Terrestrial Vegetation Drives Methane Production in the Sediments of two German Reservoirs

Jörg Tittel1*, Matthias Hüls2 & Matthias Koschorreck1

Inland waters and reservoirs in particular are significant sources of methane to the atmosphere. However, little information is available on the extent to which organic carbon from terrestrial vegetation or from internal photosynthesis fuels the methane production. This limits our ability to constrain methane emissions efficiently. We studied the isotopic composition ($^{13}C$, $^{14}C$) of pelagic and sedimentary carbon sources in two small German reservoirs. The methane was enriched by radiocarbon with isotopic ranges ($\Delta^{14}C$ 5‰ to 31‰) near to fresh terrestrial organic carbon (OC, 17‰ to 26‰). In contrast, potential source OC produced by internal photosynthesis was characterized by negative $\Delta^{14}C$ values ($\sim$ 30‰ and $\sim$ 25‰) as derived from signatures of inorganic carbon in the reservoirs. The particulate OC in stream supplies (terrestrial OC) was also $^{14}C$ depleted in almost all cases, but highly variable in $\Delta^{14}C$ ($\sim$ 131‰ to 42‰). Although the import of terrestrial OC was lower than the amount of OC produced by reservoir-internal photosynthesis, we conclude that the methane production was predominantly fuelled by catchment vegetation. The utilized terrestrial OC was of contemporary origin, fixed within years to decades before sampling and supplemented with reservoir-internal or aged terrestrial OC. Our results indicate that terrestrial biomass is an important driver of methane production in reservoirs receiving significant imports of terrestrial OC.

Production of methane in lakes and reservoirs is an important process in the global carbon cycle. Although carbon dioxide (CO$_2$) emissions of surface waters are sustained by catchment soil respiration, methane released from reservoirs and lakes is generated in these systems itself. The methane is mainly produced at anoxic sites, mostly in the sediments, as a product of the mineralization of organic matter. Methanogenesis is the thermodynamically least efficient pathway of organic matter mineralization and usually starts, when other electron acceptors (oxygen, nitrate, iron, sulphate) are depleted.

There are two pathways of methane production: acetate fermentation and CO$_2$ reduction. In freshwater systems fermentation is the dominant process. It depends on the supply of available organic carbon (OC). There are two sources of OC in lakes and reservoirs: (a) input of dissolved organic carbon (DOC) and particulate organic carbon (POC) from terrestrial sources of the catchment, and (b) autochthonous (internal) production by photosynthetic organisms in the water body itself. Here, we use the term ‘autochthonous’ with respect to the reservoir while ‘terrestrial’ refers to carbon imports by streams. While many studies have examined how much these sources contribute to internal carbon cycling and whether the primary substrate of methanogenic bacteria is acetate or H$_2$ plus CO$_2$, less is known about the significance of autochthonous versus terrestrial OC as substrates for methane production. However, cost-effective measures in order to limit methane emissions should essentially focus on the predominant OC source. A better knowledge of the specific sources of methane would help choosing a purposeful strategy to reduce methane emissions from reservoirs.

Organic matter is a mixture of substances with varying degradability. If exposed to suitable conditions, easily biodegradable compounds are preferably mineralized by microbes. Autochthonous OC mostly originates from planktonic photosynthesis and was found to have a high biolability. Recent studies show a positive correlation between trophic state and methane emissions suggesting that autochthonous carbon is a relevant carbon source.

---

1Helmholtz Centre for Environmental Research - UFZ, Department Lake Research, Brückstraße 3a, D-39114, Magdeburg, Germany. 2Leibniz-Laboratory for Radiometric Dating and Stable Isotope Research, Max-Eyth-Str. 11-13, D-24118, Kiel, Germany. *email: joerg.tittel@ufz.de
for methane production in reservoirs' sediments. Terrestrial OC derives from plants and soils in the catchment. Unlike autochthonous material, it is often older and thus, already partly degraded. As a result, terrestrial OC tends to be more biorefractory, i.e. it is less available for microorganisms compared to autochthonous material and sediments receiving high inputs of terrestrial OC tend to have higher OC burial efficiencies.

Terrestrial OC can be either dissolved or particulate, and it is the dominant fraction of organic matter in nutrient poor lakes. In contrast to lakes, in reservoirs, terrestrial OC originates from two sources: (a) soil and plant material remaining from the time before reservoir construction and (b) input during reservoir filling and operation. In tropical reservoirs the degradation of terrestrial material originating from pre-impoundment times is the dominant carbon source of methane production during the first years of reservoir operation. Reservoirs are known to be aquatic methane emission hotspots because they trap organic material originating from the catchment. However, the close link between sedimentary methane production and terrestrial carbon input has been established from indirect evidence, like carbon budgets or correlations between methane emission and sediment quantity and composition. Direct evidence showing that sedimentary methane originates from terrestrial carbon sources is scarce. Experiments in which sediment samples were amended with different types of organic carbon verified terrestrial carbon as an important substrate for methane production in sediments. The analysis of the isotopic composition of different carbon species in the environment offers the possibility to directly link substrates with reaction products in situ. Unfortunately, the results of studies using exclusively the stable isotope δ13C were not unambiguous. There was no relation between the δ13C of sediment organic matter and methane in 2 European lakes.

Radiocarbon is a powerful but underused tool in geochemical studies. Compared to the stable isotope δ13C, the natural abundances of the isotope 14C vary dynamically over a much broader range, which increases the probability that different sources can be distinguished. Radiocarbon in methane (radiomethane), in particular, has been analysed to explore the significance of old vs. recent carbon sources for methane production in various environments. However, there are only a few radiomethane studies from lakes or reservoirs. Methane older than 20,000 years was found in lake Kivu, an African rift lake, where the methane was predominantly formed from geogenic sources. The sediment but no sodium chloride was added to the nitrogen-filled jar before it was closed with crimp caps (20 mm, aluminium and butyl/PTFE). For analysis of 13C- and 14C-POC-Out isotopes 1 L volume, acid rinsed and baked flasks were used. Table 2 gives an overview of parameters and sampling.

We used a multi-isotope approach to identify the carbon source of methane produced in the sediment of two drinking water reservoirs. We chose two reservoirs that were similar in size and catchment area but different in nutrient supply and trophic status. Due to the small size of the studied reservoirs the spatial gradients of OC supplied by streams are presumably less significant as in larger systems. By analysing the δ13C and δ14C content of the methane and of the potential carbon sources in the reservoirs and in the catchments we aimed to disentangle the role of autochthonous versus terrestrial sources for methane production.

