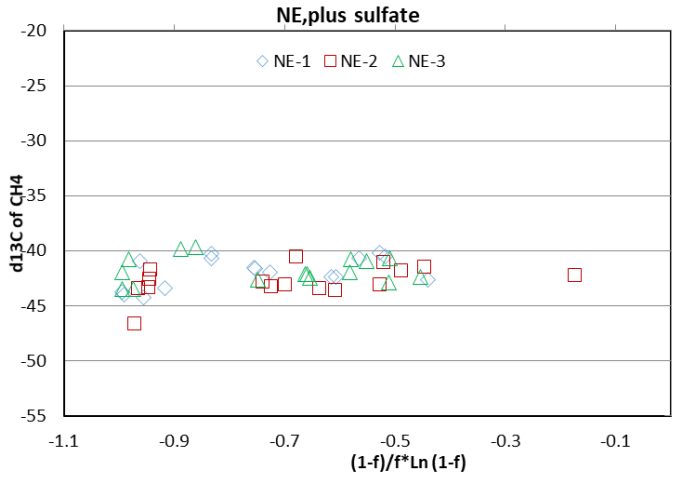
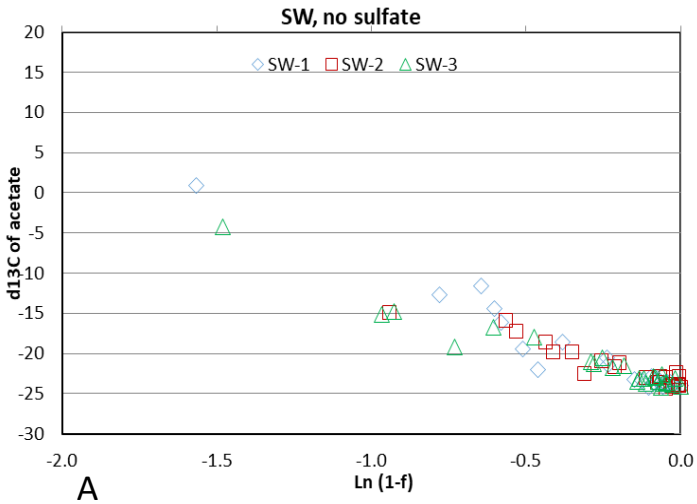


D

Fig. S14

Fuchskuhle – **SW**



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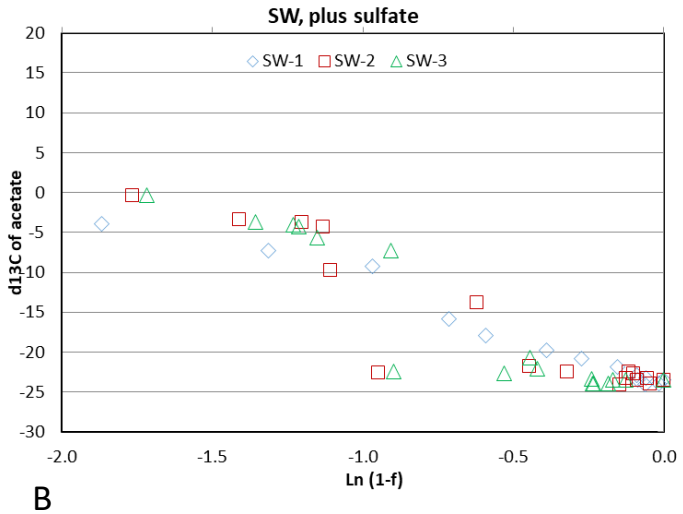

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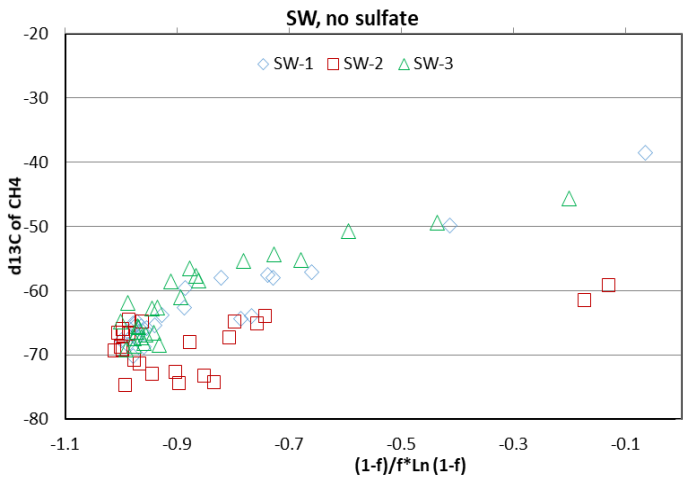

