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Stable and Radio Isotope Analysis to Identify Methane Sources During a Remedial Action

Richard Coffin - Strategic Carbon LLC (rcoffin@strategic-carbon.com)
Jim Mueller (jim.mueller@provectusenv.com)

Background/Objectives: Methanogens/Archaea may be dominant microbes in reduced environments, and methanogenesis can be a component of anaerobic bioremediation. If Archaea are not controlled, then *in situ* remedial actions employing conventional (*i.e.,* no active control of Archaea) amendments such as [emulsified] oils/lecithins, lactates/sugars, simple hydrogen release compounds, conventional/original ISCR reagents, etc. can generate excessive amounts of methane (CH_4) during the reduction of carbon dioxide (CO_2). Methane can also be associated with deep earth deposits of crude oil, acquired crude oil in leaking tanks and pipelines or the degradation of petroleum carbon and subsequent reduction of CO_2 that is respired during petroleum degradation. The origin of CH_4 is not always clear.

Such was the situation where Provect-IR® antimethanogenic ISCR amendment (contains fermentable carbon sources, zero-valent iron, methane control technology, and other materials) was applied to treat chlorinated solvents in groundwater at a former dry cleaner facility. The technology rapidly and effectively reduced the concentration of PCE in groundwater thus meeting the project goals without the accumulation of intermediates (TCE, DCE or VC) and without excessive methanogenesis (*e.g.,* <2 mg/L) in the treated area. However, approximately nine months post-treatment, methane (5 to <10 mg/L) was quantified downgradient of the treated areas (See **Figure 1**). The possible sources of CH_4 are the carbon present in the initial amendments applied to enhance PCE degradation, petroleum hydrocarbons associated with previously identified underground storage tanks, or leaking sewage pipeline present through this study region (Figure 1). Alternatively the CH_4 was not a direct result of the remedial action, noting: i) the area of interest was directly associated with gasoline/Diesel USTs (removed but no active remediation was performed); ii) the soil gas contained CH_4 along with BTEX and other gasoline constituents and CH_4 is often generated (in large quantities) during the biodegradation of these compounds under hypoxic conditions; and iii) there was <250 ppm TOC in groundwater emanating from the upgradient active treatment area which was unlikely sufficient to generate large amounts of CH_4. In addition, there were sewer lines, utilities, multiple gasoline stations, and operational industrial activities in the immediate area which could also represents sources of TOC/ CH_4.