Materials and Methods

Study sites and sampling. Rappbode and Hassel are adjacent reservoirs located upstream in a system of dams used for drinking water production. They are situated in the lower part of the Harz mountains, Germany (51.7092°N, 10.7981°E; 51.7091°N, 10.8319°E; respectively). Both reservoirs are small (Table 1) and with a total length of 1.6 to 1.8 km comparable in size to the river-reservoir transition zone of larger reservoirs. They receive inflows each from one stream but differ with respect to nutrient imports. The Rappbode reservoir catchment is dominated by forest while there is some influence of agriculture on the more eutrophic Hassel reservoir.

Both bodies of water regularly develop an anoxic hypolimnion during summer stratification. At the time of sampling, dissolved oxygen was absent below 12 m depth. The thermocline was established at 9 m depth in Rappbode reservoir and at 7 m depth in Hassel reservoir. Both reservoirs showed only minor longitudinal gradients with respect to methane and CO2 concentrations, emissions and hydrochemistry. Plankton concentrations are typically increasing from the inflow to the dam. Due to the mostly steep and rocky shore few aquatic macrophytes were present at the inflow area. The sediment organic matter content ranged between 13 and 26% loss on ignition and tended to be highest at the deepest points. The sampling stations were located at the deepest points 50 m away from the spillover. In Hassel reservoir, one additional sample was taken at a station near to the inflow at 4 m depth.

Samples characterizing the total inorganic carbon (TIC) in the surface layer (TIC-Sur) as well as the outflow POC (POC-Out) were both obtained using a boat near the spillover from 0.5 to 1.0 m depth. The samples were collected weekly from 21.02.12 until 19.02.13. We used a Limnos water sampler (Turku, Finland) and 100 mL (TIC) and 200 mL (POC) acid-rinsed, brown glass flasks with solid ground glass stoppers. For 13C- and 14C-TIC isotopes, every second week we collected water samples of 200 mL and 1 L volume, respectively in acid-rinsed and baked (500 °C, 4 h) glass bottles. We added 0.2 mL of a HgCl2 solution (1%) to the 13C samples before they were baked (500 °C, 4 h) glass bottles. We added 0.2 mL of a HgCl2 solution (1%) to the 13C samples before they were added to the 13C samples before they were analysed to explore the significance of old vs. recent carbon sources for methane production in various environments. However, there are only a few radiomethane studies from lakes or reservoirs. Methane older than 20,000 years was found in lake Kivu, an African rift lake, where the methane was predominantly formed from geogenic sources of CO2 and H2. The sediment of a reservoir in the northern boreal area of Finland contained methane that was produced from recently fixed or from older sources fixed 670 years before present (BP). We used a multi-isotope approach to identify the carbon source of methane produced in the sediment of two drinking water reservoirs. We chose two reservoirs that were similar in size and catchment area but different in nutrient supply and trophic status. Due to the small size of the studied reservoirs the spatial gradients of OC supplied by streams are presumably less significant as in larger systems. By analysing the δ13C and δ14C content of the methane and of the potential carbon sources in the reservoirs and in the catchments we aimed to disentangle the role of autochthonous versus terrestrial sources for methane production.
cores for 14C-CH4-Sed measurement were taken in Hassel reservoir, one from the deepest point and one from the shallow station near the inflow. In addition to Hassel and Rappbode, two other reservoirs were sampled once for 14C-TIC-Sur and 14C-CH4-Sed, i.e. Bautzen (Germany, 51.2175°N, 14.4665°E) and Sau (Spain, 41.9705°N, 2.3936°E).

Sample processing in the laboratory. The 13C and 14C TIC-Sur and TIC-autumn samples were processed as described earlier41. To collect CO2 for 14C analysis within 24 hours the 14C samples were acidified (HCl 37%, pH 2) and outstripped with UHP nitrogen for 4 hours. The inorganic carbon was precipitated as carbonates in a saturated and pre-filtered barium hydroxide solution. The precipitates were washed three times with nitrogen bubbled deionized water to remove the remaining barium hydroxide and then dried at 60°C under continuous nitrogen supply in a throughflow system. The carbonates were stored in UHP nitrogen flushed vials closed by crimp caps (20 mm, aluminium and butyl/PTFE). To characterise the isotopic composition of TIC present in the surface layers during the annual cycle (TIC-Sur) the samples from a particular reservoir were combined to a bulk sample. Therefore, the analyses of our time-integrating samples are representative for the TIC near the dam that was exported from the reservoir during the sampling year. Samples were processed in a glove box under an argon atmosphere. The samples for POC-Out concentration as well as for 13C-POC-Out and 14C-POC-Out analyses were filtered (combusted GF/F) and acid-treated39. From every 14C-POC-Out sample one defined piece of filter was separated. The weight of pieces was proportional to the POC yields at individual sampling days. The pieces were combined to a bulk sample. The same was applied to the 14C-POC-Out samples. These time-integrating samples characterize the 13C and 14C of the POCSed exported from the reservoirs via the near spillover (POC-Out). Glassware used for isotope analysis was rinsed twice with 0.1 N HCl and baked at 500°C for four hours.

From the jar containing the suspended sediment of the first core we collected gas from the headspace using a syringe after repeated cycles of shaking and sediment settling. We did not acidify the sediment samples to avoid a dissolution of carbonates such as calcite precipitates. The gas was directly injected into the gas chromatograph to measure the concentrations of CH4-Sed and of CO2-Sed (see Isotope and water chemistry analyses below). In addition, depending on concentrations 4–12 mL gas was transferred into UHP nitrogen flushed vials (Extetainer 12 mL, Labco, Lampeter, UK) crimped with septum caps (butyl/PTFE) for later analysis of 3H-CH4-Sed.

