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# Carbon and Hydrogen Isotopes as Tracers of Methane Dynamic in Wetlands

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## Abstract

**This work presents a review of the main processes leading to the production and sinking of methane in wetlands and how they affect the stable isotope composition of carbon and hydrogen. Isotope fractionation factors associated to diffusion, ebullition, bacterial oxidation, etc., have been gathered from relevant literature in an intent of providing researchers in this thematic with practical procedures and tools for the interpretation of experimental data. Also it is presented guidelines of the most reliable field and laboratory methods used at present for the correct sampling and analyzing methane in different stages of occurrence, as well the most common tools used in their interpretation. Each statement is fully referenced to a long updated list of publications.**

## Keywords

## 1. Introduction

$CH_4$ is a chemically and radioactively important atmospheric trace gas and is the second most important greenhouse gas after $CO_2$. $CH_4$ concentration increased from ~800 ppb in the 1850s to ~1700 ppb in the late 2000s [1], suggesting an imbalance between estimated global emissions and removals. Decadal budgets for $CH_4$ sources and sinks between 1980 and 2010 reflect average imbalance of 6 Tg $CH_4$ $a^{-1}$ in the 2000s, 17 Tg $CH_4$ $a^{-1}$ in the 1990s and 34 Tg $CH_4$ $a^{-1}$ in the 1980s [2]. The total amount of global $CH_4$ emissions is currently estimated as 500 - 600 Tg $a^{-1}$ [3] and this $CH_4$ is emitted from a range of natural and anthropogenic sources as a result of the anaerobic decomposition of organic matter, land use changes and fossil fuel related emissions. Roughly, natural $CH_4$ sources (wetlands, termite activity, other wild ruminants, geological, fresh water systems, permafrost, hydrates, wild fires) are considered responsible for 40% of total emissions while 60% of emissions are related to

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human activities (waste decomposition, fossil fuels, rice cultivation, agriculture, livestock and the burning of biomass) [4]. Recently, living vegetation has also been suggested as an important natural source of $CH_4$ [5]. On the other hand, $CH_4$ is removed from the atmosphere by a variety of chemical and biological processes, which occur in different regions of the atmosphere. These include tropospheric and stratospheric oxidation and uptake by soils.

Although most sources and sinks of $CH_4$ have been identified, their relative contributions to atmospheric $CH_4$ levels are still highly uncertain. The quantification of individual contributions to global emissions has proved to be difficult, despite they are crucial to predict climate change and managing Earth's carbon reservoirs. The net rate of $CH_4$ emissions is generally estimated from three approaches: 1) extrapolation from direct flux measurements and observations; 2) process-based modelling (bottom-up approach) and 3) inverse modelling that relies on spatially distributed, temporally continuous observations of concentration, and in some cases isotopic composition in the atmosphere (top-down approach) [6]-[9]. Observations of the $^{13}C/^{12}C$ ratio of atmospheric $CH_4$ was used in isotope mass balance models to define the magnitudes of different sources and sinks and to constrain the present-day $CH_4$ budget using optimal estimation. Three $CH_4$ emission scenarios are estimated: 64% - 76% biogenic, 19% - 30% fossil, and 4% - 6% pyrogenic sources [10]. Biogenic sources contain $CH_4$-generating microbes (methanogens) and comprise anaerobic environments such as natural wetlands and rice paddies, oxygen-poor freshwater systems (lakes, rivers, streams, reservoirs such as dams), digestive systems of ruminants and termites, and organic waste deposits (such as manure, sewage and landfills). Thermogenic $CH_4$, formed over millions of years through geological processes, is a fossil fuel. It is vented from the subsurface into the atmosphere through natural features (such as terrestrial seeps, marine seeps and mud volcanoes), and through the exploitation of fossil fuels, that is, through the exploitation of coal, oil and natural gas. Pyrogenic $CH_4$ is produced by the incomplete combustion of biomass and soil carbon during wildfires, and of biofuels and fossil fuels. **Table 1** gives a data set of the main isotopic values of carbon that involved $CH_4$ sources.

Wetlands contribute as much as 70% of the total natural emissions [16] [17]. Their emissions from most types (marshes, swamps, bogs, and fens according to reference [18]) can vary by a few orders of magnitude over just a small number of meters, because they are influenced by a wide range of environmental parameters. These include soil characteristics such as the availability of organic carbon and nutrients, plant physiology, community composition and cover and water table depth and soil temperature. Seasonal and inter-annual variations of $CH_4$ wetland emissions are reported by focusing on three determinants keys: temperature, substrate availability and water table's level [19]-[21]. Besides these factors, the actual amount of $CH_4$ emitted to the atmosphere or rates of $CH_4$ emission depends on the balance between $CH_4$ production and consumption and the mode of methane transport. Wetlands transfer $CH_4$ to the atmosphere by diffusion, ebullition, and by transport through arenchymous vascular plants but only part of the $CH_4$ produced is emitted to the atmosphere [22]. Considerable $CH_4$ amounts are consumed or oxidized by methane-trophic bacteria in the rhizosphere and in surficial oxic layers during diffusive transport to the soil surface [23] [24].

