Supporting Information for The behaviour of low molecular weight organic carbon-14 containing compounds in contaminated groundwater during denitrification and iron-reduction.

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This consists of five sections, S1, S2, S3, S4 and S5.

S1. Method for ^{14}C measurement in solid fraction

In to a 250 mL reaction vessel 1 ±0.01g of sediment was added with a magnetic stirrer. The system was sparged with N_2(g) prior to and during the extraction process, gas flow rate was set to 40 mL/min. Once sparged the reaction vessel was attached to two 125 mL gas washing bottles, the first of which contained 100 mL of Carbo-Sorb E and the second 100 mL of 1 M NaOH (acting as a safety bottle to ensure no release of $^{14}CO_2$(g)). 20 mL of 2 M H_2SO_4 was added to the reaction vessel to achieve a pH ~ 3 when buffered by sediment. It was left to react for 30 minutes before disconnecting the gas washing bottles and exchanging for duplicate wash bottles. For the oxidation step 20 mL of 5% potassium persulfate and 4 mL 4% $AgNO_3$ were added to the container and heated to 80-90 °C. Further additions of $K_2S_2O_8$ and $AgNO_3$ at the same concentration and volume were made after one and two hours of reaction. The system was left for another hour to give a total oxidation reaction time of 3 hours. Triplicate samples of 1 mL were collected from wash bottles containing Carbo-Sorb E and mixed with 10 mL of PermaFluor E (Ahn et al., 2003; Magnusson et al., 2007). All samples were left to dark adjust for 24 hours prior to counting on the liquid scintillation counter.

S2. Experimental results for complete run

Experiments were run until a minimum of 80% of any ^{14}C-labelled LMWO was removed from solution. The complete data sets from addition of LMWO to the completion of the experiments for each condition are shown below.

S2.1 Nitrate amended microcosms, 7 to 22 days.

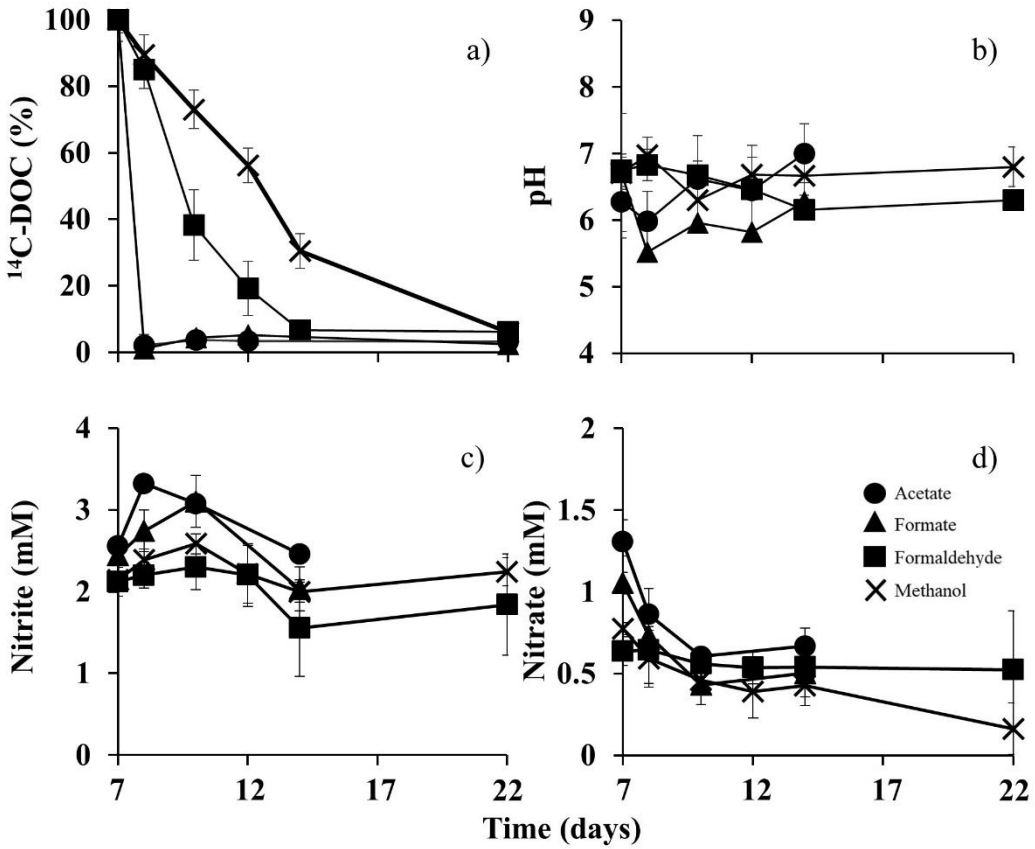

Figure S1 The results of the complete run of denitrification experiments (7 to 22 days) a) the percentage of ^{14}C in solution; b) pH; c) concentration of nitrite in solution; d) concentration of nitrate in solution (original nitrate addition = 10 mM, t=7 indicates time of ^{14}C addition). Error bars denote one standard deviation of triplicate measurements; where not shown error bars are less than the size of the symbol used.