Table 1. Morphometry, trophic status and carbon fluxes of Hassel and Rappbode reservoirs. Residence time, carbon import and export values as well as stream total phosphorus (TP) refer to the sampling year (20.02.2012–19.02.2013). *Friese, et al.32.  †Means of weekly samples of TP in inflowing streams Hassel and Rappbode, methods see Friese, et al.32.  ‡Means of biweekly samples from 2 m depth, methods see Friese, et al.25.  §Means of biweekly samples from 2 m depth, unpublished data, methods for phytoplankton biomass estimation see Friese, et al.32, a specific carbon content of 0.23 mg C mm−2 was assumed.  ¶Estimated on the basis of phytoplankton biomass in 2012 (see above ‡) and biomass-specific photosynthetic production. The latter was derived from net primary production (2013 in Hassel reservoir, 2014 in Rappbode reservoir; Morling, et al.38) and corresponding phytoplankton biomass during production measurements.  ††Tittel et al.34, therein reservoir imports were referred to as ‘yields’ with respect to catchment areas. Here, the imports were related to the reservoir surface area.  ‡‡See POC-In of Table 3.  ‡‡‡Flux of methane or CO2 out of the sediment into anoxic hypolimnetic water measured in 2011. Fluxes were estimated from accumulation rates in the anoxic hypolimnion during summer44.  ‡‡§Methane flux between reservoir and atmosphere measured seasonally in 2015 with floating chambers (Table S2).

|                       | Rappbode reservoir | Hassel reservoir |
|-----------------------|--------------------|-----------------|
| Catchment area (km²)  | 47.6               | 44.6            |
| Reservoir surface area (km²) | 0.22               | 0.29            |
| Mean depth (m)        | 5.3                | 5.0             |
| Maximum depth (m)     | 17                 | 14              |
| Residence time (days) | 34                 | 51              |

Pools

| Inflow total phosphorus (µg P L⁻¹)  | 23     | 47           |
| Chlorophyll a (µg L⁻¹)             | 8.6    | 16.3         |
| Phytoplankton biomass (g C m⁻²)    | 4.9    | 12.3         |

Process rates

| Internal photosynthesis (g C m⁻²·year⁻¹) | 46     | 130          |
| DOC terrestrial import (g C m⁻²·year⁻¹) | 207    | 185          |
| POC terrestrial import (POC-In) (g C m⁻²·year⁻¹) | 34    | 32           |
| Benthic CH₄ flux (g C m⁻²·year⁻¹)      | 6      | 8            |
| Benthic CO₂ flux (g C m⁻²·year⁻¹)      | 38     | 19           |
| CH₄ emission (g C m⁻²·year⁻¹)          | 2.0    | 1.2          |
The radiocarbon age (CRA) relative to the year 1950 AD as year 0 BP and on the basis of a 14C half-life of 5568 years.

Gas chromatography (SRI 8610 with flame ionisation detector and methaniser, SRI Instruments, Torrance, USA) and headspace analysis (Elementar, Hanau, Germany). We related the POC-Sed values to the volume of the sediment using the measured bulk density of the subsamples (see below). The CH4-Sed and CO2-Sed concentrations were analysed by headspace gas chromatography (SRI 8610 with flame ionisation detector and methaniser, SRI Instruments, Torrance, USA). We calculated the conventional radiocarbon age (CRA) relative to the year 1950 AD as year 0 BP and on the basis of a 14C half-life of 5568 years.

| Parameter description | Sampling frequency | Data source |
|-----------------------|--------------------|-------------|
| **Catchment**         |                    |             |
| Δ¹³C-POC-Needles      | 14C isotope concentration in tree needles characterising fresh terrestrial OC | 2 samples | * |
| Δ¹³C-POC-Soil         | 14C isotope concentration in soil OC characterising aged terrestrial OC | 3 samples | * |
| **Stream inflow (reservoir import)** |                    |             |
| POC-In                | Concentration or flux of terrestrial POC | weekly | * |
| Δ²H-POC-In            | 2H isotope concentration in terrestrial POC | 4 samplings | this study |
| Δ¹³C-POC-In           | 13C isotope concentration in terrestrial POC | 5–7 samplings | * |
| **Outflow (reservoir export)** |                    |             |
| POC-Out               | POC concentration or flux | weekly | this study |
| Δ²H-POC-Out           | 2H isotope concentration in POC, time-integrated | every 2 weeks | this study |
| Δ¹³C-POC-Out          | 13C isotope concentration in POC, time-integrated | every 2 weeks | this study |
| **Surface layer of reservoir** |                    |             |
| TIC-Sur               | TIC concentration | weekly | this study |
| Δ¹³C-Sur              | 13C isotope concentration in TIC, time-integrated | every 2 weeks | this study |
| Δ¹⁴C-Sur              | 14C isotope concentration in TIC, time-integrated, represents Δ¹³C of autochthonous OC | every 2 weeks | this study |
| Δ¹³C-autumn           | 13C isotope concentration in TIC, end of summer stratification | single sample | this study |
| Δ¹⁴C-autumn           | 14C isotope concentration in TIC, end of summer stratification, represents Δ¹³C of autochthonous OC | single sample | this study |
| **Sediment of reservoir** |                    |             |
| CO₂-Sed               | CO₂ concentration relative to sediment volume | single sample | this study |
| Δ¹³C-CO₂-Sed          | 13C isotope concentration in CO₂ | single sample | this study |
| Δ¹³C-CO₂-Sed          | 13C isotope concentration in CO₂ | single sample | this study |
| POC-Sed               | POC concentration relative to sediment volume | single sample | this study |
| Δ²H-POC-Sed           | 2H isotope concentration in POC | single sample | this study |
| Δ¹³C-POC-Sed          | 13C isotope concentration in POC | single sample | this study |
| CH₄-Sed               | CH₄ concentration relative to sediment volume | single sample | this study |
| Δ²H-CH₄-Sed           | 2H isotope concentration in CH₄ | single sample | this study |
| Δ¹³C-CH₄-Sed          | 13C isotope concentration in CH₄ | single sample | this study |
| Δ¹³C-CH₃-Sed          | 13C isotope concentration in CH₃ | 1–3 samples | this study |

Table 2. Description of parameters used in this manuscript. *See Methods. Terrestrial: import into the reservoir by streams, autochthonous: internal production in the reservoir by photosynthesis.

13C-CH₄-Sed and 13C-CO₂-Sed (see below). To collect the methane for 14C analysis, we used two sharpened metal tubes (1 mm inner diameter) that were guided to the two septa of the lid (see above). One tube only reached the gas headspace, the second was moved into the liquid layer near to the bottom of the jar. The gas of the headspace was flushed by a stream of UHP nitrogen and the methane was purified with the help of a liquid nitrogen trap removing CO₂ and water vapor and then oxidized at 870°C under continuous oxygen supply. The produced CO₂ was reduced with H₂ at 600 °C to graphite for accelerator mass spectrometry (AMS). Finally, a portion of the sediment from the first core was centrifugated (3750 rpm, 20 min) and dried at 105 °C to estimate the bulk density.