The accuracy of predictions of $CH_4$ and ultimately the extent of climate change that can be expected in the earth system depend on a better understanding of $CH_4$ dynamics from wetlands for each region. Most studied, considering all processes, were done in wetlands from Northern Hemisphere [25]. However, until quite recently, ebullition was considered to be only locally important, and most attention was focused on matrix diffusion of dissolved $CH_4$ and plant-mediated transport. For instance, reference [26] used measurements to argue that ebullition has been temporally and spatially very variable and it could exceed diffusive fluxes by two orders of magnitude, stating that heterogeneity in ebullition (bubbling) is a major contribution to variability of emissions. So, it is necessary to study these systems at different regions as well as to know the suitable tools that would meet this purpose. In this review we present literature data that show how stable carbon isotopic signatures, in combination with other parameters, can be used to quantify the relative contribution of the major methanogenic
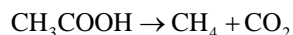
**Table 1.** Isotopic composition range from different sources of $CH_4$.

| Biogenic $CH_4$ (‰) | Thermogenic $CH_4$ (‰) | Pyrogenic $CH_4$ (‰) |
|---|---|---|
| $\delta^{13}C$-$CH_4$: −110 to −50 <br> $\delta^2H$-$CH_4$: −275 to −100 | $\delta^{13}C$-$CH_4$: −50 to −20 <br> $\delta^2H$-$CH_4$: −275 to −100 | $\delta^{13}C$-$CH_4$: −25 to −13 |
| [11] [12] [13] | [11] [13] | [14] [15] |

pathways, $CH_4$ oxidation and transport mechanisms.

## 2. Why to Apply the Stable Isotopes?

Wetlands, where conditions are more favourable to the accumulation of organic matter than to its decay, are characterized by a subsurface, anaerobic zone of $CH_4$ production, and organic soils (as peat) often submerged or water-saturated. The generation of $CH_4$ by bacteria requires a fully saturated environments that excludes atmospheric $O_2$ in the absence of other free-energy electron-acceptors such as $NO_3^-$ and $SO_4^{2-}$ and low redox (Eh < −200 mV), as can be observed in wetlands [27]. Methane-genesis is the final step in anaerobic decomposition and represents a complex process that proceed via two major metabolic pathways: acetate fermentation (1) and $CO_2$ reduction (2)

$$CH_3COOH \rightarrow CH_4 + CO_2$$

$$CO_2 + 4H_2 \rightarrow 2H_2O + CH_4 \ \text{ or } \ HCO_3^- + 4H_2 \rightarrow 2H_2O + CH_4 + OH^-$$

The measurement of stable isotopes of carbon on $CH_4$ and $CO_2$ is an effective way to identify the different phases of biodegradation of organic matter [28] [29]. According to these authors, $CO_2$ is isotopically light during the initial aerobic and anaerobic oxidation phases of biodegradation with $\delta 13C$ values that varies from −35‰ to −10‰, thus covering the range of most terrestrial plants [27]. The initial input of isotopically light $CO_2$ associated with earlier biodegradation phases is soon overcome during the methanogen phase by the constant input of isotopically heavy $CO_2$ associated with acetate fermentation and microbial $CO_2$ reduction (the two primary metabolic pathways by which microbial $CH_4$ is produced) (**Table 2**). During methanogenesis, $CH_4$ is enriched in the lighter carbon isotope ($^{12}C$) and the $CO_2$ associated with microbial $CH_4$ production is enriched in the heavier isotope ($^{13}C$). Thus, in a semiclosed environment such as a wetland, the $\delta^{13}C$ of $CO_2$ is strongly affected by methanogen reactions with reported values between −10‰ and +20‰. Values of $\delta^{13}C\text{-}CH_4$ due to fermentation of acetate and $CO_2$ reduction are −65‰ to −50‰ and −110‰ to −60‰, respectively [11] [30].