S2.2 Iron-reducing microcosms, 28 to 60 days

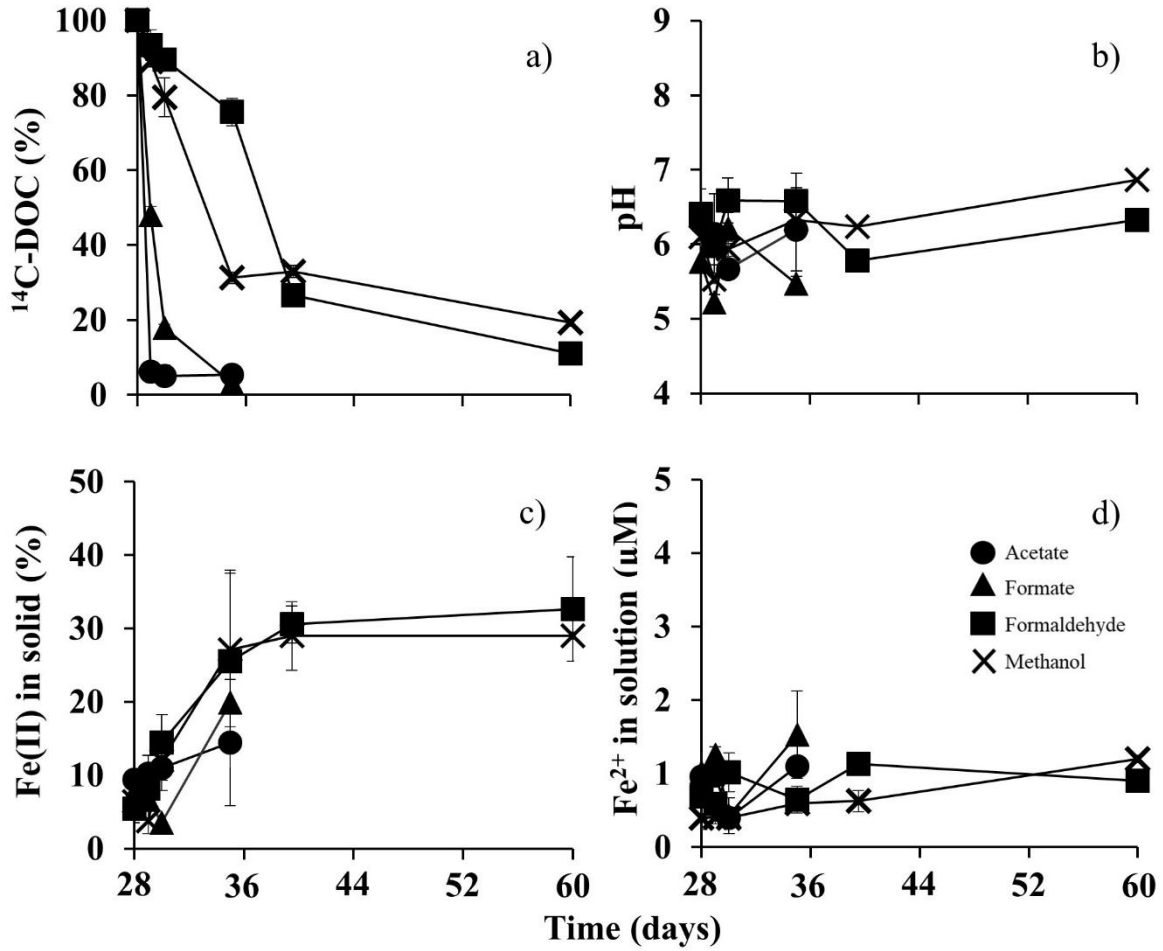

Figure S2 The results of the complete run of iron-reducing experiments (28 to 60 days) a) the percentage of ^{14}C in solution; b) pH; c) fraction of Fe(II) in solids; d) concentration of Fe^{2+} in solution. Error bars denote one standard deviation of triplicate measurements; where not shown error bars are less than the size of the symbol used.