Approach: On September 16, 2017 (approximately 12 months after upgradient remedial action) field screening using a RKI model GK-2012 portable gas monitor (CO_2, CH_4, CO, H_2S) identified at least two wells (MW-15D and MW-16D) downgradient from the ISCR treatment area that contained elevated methane concentrations. Groundwater was collected from these wells (purple triangles – Figure 1), preserved on site accordingly to established methods, and transported to TAMU-CC on ice under standard chain-of-custody procedures for isotopic analyses (Coffin et al., 2008; 2015). To determine the carbon source in dissolved CH_4 and CO_2 the samples were radiocarbon ($\Delta^{14}C$) and stable carbon ($\delta^{13}C$) isotope analyses at the National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) facility, at Woods Hole Oceanographic Institution, MA (WHOI). CH_4 samples were oxidized to CO_2 for analysis at NOSAMS in the laboratory of Dr. Jeff Chanton (Florida State University).
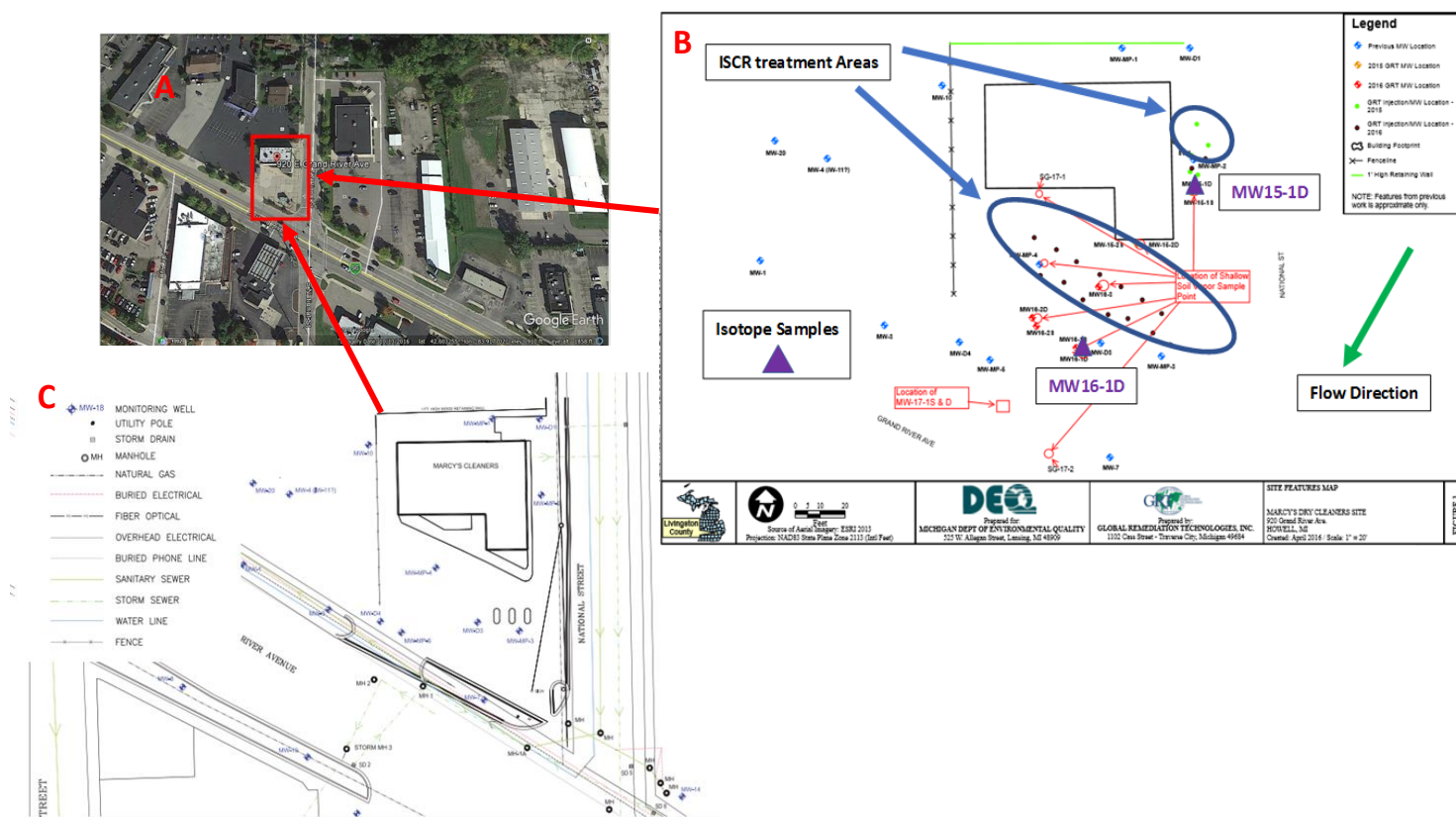
Figure 1: An overview of the sample location including; A) location of the dry cleaning facility; B) location of the monitoring wells including the sample wells reviewed in this study (purple triangles); C) distribution of sewer lines, storm water drains and utilities below ground in the study area.

Review of Field Sampling:

1. <u>Initial well assessment</u>: The portable gas monitor was used to measure CH_4, O_2, CO_2 and H_2S gas concentrations inside various monitoring wells (**Table 1, Figure 1**) and compared to atmospheric background to observe levels of spatial variation in the gas concentrations. In addition, depth to groundwater measurements determined that the shallow wells (screened from *ca*. 3 to 8 ft bgs) did not consistently contain water. Based on these data, groundwater samples were collected from wells MW16-1D and MW15-1D (screened from 9 to 14 ft bgs) for isotope analyses.

Table 1: Methane, oxygen, carbon dioxide, and sulfide overview with use of a handheld gas monitoring instrument. Note, %CH_4, %O_2 and CO_2 concentrations are estimates of values in the well water and were used to confirm the sample selections but does not represent sample concentrations.