$CH_4$ production is regulated mainly by $O_2$ concentration as mentioned above, but also of pH, temperature, salinity, organic substrates and nutrient availability [31]. Some authors think that once anaerobic conditions are attained, the quality and supply of substrates is the major factor in $CH_4$ production: substantial amounts of $CH_4$ are only produced when labile carbon substrates are largely available and old (recalcitrant) wetland plays only a subordinate role as a substrate for $CH_4$ production [32]. Reach conclusions about the relative importance of methanogen pathways for $CH_4$ production are difficult because $\delta^{13}C\text{-}CH_4$ values of the upper organic horizon (above 1 m) are substantiality affected by the processes of $CH_4$ transport and oxidation [33]. So, based on differences in $\delta^{13}C\text{-}CH_4$ among organic layer and isotopic fractionation factors associated with gas transport and oxidation, these authors assessed the potential quotas of transported/oxidized $CH_4$ Also, reference [34] used a simple mixing model to estimate the relative proportion of the acetate fermentation and $CO_2$ reduction pathway.

$CH_4$ is produced at depth and lost to the atmosphere from wetlands via three mechanisms: 1) diffusion through pore water to the water table and hence through the zone above the water table (if one exists) to the wetland surface; 2) diffusion or active transport through vascular plants; and 3) ebullition, that refers to methane released to the atmosphere in form of bubbles moving to the wetland surface. $CH_4$ bubbles commonly occur in water saturated organic layers where they remain trapped and grow in size. $CH_4$ is only sparingly soluble in

**Table 2.** Enrichment factors ($\varepsilon$) to different processes for carbon.

| Processes | Enrichment factors ($\varepsilon$) | Reference cites |
|---|---|---|
| Acetate fermentation pathway | $\varepsilon = 40‰ \text{ - } 50‰$ | [11] |
| $CO_2$ reduction pathway | $\varepsilon = 55‰ \text{ - } 90‰$ | [11] |
| $CH_4$ oxidation | $\varepsilon = 3‰ \text{ - } 31‰$ <br> $\varepsilon = 22‰$ | [54] <br> [55] |
| Diffusion $CH_4$ across | $\varepsilon = 20‰$ (cover soil) <br> $\varepsilon = 18‰$ (cover soil) <br> $\varepsilon = 2‰ \text{ - } 3‰$ (interface water-air) | [56] <br> [49] <br> [57] |
| Ebullition | No fractionation | [53] |

water [35] and Henry's law predicts that their solubility in a solvent is directly proportional to the partial pressure. Other gases, particularly nitrogen, could contribute with their partial pressure and exceed the total hydrostatic force. If this pressure is exceeded, gas bubbles, consisting of a mixture of $CH_4$, $CO_2$ and $N_2$, could travel upward toward groundwater surface, perhaps along fractures [36]. This process of ebullition by which bubbles transport $CH_4$ upward, occurs when the buoyancy of bubbles overcomes the forces that keep them in place (in particular, surface tension) when a certain threshold pressure is reached. Often this release is associated with changes in water level [37]-[39], barometric pressure [40]-[42] and temperature [43], fracture of the confining layer as well as mechanical disturbance [44]. The rapid transfer of $CH_4$ bubbles through the aerobic near surface layer means there will be little or no consumption by methanotrophs. Some ebullition conceptual models suggest that bubbles form more readily in shallow layers-within 1 m-[45], while others as sure that they form at greater depth and accumulate within the wetland profile according physical properties of subsoil [26] or presence of zones of overpressure due to transient confining layers [46] [47]. Also, these last authors affirm the occurrence of $CH_4$ deep ebullition because they found large concentrations of dissolved $CH_4$ and inorganic carbon (DIC) in the deeper pore water related to $CO_2$ produced by fermentative and methanogen bacteria. Others suggest that bubbles are not trapped in subsoil but are lost directly to the water table, after which the gas moves slowly via diffusion through the zone above the water table where $CH_4$ is oxidized [48].

Molecular diffusion occurs through the water or air filled soil pores and the atmosphere and depends on the vertical $CH_4$ concentration gradient following Fick's Law: $J = -D\dfrac{dC}{dz}$, where $J$ is diffusive flux, $D$ is the diffusion coefficient, $C$ the solute concentration and $Z$ the depth. Fractionation ($\alpha$) during diffusion is caused by differences the diffusion coefficients of the two isotope species. The stable $CH_4$ isotopes ($^{12}CH_4$, $^{13}CH_4^{12}$ and $CH_3^2H$) differ in molecular weight and their diffusion coefficient, faster diffusive transport of the lighter isotope on hence in an enrichment of the heavier isotope in the remaining gas phase. Reference [49] demonstrated that diffusion is an important transport mechanism for $CH_4$ cover soils. They found that the fractionation factor due to transport, $\alpha$ transport, can be as high as 1.0178, due to the difference in molecular diffusion coefficients of $CH_4$ isotopes ($\varepsilon = 18‰$). Diffusive transport of $CH_4$ across the air-water interface was associated with anisotopic fractionation to 2‰ - 3‰ or $\varepsilon = 2‰ - 3‰$ [50].