S3. Results of control experiments

Control experiments were run concurrently with active microcosms with the same incubation time to establish the desired redox condition. After the incubation time (7 days and 28 days for denitrification and iron reduction respectively) the microcosms were autoclaved at 120˚ for 30 minutes to eliminate microbial activity and the LMWO was added. The experiments were then run over the subsequent seven days to determine any abiotic processes which may affect the aqueous ^{14}C concentration of the LMWO. The results of these experiments are shown below.

S3.1 Nitrate amended control microcosms

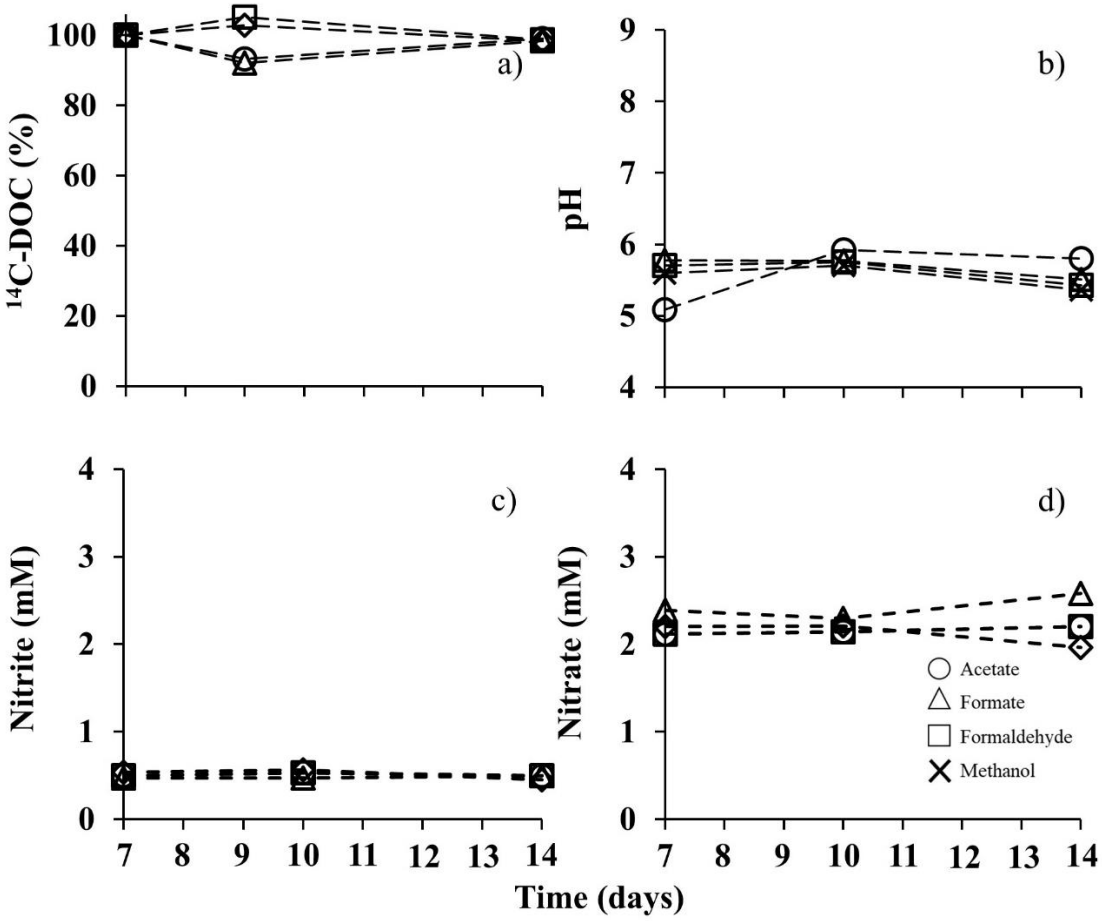

Figure S3 The results of the denitrification control experiments a) the percentage of ^{14}C in solution; b) pH; c) concentration of nitrite in solution; d) concentration of nitrate in solution (original nitrate addition = 10 mM, t=7 indicates time of LMWO addition). Experiment duration from day 7 to day 14.