WELL ID	Proximity to Treated Area	%CH_4	%O_2	CO_2 ppm
ATMS Background	Standards	0	20.9	0
MW16-1S	Downgradient ca. 20 ft	19	0	9
MW16-1D	Downgradient ca. 20 ft	27	0	212
MW MP-2	Within treated area	7	18	90
MW17-1S/D	Downgradient ca. 45 ft	1	12	0
MW15-1D	Downgradient ca. 10 ft	47	0	380

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2. <u>Sampling:</u> A peristaltic pump was used for down-well sampling (**Figure 2).** Samples were collected in glass serum bottles fitted with air-tight butyl rubber septa. For each well, four 500 ml bottles were filled 2x their volume and fixed with 5 ml of saturated $CuSO_4$. Sealed bottles were shipped via overnight courier at 5°C.

Figure 2: Overview of field sampling; A) handheld GX 2012 applied to down well air gas analysis, B) well 16-1 presented as an example of the wells sampled, C) open shallow and deep well, well pump used is included, D) well water samples.

Carbon Isotope Analysis:

Analysis of CO_2 $\Delta^{14}C$ and $\delta^{13}C$ was conducted at Woods Hole National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS, http://www.whoi.edu/nosams/Sample_Processes). Oxidation of CH_4 to CO_2 was with a closed inline combustion protocol at 580 °C followed by cryogenic distillation (Florida State University, Chanton et al., 2012). The evolved CO_2 gas (37.3 to 55.7 µmol) was transferred to individual break seals and sent to the Woods Hole National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) facility for $\Delta^{14}C$ and $\delta^{13}C$ analyses.

Data Interpretation:

Historic data demonstrate the following:

1. Petroleum based methane does not contain radiocarbon; petroleum age extends past the time for radiocarbon decay (Boyd *et al*., 2013; Coffin *et al.,* 2008, 2015).

2. Sewage carbon's ^{14}C content is a function of the CO_2 source assimilated in resource production at the bottom of the food chain. Previous reports indicate that food sources from marine to land environments is a modern carbon age (^{14}C rich, Colmen *et al*., 1995). The current $\Delta^{14}C$ of atmospheric CO_2 is near 0‰ (Levine *et al.,* 2008),

3. Carbon dioxide respired during a mix of petroleum based and bioenhancer degradation will have an intermediate radiocarbon content showing a mix of old and modern carbon. Through the remediation timeline, modern CO_2 from the bioenhancement with ISCR amendment will decline and petroleum sourcing will create CO_2 with ^{14}C-depleted (or ancient) signature (Coffin *et al*., 2008).

4. Stable carbon isotope data from ~-60‰ and lower is a source from biological CH_4 production (Whiticar, M. J. 1999).

$\delta^{13}C$ and $\Delta^{14}C$ of CH_4 and CO_2 are provided in the data review table inserted in Figure 2. Samples were taken with the understanding that their origin could be from:

1. Petroleum hydrocarbons from historic operations on site (USTs abandoned in place);
2. A source of CH_4 directly from sewage lines, and/or;
3. The ISCR amendment containing plant-based carbon.

While the ISCR amendment did focus on chlorinated compound (PCE and recognized catabolites) degradation, the contaminant concentration from *ca*. <1 to 70 mg/L total CVOCs hence the amount of carbon that could potentially be converted to methane or CO_2 (UMBB) was presumed to be insignificant in terms of its ability to impact these results.

The $\Delta^{14}C$ analysis assumes petroleum is "old carbon" and will therefore not have ^{14}C (~-999‰). Conversely, the ISCR amendment and sewage are comprised of new carbon and will show a "modern" enriched ^{14}C value (-200 to 0‰). More positive data values therefore indicate the CH_4 source is the sewage lines or ISCR amendment (*i.e.,* modern carbon) while more negative values reflect depleted "old" carbon indicating that the source is degradation of petroleum. An intermediate value would indicate both sources contribute to CH_4.