Diffusion affects isotopic composition of $CH_4$ differently than ebullition and $CH_4$ transport from anoxic sediments and flooded soils by emergent plants. $\delta^{13}C$-$CH_4$ value of released bubbles is not different from the reservoir of bubble gas held within the sediment because ebullition bypasses oxidation in the aerobic zone [33]. Many wetland plants possess aerenchymous tissue that allows for transport of oxygen into the root zone as an adaptation to rooting in waterlogged soils. Whereas this oxygen allows for oxidation of $CH_4$ in the root zone [51], at the same time, $CH_4$ is transported through the aerenchyma out into the atmosphere. It was influenced by the type species and functional plant groups that affect these processes differently [52]. In vegetated wetlands, plant-mediated transport involves several steps that may further fractionate isotopes, $i.e.$ diffusion to the roots, partial oxidation by methanotrophic bacteria in rhizosphere, diffusion into the roots, transport by convective bulk flow and emission [53]. At the moment, only the isotope effects of CH4 oxidation and convective bulk flow have been well studied. The last one, results in the little isotopic fractionation [53] whereas $CH_4$ oxidation in the rhizosphere and in surficial oxic layers during diffusive transport to the soil surface (0 - 1 m) is notorious. Previous reports indicate that almost 60% of $CH_4$ production is oxidized to $CO_2$ [48]. Bacteria oxidize the $^{12}C$-isotopes slightly faster than the $^{13}C$-isotope, then, the result is an increase of the $^{13}C/^{12}C$ ratio of the remaining $CH_4$. The enrichment factors can be seen at **Table 2**. This increase can be used to estimate $CH_4$ oxidation. By the other way, variations on $\delta^{13}C$-DIC of water can reveal the importance of different biochemical pathways of methanogenesis and $CH_4$ oxidation [27]. It was remarked that acetate fermentation that occur chiefly in fresh water environment is responsible for the great fractionation of $^{13}C$ between $CO_2$ and $CH_4$. $CO_2$ with much enriched signature incorporates to the DIC, but this signal is often masked by the simultaneous oxidation processes releasing isotope depleted $CO_2$ to surrounding water.

## 3. Measurements and Analytical Methods

### 3.1. CH$_4$ and CO$_2$ Fluxes and Isotopes

Closed chamber methods are used to measure surface emissions. These methods are independent of the physical properties of soil and allow measuring the $CH_4$ and $CO_2$ released to the atmosphere. The closed chamber me-

thods are applied during fieldwork, after calibration in laboratory [58] [59]. The accumulation chamber is placed on a collar that had been previously installed on the ground. Surface emissions are estimated as the increase of $CH_4$ and $CO_2$ concentrations over time. Flux rates are calculated fitting by linear regression the variation of concentration ($C$) over time and adjusting for chamber volume and covered area, according to the following equation: $F = VA\dfrac{dC}{dt}$ where $F$ is the surface emission rate ($mg \cdot m^{-2} \cdot day^{-1}$), $V$ is the chamber volume and $A$ is the soil area under the chamber and $dC/dt$ is the variation of $C$ with $t$ within the chamber. In the closed static chamber method, soil gases are extracted with plastic syringes and analyzed by gas chromatography (GC). In flooded soil the fluxes are estimated using floating static chambers. Sometimes chambers are covered with reflective material to minimize the increase of inside temperature during deployments. Gas samples are then stored by needle injection in pre-evacuated glass flasks sealed with butyl rubber stoppers for later concentration determination.

## 3.2. Methane Concentration in Sediment Gas Bubbles and Water

Bubbles are sampled by stirring up the sediments. Arising bubbles are collected with an entirely submerged 25-cm diameter inverted plastic funnel at sites devoid of vascular vegetation. The open narrow end of the funnel was sealed with a rubber stopper of 3-cm diameter. With a needle, bubble gas samples are pumped into a plastic syringe adapted with a luer-lock valve and thereafter injected in pre-evacuated glass flasks sealed with butyl rubber stoppers for later GC analysis ($CH_4$, $N_2$, $CO_2$ concentration). Also, bubbles samples from natural ebullition are collected into floating traps without disturbing sediments [34]. Bubbles traps (~1 diameter) placed permanently under the surface of water bodies. Bubble $N_2$ content may be indicative of rates of ebullition from sediments [53]. As increasing volumes of $CH_4$ bubble from the sediments, they strip out $N_2$, a gas that is not produced within the sediments. Thus, bubbles with low $N_2$ concentration indicate high rates of bubble stripping or high ebullition. Continuous bubble fluxes of $CH_4$ ($mg\ CH_4\ m^{-2} \cdot d^{-1}$) at shallow depths (20, 40, 60 cm below the surface and water table) are measured by gas trap funnels [45]. Other method to measure $CH_4$ entrapped can be seen in reference [60].