The sediment of the second core was flushed with UHP nitrogen using a septum lid and the stripped CO₂ was precipitated in a barium hydroxide solution for 14C measurement by AMS. We dried the sediment for estimation of the bulk density.

The sediment sampled by the third core was dried at 60 °C and checked for roots and impurities with the help of a dissecting microscope. Subsamples were weighed and treated with HCl (37%) to remove inorganic carbon, dried again at 60 °C for 4 hours, crimped in silver boats and stored in a desiccator until POC-Sed analysis. Organic carbon for isotope analysis was extracted by the acid-base-acid method. Subsamples were combusted at 900 °C in presence of CuO and a silver catalyst and graphitized for AMS. Aliquots were shipped in tin boats for 13C analysis by mass spectrometry.

**Isotope and water chemistry analyses, calculations.** The TIC-Sur was quantified using a Dimatoc 2000 analyser (Analyseotechnik, Essen, Germany). For POC-Out and POC-Sed a Vario EL analyser was used (Elementar, Hanau, Germany). We related the POC-Sed values to the volume of the sediment using the measured bulk density of the subsamples (see below). The CH₄-Sed and CO₂-Sed concentrations were analysed by headspace gas chromatography (SRI 8610 with flame ionisation detector and methaniser, SRI Instruments, Torrance, USA). Radiocarbon was analysed by AMS (3 MV HVEE Tandetron 4130)32. Values of Δ¹³C express the carbon isotope ratio as deviations in per mil (%) from the oxalic acid II standard (SRM 4990 C). They were corrected for process and instrument blanks and for isotope fractionation by the AMS system. We calculated the conventional radiocarbon age (CRA) relative to the year 1950 AD as year 0 BP and on the basis of a 14C half-life of 5568 years.
Gas isotope samples ($^{2}$H–CH$_{3}$-Sed, $^{13}$C–CH$_{3}$-Sed and $^{13}$C-CO$_{2}$-Sed) were sent in duplicates to the Stable Isotope Facility at the University of California, Davis, USA. Analyses were performed using a Thermo Scientific GasBenchII plus PreCon(centration) device coupled to a Thermo Scientific Delta V Plus isotope-ratio mass spectrometer (IRMS). The $^{2}$H abundances were expressed with respect to V-SMOW (Vienna Standard Mean Ocean Water). The $^{13}$C values were expressed relative to standard Vienna PeeDee Belemnite. The results were not corrected for headspace fractionation as the gases were thoroughly transferred to the headspace in the saturated sodium chloride solution (see above). Furthermore, the $^{13}$C-CO$_{2}$-Sed were not corrected for fractionation within the bicarbonate system. Depending on pH, the CO$_{2}$ can be 0–9‰ more depleted than the dissolved inorganic carbon$^{37}$. This introduces a small uncertainty in our $^{13}$C-CO$_{2}$-Sed estimates, which does not affect our conclusions. The $^{13}$C abundances of TIC-Sur as well as of POC-Out were analysed by MS (Thermo Scientific Delta V IRMS) at the Colorado Plateau Stable Isotope Laboratory, Flagstaff, USA. Error ranges of analytical methods are given in Table S1.

We also measured the bulk density of the first and second sediment core. We calculated the water content from the difference of the net weight of the sample and the dry weight (DW). The volume of the sediment was estimated as water content minus 0.5. DW assuming a specific density of the solid fraction of 2.0 g mL$^{-1}$.

### Results and Discussion

### Isotopic composition of methane and of carbon sources.

The methane in the sediments of both reservoirs was of modern origin ($\Delta^{14}$C-CH$_{4}$-Sed > 0‰, Fig. 1, Table 3). This means that it included $^{14}$C-enriched carbon released by nuclear testing after 1950. The samples from Hassel reservoir yielded $\Delta^{14}$C values of 5 ± 3‰ and 13 ± 3‰ from two cores taken at the deepest station as well as of 30 ± 3‰ from the core of the shallow site (single measurements ± analytical errors). The Rappbode reservoir sample contained methane with a $\Delta^{14}$C of 31 ± 3‰.

To assess the origin of OC supporting methane production it is crucial to constrain the isotopic signatures of potential carbon sources. The photosynthetically fixed carbon in all algal species carries a uniform $\Delta^{13}$C signature, which is the $\Delta^{13}$C value of the CO$_{2}$ in the photic zone. According to standard practice $\Delta^{13}$C values are corrected for fractionation by convention$^{35}$, i.e. for fractionation within the system of inorganic carbon species or during photosynthesis. Therefore, the $\Delta^{14}$C–TIC-Sur constrains the radiocarbon signal of autochthonous OC and of methane deriving from this source (blue boxes in Fig. 1).

To estimate the $\Delta^{14}$C of CO$_{2}$ available for photosynthesis, we used time-integrating samples from the surface layers of the reservoirs covering the sampling year ($\Delta^{14}$C-TIC-Sur, Methods). In both reservoirs, they contained radiocarbon depleted TIC ($\Delta^{14}$C ~ 30 ± 4‰ and ~ 25 ± 2‰, means ± 0.5 ranges of duplicate measurements, Rappbode and Hassel reservoirs, respectively; Fig. 1, Table 3). These results agree well with those from individual samples of surface layers measured at the end of summer stratification ($\Delta^{14}$C-TIC-summer, ~25‰ and ~22‰).