As both sewage and the ISCR amendment contain modern $\Delta^{14}C$, this analysis alone is insufficient to identify multiple origins of modern CH_4. To delineate between these potential sources of carbon it is also necessary to evaluate $\Delta^{14}C$ of the groundwater CO_2. Microbial degradation of the ISCR amendment will first yield CO_2 into the groundwater which would be subsequently reduced to CH_4. Hence, the groundwater CH_4 $\Delta^{14}C$ would be observed to be similar to the value of CO_2; not more ^{14}C enriched (Coffin *et al*., 2015). In contrast, pipeline sewage CH_4 with a more modern signature will be introduced directly into the groundwater. Oxidation of sewage CH_4 in the ground water will result in an enriched groundwater CO_2 $\Delta^{14}C$. Stable carbon isotope $\delta^{13}C$ data provides further assessment of the CH_4 source where $\delta^{13}C$ between -60 and -100‰ indicates a biogenic source and higher values (-40 to -60 ‰) indicate a petroleum source.

Results:

Methane Originates from Modern Microbially-Produced Carbon: Methane produced by bacteria that cycle modern atmospheric carbon will typically have a $\delta^{13}C$ of -60‰ and a $\Delta^{14}C$ of near 0‰. For this study $\delta^{13}C$ CH_4 values of –57.96‰ to -60.61‰ show the gas source is biogenic, produced from organic carbon biodegradation (**Figure 2A**). Considering the $\Delta^{14}C$ data, CH_4 in groundwater from both well locations was likely produced from modern carbon as high as -40.95‰ (**Figure 2B**), hence there was no discernible contribution from petroleum gas or biodegraded petroleum which would contain older carbon (-999‰, **Figure 2C**). **Hence, the potential sources of enriched $\Delta^{14}C$ at this study site are ISCR amendment degradation and a leaking sewage line.**

$\Delta^{14}C$ and $\delta^{13}C$ Data Review

Stable Carbon and Radiocarbon Data Summary

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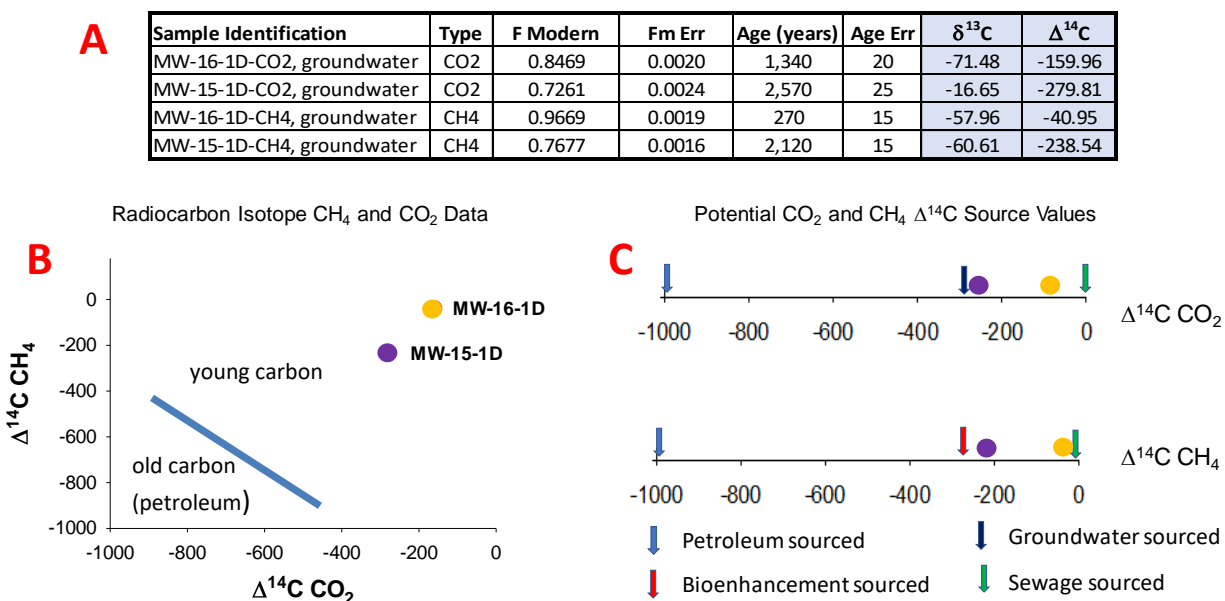
Sample Identification	Type	F Modern	Fm Err	Age (years)	Age Err	$\delta^{13}C$	$\Delta^{14}C$
MW-16-1D-CO2, groundwater	CO2	0.8469	0.0020	1,340	20	-71.48	-159.96
MW-15-1D-CO2, groundwater	CO2	0.7261	0.0024	2,570	25	-16.65	-279.81
MW-16-1D-CH4, groundwater	CH4	0.9669	0.0019	270	15	-57.96	-40.95
MW-15-1D-CH4, groundwater	CH4	0.7677	0.0016	2,120	15	-60.61	-238.54

