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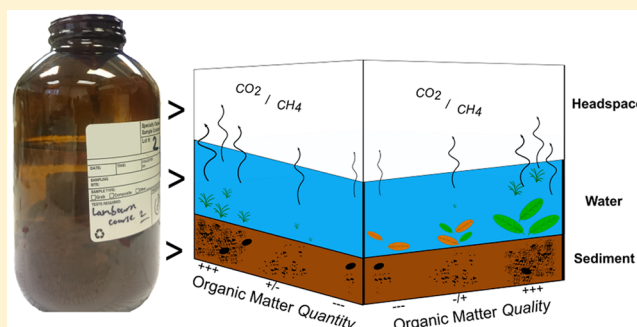
Streambed Organic Matter Controls on Carbon Dioxide and Methane Emissions from Streams

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Supporting Information

ABSTRACT: Greenhouse gas (GHG) emissions of carbon dioxide (CO₂) and methane (CH₄) from streambeds are currently understudied. There is a paucity of research exploring organic matter (OM) controls on GHG production by microbial metabolic activity in streambeds, which is a major knowledge gap given the increased inputs of allochthonous carbon to streams, especially in agricultural catchments. This study aims to contribute to closing this knowledge gap by quantifying how contrasting OM contents in different sediments affect streambed GHG production and associated microbial metabolic activity. We demonstrate, by means of an incubation experiment, that streambed sediments have the potential to produce substantial amounts of GHG, controlled by sediment OM quantity and quality. We observed streambed CO₂ production rates that can account for 35% of total stream evasion estimated in previous studies, ranging between 1.4 and 86% under optimal conditions. Methane production varied stronger than CO₂ between different geologic backgrounds, suggesting OM quality controls between streambed sediments. Moreover, our results indicate that streambed sediments may produce much more CO₂ than quantified to date, depending on the quantity and quality of the organic matter, which has direct implications for global estimates of C fluxes in stream ecosystems.



INTRODUCTION

River corridors, in particular near the interface between groundwater and surface water, provide crucial ecosystem functions such as nutrient spiralling, pollutant attenuation, organism distribution, and fish spawning.^{1–8} In terms of biogeochemical functions, freshwater sediments at groundwater–surface water interfaces in streambeds have been identified as significant contributors to both the global carbon (C) cycle and GHG production.^{9–16} Streambed and river C cycling is strongly affected by the composition and turnover of organic matter (OM) in these environments.^{3,6,9,15,16} Despite early findings in forest streams that streambed sediment OM can be an important driver in sediment respiration,⁹ GHG production as a result of aerobic or anaerobic respiration in streambed sediments has remained understudied in nutrient-rich, agricultural lowland streams.^{17–22} Enhanced nutrient and C loads (and often larger streambed residence time) in lowland agricultural streams offer the potential to significantly alter aquatic ecosystems worldwide,^{23–25} most critically by increasing GHG production.^{20,26–29}

The drivers and controls of GHG emissions from agricultural streams less than 100 m wide, in particular those less than 10 m wide, draining nutrient-enriched catchments remain unknown,^{30–32} despite these systems showing some of the highest CO₂^{16,22} and CH₄^{26,33} outgassing per surface area. One reason for this is the limited knowledge of the impacts of heterogeneous

spatial patterns in geologies, streambed substrates, and, in turn, sediment chemical conditions.^{34–37} Microbial communities in the streambed vary strongly between different substrates,^{38–41} along the river length^{11,42} and with surrounding land use,⁴³ suggesting spatially heterogeneous patterns in GHG production. Streambed CO₂ production is generally associated with aerobic microbial metabolic activity (MMA) as it is a product of the breakdown of organic matter.^{10,44} CH₄ production is typically associated with anaerobic fermentation of organic matter by methanogenic archaea in areas with relatively finer sediment types.^{45–47} Aerobic CO₂ and anaerobic CH₄ production may occur simultaneously in the streambed in discrete microzones; therefore, CH₄ production is not strictly limited to completely anaerobic events if anaerobic microzones are present.⁴⁶ Understanding the relevance and spatial patterns of GHG production from lowland river streambeds requires consideration of variability in OM quantity (as prime control of lowland river respiration processes) as well as the quality of the OM (as control for OM turnover efficiency).