S3.2 Iron-reducing control microcosms

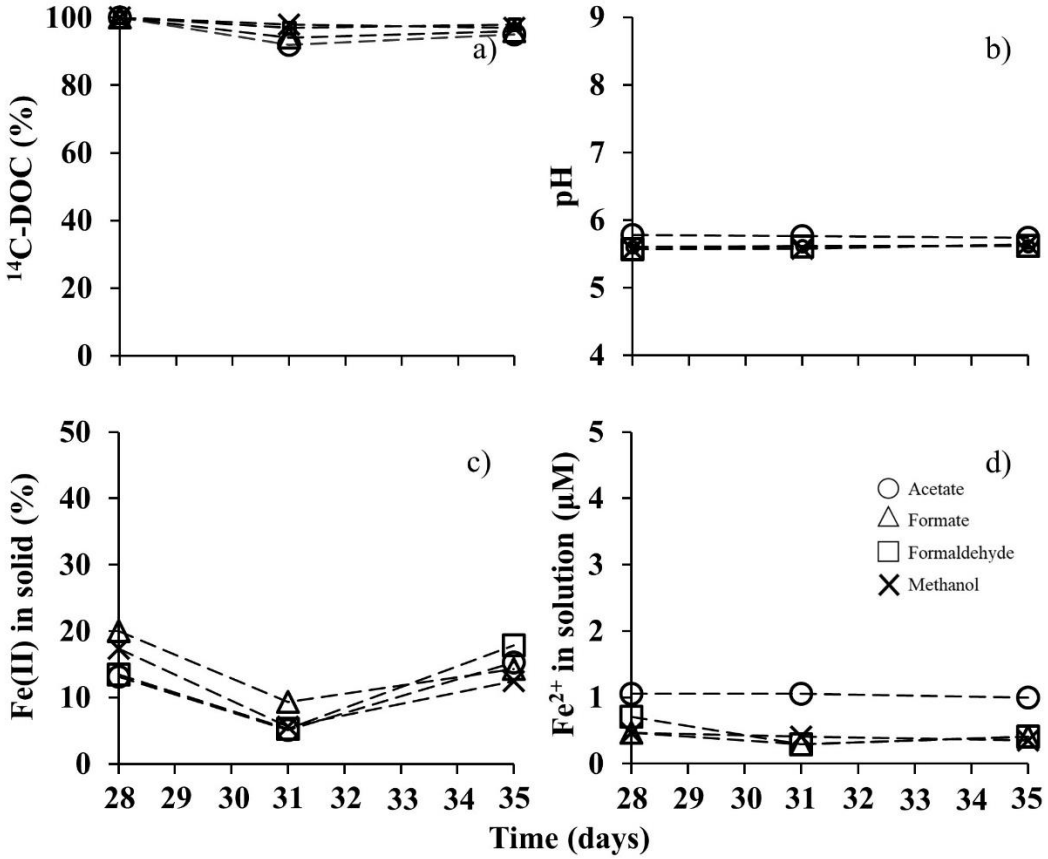

Figure S4 The results of the iron-reducing control experiments a) the percentage of ^{14}C in solution; b) pH; c) fraction of Fe(II) in solids; d) concentration of Fe^{2+} in solution. Experiment duration from day 28 to day 35.

S4. OTU table

Table S1 Summary OTU table with all identified phylum and significant orders (italics) for all samples