Figure 2: This figure provides an overview of our data interpretation. A) $\Delta^{14}C$ and $\delta^{13}C$ for CH_4 and CO_2 samples taken at the well locations and discussed in this summary are highlighted in blue. Radiocarbon data are listed as fraction modern and error, carbon age and error [for external reference], and $\Delta^{14}C$ to provide capability to compare these data with other studies. B) Radiocarbon ($\Delta^{14}C$) is compared for CH_4 and CO_2 samples taken from MW-16-1D and MW-15-1D (Figure 1). C) $\Delta^{14}C$ CO_2 and CH_4 data (section B in this figure) are compared with potential endmembers from this study site. Petroleum carbon $\Delta^{14}C$ will be -999‰, with no measureable ^{14}C present (Coleman *et al.*, 1995). Groundwater CO_2 will be moderately depleted in ^{14}C with a value of -279.81‰ (e.g., Coffin *et al.,* 2008); this value varies between ecosystems, depending on pavement capping vs gas flux from the atmosphere and plant growth vs. industrial activity. Sewage CH_4 and CO_2 found in the groundwater wells would come from leaking pipelines and $\Delta^{14}C$ would be modern, originating from recent carbon production.

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The ISCR Amendment was the Primary Source of CH_4 near MW15-1D: The well MW15-1D was located near the former dry-cleaning facility. Approximately 9 months after treatment with the antimethanogenic ISCR amendment elevated levels of CH_4 (47%) and CO_2 (380 ppm) were observed in the headspace gasses of this well (**Figure 1, Table 1**). The $\Delta^{14}C$ measured at this location for CH_4 and CO_2 were -238.54‰ and -279.81‰, respectively (**Figure 2A, C**). In addition, MW15-1D was observed to have a CO_2 $\delta^{13}C$ of -16.65‰. The typical range for freshwater $\delta^{13}C$ CO_2 is -30‰ to 0‰ (Boutton, 1991). Considering wheat as the primary labile carbon in the ISCR amendment with a $\delta^{13}C$ value in the range of -27‰ (Wang et al., 2015), the CO_2 isotopic signature at MW15-1D reflects a mix of ambient groundwater CO_2 and ISCR amendment carbon. This site did contain the highest CO_2 concentration and $\%CH_4$ for of the wells tested (Table 1). With active microbial degradation of ISCR amendment an anoxic environment was established and CH_4 production occurred through methanogenesis and/or CO_2 reduction. The $\Delta^{14}C$ of CO_2 in the groundwater was -279.81‰ and subsequent CH_4 was -238.54‰ (**Figure 2B, C**). The moderate enrichment in this $\Delta^{14}C$ could be a result of ISCR carbon contribution to groundwater CO_2.

Sewage Carbon was the Primary Source of CH_4 near MW16-1D: The most modern CH_4 $\Delta^{14}C$ value of -40.95‰ was observed at well MW16-1D, which was downgradient of the ISCR-treated area. This indicated that CH_4 was produced from methanogenic activity and/or microbial reduction of CO_2 during the degradation of very modern carbon which is presumably sewage from the adjacent pipelines that, subsequently, leaked into study region groundwater (**Figure 2C**). This well was in close proximity to the sewage line (**Figure 1**). This statement is further supported with observation of the CO_2 $\Delta^{14}C$ and $\delta^{13}C$ at MW16-1D. At this well location CO_2 $\delta^{13}C$ was lowered to -71.48‰ representative of oxidation of CH_4 ($\delta^{13}C$ -57.95‰). In addition, the CO_2 $\Delta^{14}C$ value of -159.96‰ indicated the oxidation of modern sewage CH_4 and mixing with an older $\Delta^{14}C$ value (-279.81‰, MW15-1D). Lastly, the modern $\Delta^{14}C$ value for atmospheric CO_2 is approximately 0‰ (Levine et al., 2008) so the slight CH_4 $\Delta^{14}C$ depletion (-40.95‰ vs. 0‰) at MW16-1D indicated subsequent, moderate contribution of CH_4 production in the groundwater. This would be a result of continued sewage degradation and CH_4 production outside of the sewage pipeline.