In contrast to autochthonous OC produced by photosynthesis, terrestrial OC constitutes a mix of compounds derived from various terrestrial sources (e.g. fresh leaves, aged soil OC) with varying ages and hence radiocarbon contents. The $\Delta^{14}$C of compounds used for methane production can differ from the average $\Delta^{14}$C of all compounds of a bulk sample. The individual POC inflow samples (POC-In) covered a wide range in $\Delta^{14}$C between 43‰ and −94‰ in Rappbode reservoir as well as between 42‰ and −131‰ in Hassel reservoir (Fig. 1). The most negative $\Delta^{14}$C here corresponds to a CRA of 1067 years BP. Previous studies had shown that during the stratified period at our study sites the diffusive flux of DOC was always directed from the sediment into the

### Table 3. Size and Isotopic composition of carbon pools. For description of parameters see Table 2.

|                           | Rappbode reservoir | Hassel reservoir |
|---------------------------|--------------------|-----------------|
|                           | $\Delta^{14}$C (%) | $\delta^{13}$C (%) | $\Delta^{14}$C (%) | $\delta^{13}$C (%) |
| **Stream inflow (reservoir import)** |                    |                 |                   |                   |
| POC-In$^{a}$              | 0.5 ± 0.3 mg C L$^{-1}$ | −86.8 ± 3.2   | −30.6 ± 28.4      | 0.8 ± 0.4 mg C L$^{-1}$ | −131 ± 42     | −30.2 ± 29.2   |
| **Outflow (reservoir export)** |                    |                 |                   |                   |
| POC-Out$^{b}$             | 0.8 ± 0.3 mg C L$^{-1}$ | −41.8 ± 7.2   | n.d.              | 1.2 ± 0.7 mg C L$^{-1}$ | −43.1 ± 6.7   | n.d.            |
| **Surface layer of reservoir** |                    |                 |                   |                   |
| TIC-Sur$^{c}$             | 8.6 ± 2.7 mg C L$^{-1}$ | −29.8 ± 3.8   | −12.1 ± 0.0       | 9.6 ± 3.1 mg C L$^{-1}$ | −25.1 ± 1.7   | −9.7 ± 0.0     |
| TIC-summer$^{d}$          | 11.3 ± 2.7 mg C L$^{-1}$ | −25.4 ± 3.5   | −11.8 ± 0.0       | 13.9 mg C L$^{-1}$ | −22.0 ± 4.7   | −10.1 ± 0.2    |
| **Sediment of reservoir$^{e}$** |                    |                 |                   |                   |
| Water content             | 961 g dm$^{-3}$ | 969 g dm$^{-3}$ |                   |                   |
| Bulk density              | 78 g dm$^{-3}$ | 62 g dm$^{-3}$ |                   |                   |
| CO$_{2}$-Sed              | 0.3 mg C dm$^{-3}$ | −0.5 ± 3.7    | −14.1 ± 0.1       | 1.0 mg C dm$^{-3}$ | −2.8 ± 3.7    | −13.4 ± 0.1    |
| POC-Sed                   | 6017 mg C dm$^{-3}$ | −58.4 ± 4.4   | −30.1 ± 0.0       | 6646 mg C dm$^{-3}$ | −19.7 ± 3.9   | −29.5 ± 0.0    |
| CH$_{4}$-Sed              | 6.9 mg C dm$^{-3}$ | 31.1 ± 2.9$^{*}$ | −65.3 ± 0.5       | 1.6 mg C dm$^{-3}$ | 5.1 to 30.0$^{*}$ | −52.3 ± 0.8    |

*Concentrations: means ± SD. Isotopes: min - max values of 4–7 samplings. $^{b}$Concentrations: means ± SD. Isotopes: means ± 0.5 ranges of duplicate measurements. $^{c}$ $\Delta^{14}$C: single measurements ± analytical errors. $^{d}$ $\delta^{13}$C: duplicate measurements of one sample (means ± 0.5 ranges). $^{e}$Individual samples. $^{f}$ $\Delta^{14}$C: single measurements ± analytical errors. $^{g}$ $\delta^{13}$C: duplicate measurements (means ± 0.5 ranges). Carbon concentrations are expressed relative to total sediment volume. $^{i}$Individual isotope sample from Rappbode reservoir, range of three samples from Hassel reservoir (mean 16.0‰).
Therefore, we do not consider terrestrial DOC a relevant source of methane, although more DOC than POC was delivered by the inflows (Table 1).

The $\Delta ^{14}C$ values of 23 ± 3‰ and 20 ± 3‰ were close to those of the methane (Fig. 1). This points to fresh terrestrial plant biomass as the methane source. Freshly fixed OC can be delivered to the stream by overland flow eroding the uppermost soil layer as well as by litterfall of canopy trees.

Although the radiocarbon content of the methane nearly matched that of fresh vegetation, we need to consider the possibility that terrestrial OC with more positive $\Delta ^{14}C$ values was involved into methane production. This OC could be some years to decades older than that of the sampling year but will be more 14C enriched. The reason is that due to nuclear tests after 1950, atmospheric $\Delta ^{14}C$-CO$_2$ summer values almost doubled and then decreased from nearly 1000‰ in 1964 to 87‰ in 2000 and to 31‰ in 2012. In contrast, terrestrial plant biomass produced before 1950 is depleted in 14C (Fig. 1). More positive $\Delta ^{14}C$ values of contemporary OC could be balanced with supplies of 14C-negative sources such as aged terrestrial OC. In our catchments, aged terrestrial OC (POC-Soil) presumably derived from erosion of the stream bank. Its components spanned a gradient in $\Delta ^{14}C$ ranging potentially from moderately negative values such as −29‰ as in POC-Soil at 8 cm depth to −702‰ as in POC-Soil at 81 cm depth of the catchment soil.

For the most depleted soil OC we calculated a CRA of 9650 years corresponding to its formation after Pleistocene glaciation. Out of the three $\Delta ^{14}C$-CH$_4$-Sed measurements of the Hassel reservoir, two values (5‰, 13‰) were below the $\Delta ^{14}C$ of fresh OC (31‰, see above). Positive $\Delta ^{14}C$ values below that of fresh OC existed only transiently in the atmosphere. Therefore, a 14C-depleted source with pre-bomb 14C must have contributed to methane production. We conclude that contemporary terrestrial vegetation was the predominant carbon source, supported by 14C-depleted OC of autochthonous or of terrestrial origin.

From the methane we also obtained measurements of stable carbon and hydrogen isotopes. The $\delta ^{13}C$ of the acetate methyl group and the fractionation factors of involved methanogenic pathways. If
hydrogenotrophic methanogenesis was taking place, the $^{14}$C signature of the methane would be constrained by the $^{14}$C signature of CO$_2$. The $\Delta^{14}$C-CO$_2$-Sed values were $\sim 0$‰ and relatively near to those of the fresh terrestrial OC, which means that the CO$_2$ was likely produced from the mineralisation of terrestrial OC. If CO$_2$ was actually a methane source, fresh terrestrial OC must have been also a significant basis of methane formation. This would not change our conclusions.