	Unaltered			Denitrification				Iron-reducing			
	A	B	C	Acetate	Formate	Formaldehyde	Methanol	Acetate	Formate	Formaldehyde	Methanol
Acidobacteria	28.30	29.99	26.01	34.88	35.45	28.08	7.69	24.69	24.29	10.60	19.23
Actinobacteria	14.54	6.48	10.10	9.56	9.07	14.66	6.63	11.93	14.34	9.11	3.79
Armatimonadetes	1.23	1.06	2.03	1.01	1.00	0.86	0.09	0.23	0.28	0.45	2.28
Bacteroidetes	1.02	2.43	5.76	1.55	1.58	5.50	5.38	3.81	3.36	4.27	3.91
Chlamydiae	0.01	0.01	0.26	0.01	0.02	0.17	0.01	0.01	0.02	0.01	0.04
Chloroflexi	1.14	1.08	1.69	2.04	1.39	1.95	0.91	2.19	2.06	0.56	1.90
Deinococcus-Thermus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gemmatimonadetes	2.61	0.89	2.16	2.92	3.26	2.07	0.77	2.01	2.29	1.72	2.66
Planctomycetes	7.37	10.48	10.80	7.95	7.94	5.75	2.87	6.79	7.52	4.85	5.23
Alphaproteobacteria	9.99	12.64	12.78	10.98	11.05	9.92	6.22	13.16	11.78	13.88	9.40
Betaproteobacteria	5.67	9.16	6.76	11.52	13.10	12.19	34.77	21.51	21.60	37.62	28.58
Burkholderiales	1.47	1.72	2.29	6.44	7.33	8.15	32.16	14.60	10.64	34.41	9.48
Methylophilales	0.00	0.01	0.12	0.00	0.00	0.18	0.01	0.00	0.00	0.11	0.11
Rhodocyclales	0.01	0.01	0.10	0.01	0.00	0.01	0.13	0.19	0.27	0.01	0.02
Deltaproteobacteria	1.51	2.08	2.32	0.90	0.94	1.16	0.22	0.69	0.52	0.63	1.06
Gammaproteobacteria	3.12	2.61	3.87	3.39	3.08	4.19	30.39	2.81	2.25	7.87	10.86
Chromatiales	0.02	0.01	0.09	0.12	0.08	0.07	0.01	0.06	0.02	0.02	0.05
Legionellales	0.05	0.07	0.24	0.03	0.07	0.19	0.02	0.04	0.03	0.12	0.19
Pseudomonadales	0.46	0.52	0.63	0.59	0.45	0.55	0.25	0.38	0.40	0.40	0.26
Xanthomonadales	0.23	0.62	1.15	0.39	0.42	1.69	29.69	0.77	0.62	5.75	4.42
Proteobacteria - unclassified	3.92	2.88	3.19	2.82	2.70	2.04	0.82	2.29	1.86	0.99	1.64
Spiro	0.01	0.03	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Synergistetes	0.17	0.06	0.11	0.33	0.24	0.27	0.08	0.22	0.28	0.12	0.19
Verrucomicrobia	13.38	12.27	6.84	6.29	5.44	6.09	1.24	3.34	3.14	4.59	5.16
BRC1	0.05	0.12	0.08	0.02	0.02	0.02	0.00	0.00	0.00	0.07	0.01
candidate_division_WPS-1	0.37	0.50	0.55	0.24	0.25	0.39	0.07	0.10	0.10	0.18	0.35
candidate_division_WPS-2	0.34	0.64	0.63	0.44	0.33	0.49	0.08	0.20	0.21	0.22	0.31

Candidatus_Saccharibacteria	0.90	1.04	1.18	0.45	0.51	0.91	0.23	1.07	0.57	0.37	1.60
Cyanobacteria/Chloroplast	0.01	0.03	0.09	0.00	0.00	0.02	0.01	0.02	0.00	0.02	0.02
Firmicutes	2.02	0.92	1.64	2.03	1.89	1.35	1.22	2.40	2.99	0.64	0.85
Hydrogenedentes	0.01	0.02	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01
Latescibacteria	1.64	2.33	0.75	0.32	0.30	1.76	0.16	0.13	0.18	1.15	0.75
Microgenomates	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nitrospirae	0.65	0.25	0.27	0.34	0.43	0.16	0.15	0.38	0.35	0.05	0.17
Parcubacteria	0.01	0.00	0.08	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.02
Poribacteria	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SR1	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

S5. Siderite precipitation

Table S2 Input solution composition for siderite precipitation model

Solution composition	**g/L in DIW**
pH	6.7
Pe	0
KCl	0.006
$MgSO_4.7H_2O$	0.976
$MgCl_2.6H_2O$	0.081
NaCl	0.0094
Fe(II)	0.02
C as CO_3^{2-}	0.02

Geochemical modelling was undertaken using the PHREEQC (version 3) geochemical speciation program (Parkhurst and Appelo, 2013) and the Hatches database (version 18)(Cross and Ewart, 1991). Siderite saturation index predicted to be +0.02 under these conditions.

References

Ahn, H.J., Song, B.C., Sohn, S.C., Lee, M.H., Song, K., Jee, K.Y., 2013. Application of a wet oxidation method for the quantification of ^{3}H and ^{14}C in low-level radwastes. Applied Radiation and Isotopes 81, 62-66.

Cross, J. E., & Ewart, F. T. (1991). HATCHES—a thermodynamic database and management system. Radiochimica Acta 52(2), 421-422.

Magnusson, Å., Stenström, K., Aronsson, P.-O., 2007. ^{14}C in spent ion-exchange resins and process water from nuclear reactors: A method for quantitative determination of organic and inorganic fractions. Journal of Radioanalytical and Nuclear Chemistry 275, 261-273.

Parkhurst, D. L., & Appelo, C. A. J. (2013). *Description of input and examples for PHREEQC version 3: a computer program for speciation, batch-reaction, one-dimensional transport, and inverse geochemical calculations* (No. 6-A43). US Geological Survey.