Conclusions:

These data show with strong certainty two distinct sources of CH_4 at MW15-1D and MW16-1D (**Figure 1**). This statement is based on the following points.

1. $\Delta^{14}C$ CH_4 and CO_2 data are modern; there is no contribution from petroleum gas or microbial degraded petroleum.
2. $\delta^{13}C$ CH_4 shows the gas source at both sampling locations is biogenic, produced from organic carbon degradation.
3. Data suggested that each source is focused within its region, and there was little mixing of sources between the two wells, approximately 200 ft apart.
4. The most modern CH_4 ^{14}C signature was observed at well MW16-1D and the gas was produced from microbial reduction of CO_2 during the degradation of sewage (very young carbon) and/or subsequent leakage from the sewage lines.
5. The ISCR amendment was the primary source of carbon for CH_4 production at MW15-1D.

Recommendations:

1. These data indicate that the antimethanogenic component of the ISCR amendment persisted for approximately nine months. To reduced subsequent methanogenesis following successful destruction of targeted contaminants, lower amounts of the ISCR amendment can be considered. In addition, the ISCR amendment can be supplemented with alternative antimethanogenic compounds with increased longevity, such as an essential plant oil (garlic oil).

2. A more thorough assessment of CH_4 and CO_2 endmember concentrations and $\Delta^{14}C$ in various wells at the site would provide a more complete evaluation of the spatial range of impact for sewage and ISCR CH_4. At this time, a prescribed amount of garlic oil can be added to MW-15D and monitored for the ability to reduce the activity of methanogenic Archaea and the corresponding production of CH_4 in this area.

References:

Boyd, T.J., Pound, M.J., Lohr, D., Coffin, R.B. 2013. Radiocarbon-depleted CO2 evidence for fuel biodegradation at the Naval Air Station North Island (USA) fuel farm site. Environmental Science. Processes & Impacts; DOI:10.1039/c3em00008g.

Boutton, T.A. 1991. Stable carbon isotope ratios of natural material: II. Atmospheric, Terrestrial, Marine, and Freshwater Environment. Carbon Isotope Techniques EDS. D. Coleman and B. Fry. Academic Press.

Chanton, J.P., Cherrier, J., Wilson, R.M., Sarkodee-Adoo, J., Bosman, S., Mickle, A., Graham, W.M. 2012. Radiocarbon evidence that carbon from the Deepwater Horizon spill entered the planktonic food web of the Gulf of Mexico. Environmental Research Letters, doi:10.1088/1748-9326/7/4/045303

Coffin, R.B., Pohlman, J.W., Grabowski, K.S., Knies, D.L., Plummer, R.E., Magee, R.W., Boyd, T.J. 2008. Radiocarbon and stable carbon isotope analysis to confirm petroleum natural attenuation in the vadose zone. Environmental Forensics 9:75-84

Coffin, R.. B., Osburn, C.L., Plummer, R.E., Rose. P.S., Grabowski, K.S. 2015. Deep sediment methane incorporation into shallow sediment carbon pools in Atwater Valley, Texas-Louisiana Shelf, Gulf of Mexico. Energies *8*, 1561-1583; doi:10.3390/en8031561.

Colemen, D.D., Chao-Li, L., Hackley, K.C., Pelphrey, S.R. 1995. Isotopic Identification of Landfill Methane. Environmental Geosciences 3:95-103.

Levine, I., Hammer, S., Kromer, B., Meinhardt, F. 2008. Radiocarbon observations in atmospheric CO2: Determining fossil fuel CO2 over Europe using Jungfraujoch observations as background. Science of the Total Environment, 391:211-216.

Wang, J., Wang, X., Xu, M. Feng, G., Zhang, W., Yang, X., Huang, S. 2015. Contributions of wheat and maize residues to soil organic carbon under long-term rotation in north China. Environmental Chemistry, doi:10.1038/srep11409

Whiticar, M.J. 1999. Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. Chemical Geology, 161:291-314.

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