We also measured $^{14}$C-enriched methane in two other reservoirs (Santa Fe $\Delta^{14}$C-CH$_4$-Sed 82‰, Bautzen 9‰, not shown). The TIC-Sur was also modern (27‰ and 15‰, respectively). The methane sources of the eutrophic Bautzen reservoir cannot be distinguished as the $\Delta^{14}$C-CH$_4$-Sed was near to the $\Delta^{14}$C of autochthonous OC (TIC-Sur) and near to the $\Delta^{14}$C of fresh terrestrial OC. The values for the oligotrophic Santa Fe reservoir show that the methane contained terrestrial OC that was more enriched than the recently fixed fraction. Values equal to or higher than 82‰ occurred in atmospheric CO$_2$ in 2001 and before. The significance of terrestrial OC is consistent with the characteristics of the very small Santa Fe reservoir (7 ha) which is closely surrounded by deciduous forest. This is in line with our conclusion that the methane production was based on contemporary terrestrial biomass with supplements of aged terrestrial or autochthonous sources.

Figure 2. The carbon sources of methane. Recent OC comprised autochthonous biomass produced during the sampling year (Table 1) as well as fresh terrestrial OC ($\Delta^{14}$C-POC-Needles 17‰ to 26‰, see text). Old OC was of terrestrial origin (POC-Soil). The carbon fluxes and isotope ratios were calculated by a mass budget (Table S3). The hatched areas indicate the OC used for methane production as derived from the discussion.
it was oxidized at the sediment surface. By comparing the concentration of methane in the sediment with the methane production rates we can estimate that all methane in the sediment was produced within one year prior to sampling. The production of methane was low relative to the OC production in the reservoirs and to the import of OC from catchments (Table 1).

Although more OC was provided by internal photosynthesis than by stream POC, the methane production was based predominantly on contemporary terrestrial OC. This source represents only a small fraction of the OC theoretically available for methanogenesis (Fig. 2). Unlike the majority of the terrestrial POC, the autochthonous OC may be readily available and preferentially decomposed under aerobic conditions or by other energetically more efficient pathways than methanogenesis such as denitrification, iron und sulphate reduction. This can take place already in the water column and at the sediment-water interface. Mass balance calculations using\(^{13}\)C revealed that the degradation of autochthonous OC contributed to the TIC pool in the hypolimnion of both reservoirs. This supports the hypothesis that methanogenesis, as the terminal mineralization step, is faced with the more refractory leftovers of the other respiratory processes. This argumentation is in line with results from radiocarbon measurements from a stream draining peatland. The authors concluded that most of the younger CO\(_2\) was produced from the relatively rapid aerobic mineralization of organic matter, whereas methane production was restricted to older layers.

We found that OC originating from contemporary terrestrial vegetation was the predominant source of methane in the sediments of our reservoirs. This agrees with results of a study conducted in bogs and fens of northern Minnesota. The authors emphasized the similarity of the \(^{14}\)C values of contemporaneous atmospheric CO\(_2\) and emitted methane. The methane production in our two reservoirs was low, but in a range typical for temperate reservoirs. Between 18% and 25% of stream POC supplies were sufficient to sustain the measured methane production rates in the Rappbode and Hassel reservoirs, respectively (Table 1). The predominant use of a comparatively small and young fraction of the terrestrial POC might be related to the low methane production and interpreted as a characteristic of our systems. Rising emissions, however, do not imply that a larger fraction of terrestrial POC is required to maintain methanogenesis. This is exemplified with the temperate hydropower reservoir Lake Wohlen, where only 3% of riverine POC inputs could sustain extreme emissions that were 15 to 20 times higher than in our reservoirs. Secondly, soil organic carbon and plant material are significant sources of methane production after flooding. If it takes about 40 years until continued inputs from inflowing rivers and internal photosynthesis may become the main sources, flooded soil is not a relevant carbon source for methanogenesis in our reservoirs.

Our results seem to contradict recent studies which show that methane emissions increase with productivity of lakes and reservoirs. The large influence of allochthonous carbon in our study can be explained by the small size of the reservoirs investigated here. Our results can refer to the river-reservoir transition zone of larger reservoirs or run-off-the-river dams where large portions of the suspended matter are initially deposited and which are emission hotspots. In reservoirs larger than in this study, internal photosynthesis may become a more significant source. It has been shown that sedimentation areas at river inflows into lakes are rich in terrestrial material and exhibit high rates of methanogenesis. Our results suggest that terrestrial carbon is a major driver of such methanogenesis hotspots.

This is the first study of radiocarbon in methane together with its potential sources in a reservoir. The results demonstrate that the potential sources of methane must be isotopically well characterized, i.e. a presence of radiocarbon-enriched methane alone would not sufficiently support the conclusion that its production was based on internal photosynthesis. Although more OC was supplied by internal photosynthesis than by stream POC, the methane production was fuelled by terrestrial OC, predominantly of contemporary origin.

Received: 20 June 2019; Accepted: 12 October 2019;
Published online: 04 November 2019