If ebullition is episodic it is very difficult to quantify its importance. The role of large ebullition events needs further research and efforts to quantify this mode of emission. Methods that can quantify the distribution (and temporal variability) of free phase gas in the peat profile are needed. $CH_4$ bubbles accumulated at depth, episodically, can break through the confining layer to the surface causing very large rates of $CH_4$ loss to atmosphere (in $g \cdot m^{-2}$ as direct emission, no diffusive). Nondestructive geophysical methods that have been used in peatlands [41] such as ground penetrating radar and electrical resistivity could also be used to examine the sediment structure and gas contents within sediments. The determination of dissolved $CH_4$ in soil solution (and DIC) is an alternative non-destructive method, which simplifies the sampling procedure and allows associating the $CH_4$ and DIC dissolved with episodic ebullition (less DIC after ebullition). Reference [26] calculated large degassing events associating surface deformations to largest de-pressuring cycles.

Porewater $CH_4$ can be sampled *in situ* using modified diffusion cambers called "peepers" [33]. Gas samples collected for dissolved $CH_4$ analysis using a headspace equilibrium technique presented by reference [61], a 60-ml plastic syringe adapted with a luer-lock valve containing 30 ml of water sample and 30 ml of pure nitrogen was vigorously shaken during 2 min, transferring the dissolved phase to the nitrogen headspace. The headspace was then injected with a needle in pre-evacuated glass flasks sealed with butyl rubber stoppers for later FID-GC analysis.

## 3.3. Isotope Analysis

After the GC analysis, the remaining gas contained in the glass flasks acquired from the emitted $CH_4$ and $CO_2$ (chambers), $CH_4$ in the water column and $CH_4$ y/o $CO_2$ in the sediment bubbles are employed for $\delta^{13}C$-$CH_4$ and analysis in line with a mass spectrometer (GC-IRMS). Isotope analyses are expressed by $\delta^{13}C$ (see Notation). Water samples are analyzed for the $^{13}C/^{12}C$ ratios of their DIC component by precipitating DIC with $BaCl_2$ ($BaCO_3$).

## 3.4. Notations

Expression of results and some used formulas.

R defines the ratio between two isotopic species. Example:

$$^{13}R = \frac{^{13}C}{^{12}C};\ \ ^{2}R = \frac{^{2}H^{1}H}{^{1}H_{2}}$$

$\alpha_{A-B}$ is the fractionation factor between phases or components $A$ and $B$ $\alpha = \dfrac{R_A}{R_B}$, if > 1, $A$ is enriched in the heavy isotope respect $B$, conversely if < 1, $A$ is depleted in the heavy isotope respect to $B$. Also is used the enrichment factor (defined as $\varepsilon = 1000(\alpha - 1)$).

Results are expressed in ‰ defined as: $\delta = 1000\dfrac{R_S - R_R}{R_R}$ ‰ where $S$ denotes sample, $R$ reference standard

And the isotopic deviation in ‰, $^{2}H$ or $^{13}C$ respect the International reference Standard, V-Pee Dee Belemnitella (VPDB) for $^{13}C$ and V-Standard Mean Ocean Water for (VSMOW) for $\delta^{2}H$ [62] [63].

Since $^{2}H$ and $^{13}C$ are values actually measured, fractionation factor are calculated from $\delta$ by means of the following expression: $\alpha_{A-B} = \dfrac{1000 + \delta_A}{1000 + \delta_B}$

## 4. Conclusion

Principal processes leading to the production and sinking of $CH_4$ in wetlands are presented and discussed mainly from the stable isotopes point of view. Isotope fractionation factors associated to production, diffusion, ebullition, bacterial oxidation, etc., allow using the isotopes of carbon and hydrogen as environmental tracers of these processes. So, it can be applied to study $CH_4$ and $CO_2$ generation, distribution and consume in wetlands. This review presents an updated list of bibliographic references to support its arguments and serve as a guide to researcher in the thematic of $CH_4$ dynamic from wetland. Also it is presented guidelines of the most reliable field and laboratory methods used at present for the correct sampling and analyzing $CH_4$ in different stages of occurrence.

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