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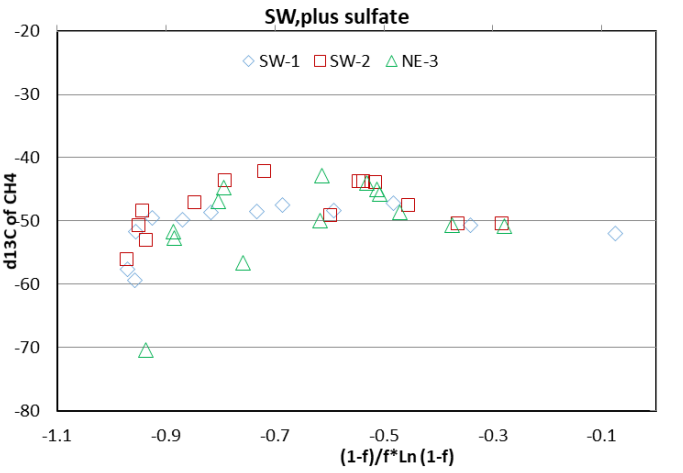
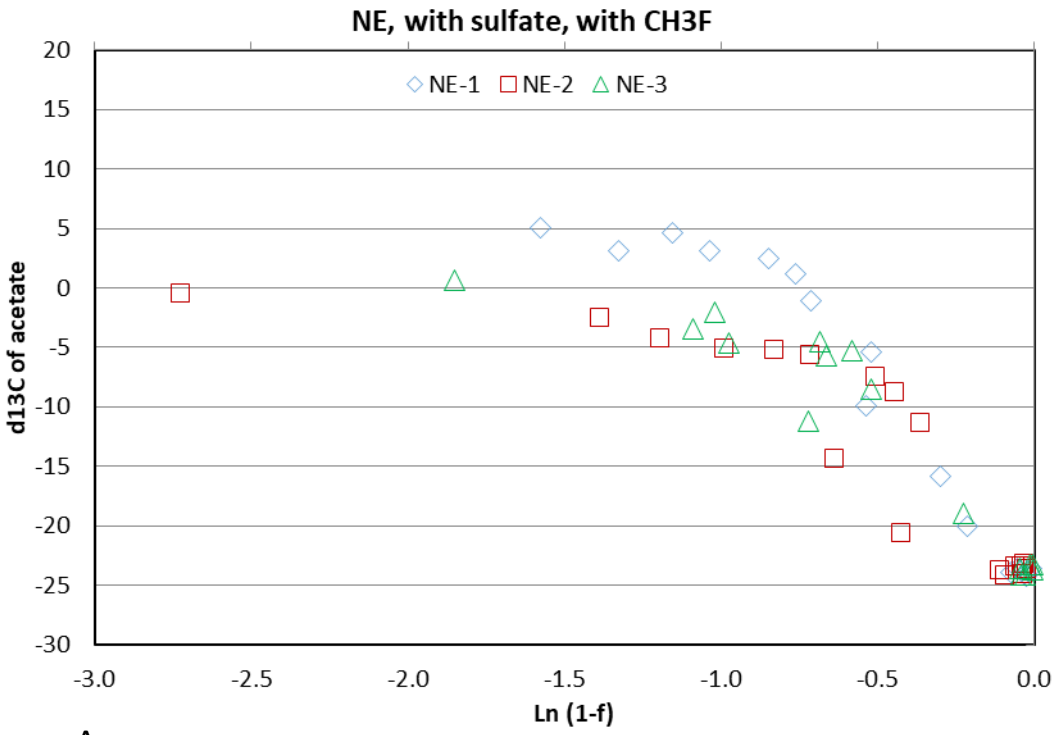


D

Fig. S15

Fuchskuhle
Incubation with sulfate and with CH3F



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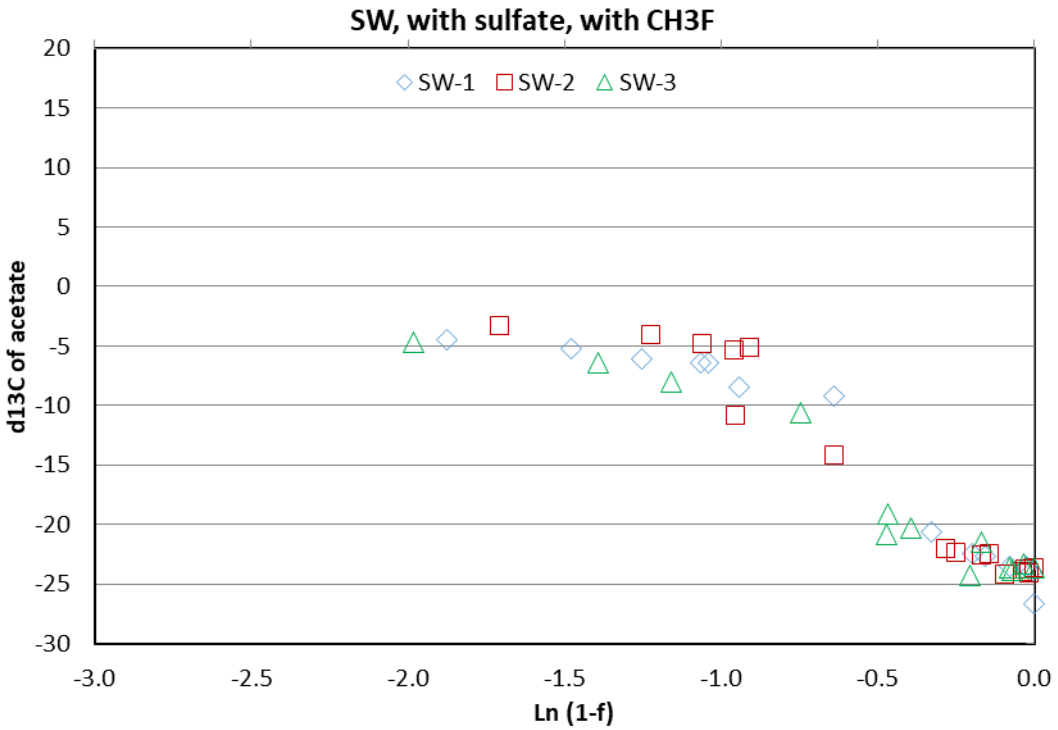

B

Fig. S16