References

1. Bastviken, D., Tranvik, L. I., Downing, J. A., Crill, P. M. & Enrich-Prast, A. Freshwater Methane Emissions Offset the Continental Carbon Sink. Science 331, 50–50, https://doi.org/10.1126/science.1196808 (2011).
2. Maberly, S. C., Barker, P. A., Stott, A. W. & De Ville, M. M. Catchment productivity controls CO\(_2\) emissions from lakes. Nat Clim Chang 3, 391–394, https://doi.org/10.1038/Nclimate1748 (2013).
3. Conrad, R. Contribution of hydrogen to methane production and control of hydrogen concentration in methanogenic soils and sediments. FEMS Microb. Ecol. 28, 193–202 (1999).
4. Bastviken, D. In Encyclopedia of Inland Waters (ed. Likens, G. E.) 783–805 (Elsevier, 2009).
5. Hanson, P., Pace, M., Carpenter, S., Cole, J. & Stanley, E. Integrating Landscape Carbon Cycling: Research Needs for Resolving Organic Carbon Budgets of Lakes. Ecosystems 18, 363–375, https://doi.org/10.1007/s10021-014-9826-9 (2015).
6. Bartels, P. et al. Terrestrial subsidies to lake food webs: an experimental approach. Oecologia 168, 807–818, https://doi.org/10.1007/s00442-011-2141-7 (2012).
7. Pace, M. et al. Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. Nature 427, 240–243 (2004).
8. Guillemette, F., McCallister, S. L. & del Giorgio, P. A. Differentiating the degradation dynamics of algal and terrestrial carbon within complex natural dissolved organic carbon in temperate lakes. J Geophys Res-Biogeo 118, 963–973, https://doi.org/10.1002/jgrg.20077 (2013).
9. Deemer, B. R. et al. Greenhouse Gas Emissions from Reservoir Water Surfaces: A New Global Synthesis. Bioscience 66, 949–966, https://doi.org/10.1093/biosci/biw117 (2016).
10. Beaulieu, J. J., DelSontro, T. & Downing, J. A. Eutrophication will increase methane emissions from lakes and impoundments during the 21st century. Nature Communications 10, 1375, https://doi.org/10.1038/s41467-019-09100-5 (2019).
11. Guillemette, F. & del Giorgio, P. A. Reconstructing the various facets of dissolved organic carbon bioavailability in freshwater ecosystems. Limnol. Oceanogr. 56, 734–748, 10.4319/lo (2011).
12. West, W. E., Coloso, J. J. & Jones, S. E. Effects of algal and terrestrial carbon on methane production rates and methanogen community structure in a temperate lake sediment. Freshwat. Biol. 57, 949–955, https://doi.org/10.1111/j.1365-2427.2012.02755.x (2012).
13. Sobek, S. et al. Organic carbon burial efficiency in lake sediments controlled by oxygen exposure time and sediment source. Limnol. Oceanogr. 54, 2243–2254 (2009).

14. Wilkinson, G. M., Pace, M. L. & Cole, J. J. Terrestrial dominance of organic matter in north temperate lakes. Global Biogeochem. Cy 27, 43–51, https://doi.org/10.1029/2012gb004453 (2013).

15. Guerin, F., Abril, G., de Junet, A. & Bonnet, M. P. Anaerobic decomposition of tropical soils and plant material: Implication for the CO2 and CH4 budget of the Petit Saut Reservoir. Appl. Geochem. 23, 2272–2283, https://doi.org/10.1016/j.apgeochem.2008.04.001 (2008).

16. Barros, N. et al. Carbon emission from hydroelectric reservoirs linked to reservoir age and latitude. Nat Geosci 4, 593–596 (2011).

17. Maek, A. et al. Sediment Trapping by Dams Creates Methane Emission Hot Spots. Environ. Sci. Technol. 47, 8130–8137, https://doi.org/10.1021/es403907 (2013).

18. DeSontro, T., McGinnis, D. F., Sobek, S., Ostrovsky, I. & Wehrli, B. Extreme Methane Emissions from a Swiss Hydropower Reservoir: Contribution from Bubbling Sediments. Environ. Sci. Technol. 44, 2419–2425, https://doi.org/10.1021/es09316 (2010).

19. Grasset, C. et al. Large but variable methane production in anoxic freshwater sediment upon addition of allochthonous and autochthonous organic matter. Limnol. Oceanogr. 63, 1488–1501, https://doi.org/10.1002/lno.1876 (2018).

20. Chanton, J. P. et al. Radiocarbon Evidence for the Substrates Supporting Methane Formation within Northern Minnesota Peatlands. Geochim. Cosmochim. Acta 59, 3663–3680, https://doi.org/10.1016/0016-7037(95)00489-Z (1995).

21. Rinke, P. et al. An inter-regional assessment of concentrations and delta C-13 values of methane and dissolved inorganic carbon in small European lakes. Aquat. Sci. 77, 667–680, https://doi.org/10.1007/s00027-015-0140-y (2015).

22. Wahlen, M. et al. C-14 in Methane Sources and in Atmospheric Methane - the Contribution from Fossil Carbon. Science 245, 286–290, https://doi.org/10.1126/science.245.4915.286 (1989).

23. Deuser, W. G., Degens, E. T., Harvey, G. R. & Rubin, M. Methane in Lake-Kivu - New Data Bearing on Its Origin. Science 181, 51–54, https://doi.org/10.1126/science.181.4944.51 (1973).

24. Huttunen, J. T. et al. Fluxes of CH4, CO2, and N2O in hydroelectric reservoirs Lokka and Porttipaata in the northern boreal zone in Finland. Global Biogeochem. Cy 16, https://doi.org/10.1029/2000gb001316 (2002).

25. Friese, K. et al. Ecological response of two hydro-morphological similar pre-dams to contrasting land-use in the Rappbode reservoir system (Germany). Int. Rev. Hydrobiol. 99, 335–349, https://doi.org/10.1002/iroh.201301672 (2014).

26. Tittel, J., Müller, C., Schultz, M., Musolf, A. & Knoller, K. Fluvial radiocarbon and its temporal variability during contrasting hydrological conditions. Biogeochemistry, 1–13, https://doi.org/10.1007/s10533-015-0137-9 (2015).

27. Rinke, K. et al. Reservoirs as sentinels of catchments: the Rappbode Reservoir Observatory (Harz Mountains, Germany). Environmental Earth Sciences 69, 523–536, https://doi.org/10.1007/s12665-013-2464-2 (2013).

28. Perz, A. Longitudinal Heterogeneity in Two Pre-Reservoirs in the Harz Mountains (Germany) master thesis, Swedish University of Agricultural Sciences (2013).

29. Dadi, T., Wendt-Pothoff, K. & Koschorreck, M. Sediment resuspension effects on dissolved organic carbon fluxes and microbial metabolic potentials in reservoirs. Aquat. Sci. 79, 749–764, https://doi.org/10.1002/asl.017-0533-4 (2017).

30. UNESCO/IHA. GHG Measurement Guidelines for Freshwater Reservoirs. 138 (UNESCO, 2010).

31. Tittel, J. et al. The age of terrestrial carbon export and rainfall intensity in a temperate river headwater system. Biogeochemistry 115, 53–63, https://doi.org/10.1007/s10533-013-9896-3 (2013).

32. Nadeau, M. J. et al. Sample throughput and data quality at the Leibniz-Labor AMS Facility. Radiocarbon 40, 239–245 (1998).

33. Grootes, P. M., Nadeau, M. J. & Rieck, A. C-AMS at the Leibniz-Labor: radiometric dating and isotope research. Nucl. Instrum. Meth. B 223, 35–61, https://doi.org/10.1016/j.nimb.2004.01.004 (2005).

34. Wendt-Pothoff, K., Kloss, C., Schultze, M. & Koschorreck, M. Anaerobic metabolism of two hydro-morphological similar pre-dams under contrasting nutrient loading (Rappbode Reservoir System, Germany). Int. Rev. Hydrobiol. 99, 350–362, https://doi.org/10.1002/iroh.201301673 (2014).

35. Fruver, M. & Polach, H. A. Reporting of 14C Data - Discussion. Radiocarbon 19, 355–363 (1977).

36. Yarnes, C. Delta C13 and delta H2 measurement of methane from ecological and geological sources by gas chromatography/combustion/pyrolysis isotope-ratio mass spectrometry: Rapid Commun. Mass Spectrom. 27, 1036–1044, https://doi.org/10.1002/rcm.6549 (2013).

37. Chanton, J. P. et al. Characteristics and reactivity of algae-produced dissolved organic carbon. J Environ Eng-Asce 131, 1574–1582, https://doi.org/10.1061/(Asce)0733-9372(2005)131:11(1574) (2005).

38. Sun, L., Deng, E. M., Meyer, J. L. & Wei, J. Use of elemental composition to predict bioavailability of dissolved organic matter in a Georgia river. Limnol. Oceanogr. 42, 714–721, https://doi.org/10.4319/lo.1997.42.4.0714 (1997).

39. Kritzig, E. S., Langenheider, S. & Lindström, E. S. Influence of dissolved organic matter source on lake bacterioplankton structure and function – implications for seasonal dynamics of community composition. FEMS Microbiol. Ecol. 56, 406–417, https://doi.org/10.1111/j.1574-6941.2006.00084.x (2006).

40. Nguyen, M. L. et al. Characteristics and reactivity of algae-produced dissolved organic carbon. J Environ Eng-Asce 131, 1574–1582, https://doi.org/10.1061/(Asce)0733-9372(2005)131:11(1574) (2005).

41. Sun, L., Deng, E. M., Meyer, J. L. & Wei, J. Use of elemental composition to predict bioavailability of dissolved organic matter in a Georgia river. Limnol. Oceanogr. 42, 714–721, https://doi.org/10.4319/lo.1997.42.4.0714 (1997).

42. Randlett, M. E. et al. Mineralization pathways of organic matter deposited in a river-lake transition of the Rhone River Delta, Lake Geneva. Environ Sci-Proc Imp 17, 370–380, https://doi.org/10.1039/cem00470a (2015).

43. Barth, J. A. C., Mader, M., Nenning, F., van Gelderen, R. & Friese, K. Stable isotope mass balances versus concentration differences of dissolved inorganic carbon – implications for tracing carbon turnover in reservoirs. Isotopes in Environmental and Health Studies 53, 413–426, https://doi.org/10.1080/10256016.2017.1282478 (2017).

44. Garnett, M. H., Hardie, S. M. L., Murray, C. & Billett, M. F. Radiocarbon dating of methane and carbon dioxide evaded from a temperate peatland stream. Biogeochemistry 114, 213–223, https://doi.org/10.1007/s10533-012-9804-2 (2013).

45. Sobek, S., DeSontro, T., Wongnian, N. & Wehrli, B. Extreme organic carbon burial fuels intense methane bubbling in a temperate reservoir. Geophys Res Lett 39, https://doi.org/10.1029/2012GL055144 (2012).

46. Eguster, W., DeSontro, T. & Sobek, S. Eddy covariance flux measurements confirm extreme CH4 emissions from a Swiss hydropower reservoir and resolve their short-term variability. Biogeochemistry 8, 2815–2831 (2011).
53. DelSontro, T., Beaulieu, J. J. & Downing, J. A. Greenhouse gas emissions from lakes and impoundments: Upscaling in the face of global change. *Limnology and Oceanography Letters* **3**, 64–75, https://doi.org/10.1002/lol2.10073 (2018).

54. DelSontro, T. *et al.* Spatial heterogeneity of methane ebullition in a large Tropical Reservoir. *Environ. Sci. Technol.* **45**, 9866–9873, https://doi.org/10.1021/Es2005545 (2011).

55. Mosher, J. J. *et al.* Spatial and temporal correlates of greenhouse gas diffusion from a hydropower reservoir in the Southern United States. *Water* **7**, 5910–5927 (2015).

56. Beaulieu, J. J., McManus, M. G. & Nietch, C. T. Estimates of reservoir methane emissions based on a spatially balanced probabilistic-survey. *Limnol. Oceanogr.* **61**, S27–S40, https://doi.org/10.1002/lo.10284 (2016).

57. Sollberger, S. *et al.* Spatial heterogeneity of benthic methane dynamics in the subaquatic canyons of the Rhone River Delta (Lake Geneva). *Aquat. Sci.* **76**, S89–S101, https://doi.org/10.1007/s00027-013-0319-2 (2014).

58. Morling, K., Herzsprung, P. & Kamjunke, N. Discharge determines production of, decomposition of and quality changes in dissolved organic carbon in pre-dams of drinking water reservoirs. *Sci Total Environ* **577**, 329–339, https://doi.org/10.1016/j.scitotenv.2016.10.192 (2017).

59. Whiticar, M. J., Faber, E. & Schoell, M. Biogenic methane formation in marine and freshwater environments: CO₂ reduction vs. acetate fermentation - Isotope evidence. *Geochim. Cosmochim. Acta.* **50**, 693–709 (1986).

Acknowledgements
We would like to thank the staff at the Stable Isotope Facility Davis and the staff at the Stable Isotope Laboratory Flagstaff for analysing the stable isotopes. Rafael Marcé, Yvonne Rosenlöcher, Erika Ruschak, Corinna Völkner and Martin Wieprecht supported the field work. We also thank Helmut Erlenkeuser who facilitated the preparation of the methane samples for AMS. Comments of two referees improved the manuscript.

Author contributions
J.T. and M.K. designed the research, did the field sampling, performed subsequent measurements and wrote the paper. M.H. prepared the methane samples for radiocarbon analysis, measured them by AMS and contributed to the writing of the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41598-019-52288-1.

Correspondence and requests for materials should be addressed to J.T.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2019