Sources of OM in streambed sediments range from agricultural erosion to local primary production within the

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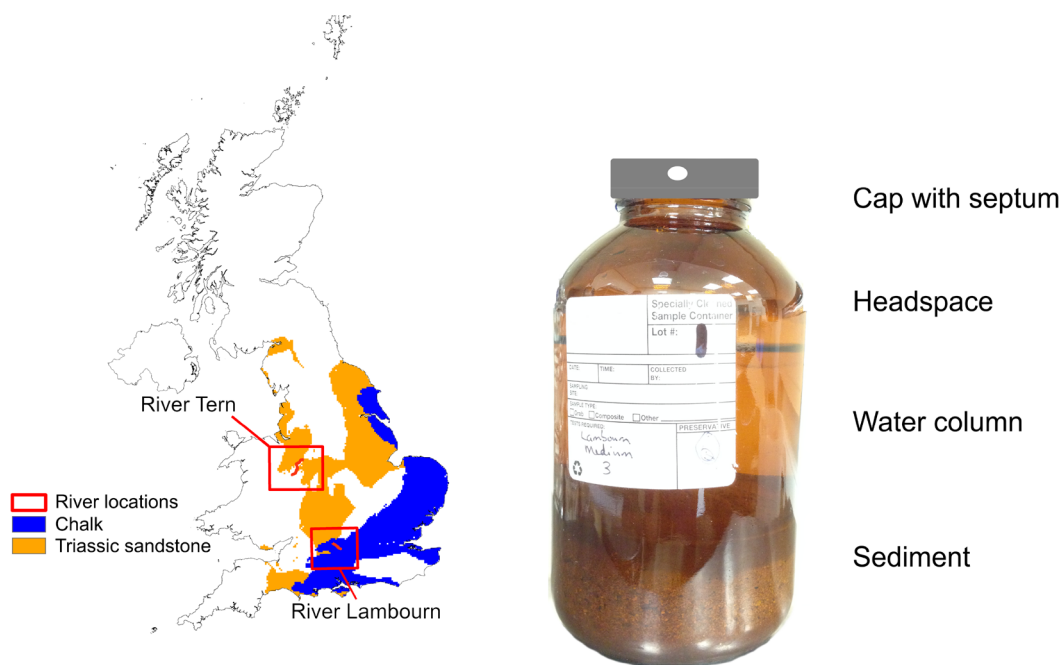


Figure 1. Sampling location of sediments used in the microcosm incubations (left). The River Tern is in a sandstone catchment, and the River Lambourn in a chalk catchment. An example of a microcosm is shown (right) indicating the experimental setup (photograph taken by authors). Map contains data from the British Geological Survey (copyright NERC, 2016) and gadm.org.

stream channel,^{48–52} all of which vary in OM quality. Therefore, C contents of streambed sediments are likely to reflect that of the surrounding erosional surfaces.^{49,53} Signatures of the underlying geology are found in stream particulate organic matter and dissolved organic matter from the headwater to the river mouth.^{39,54} There is evidence that catchment geology plays an important role in river nutrient turnover^{55,56} and ecohydrological and hydrogeological properties.^{57,58} The potential of microbial respiration and associated GHG production in soil sediments has previously been studied in detail in many contexts investigating nutrient cycling,^{59–61} organic matter decomposition,⁶² and influence of water content^{63,64} and contaminants⁶⁵ using batch incubation experiments. For streambed sediments, however, incubation studies have not been widely applied,⁶⁶ despite possible advantages of studying respiration under controlled versus field conditions. Although thermal sensitivity of streambed GHG production varies between sediments from different geologies, there is poor understanding of OM controls on streambed MMA, GHG production, and the role of OM quantity and quality.²⁹ Given that 0.47% of the earth's surface area is covered by streams,³⁰ it is important to quantify the contribution of GHG production from whole stream ecosystems, including under-researched streambed sediments.

In this context, this paper aims to quantify CO₂ and CH₄ (GHG) production from lowland streambed sediments across a gradient of streambed OM contents and different catchment geological backgrounds, focusing on sandstone and chalk. Production of CO₂ and CH₄ was measured alongside aerobic MMA in microcosms containing six different sediment types of varying OM content from UK lowland streams underlain by chalk or sandstone.

■ MATERIALS AND METHODS

Streambed sediments were incubated under controlled conditions. Rather than directly measuring C cycling and GHG production, previous studies considered total stream eva-

sion^{20,22,52} or indirectly calculated CO₂ approaches,³⁰ which may be sensitive to overestimation or underestimation.⁷⁰ Furthermore, field measurements are challenged by the difficulty of isolating the governing processes and drivers.

Streambed Sediments. Sediments used in microcosm incubations were collected from two rivers with contrasting geological substrates:⁷¹ river Lambourn and river Tern (Figure 1 and Table S1). Both rivers are in agricultural catchment areas with similar discharge.⁷² Three locations were chosen within each river based on estimated OM content to achieve a gradient (fine sediment under vegetation, nonvegetated armored streambed, and nonvegetated sand-dominated straight channel section). The total of six field-wet sediment types (Table 1) was collected in September 2015 by scooping off the top 10 cm of the streambed. Bulk sediments were sieved: fine sediments obtained from under vegetation (Chalk_{High} and Sandstone_{High}) were sieved at 0.8 cm to clear large organic debris and the others were sieved at 1.6 cm. These sediments were then homogenized and stored airtight for 5 weeks in the dark at 4.4 ± 0.8 °C until start of the experiments. Sieving is not expected to have a major influence on the results as previous studies show that microbial metabolic activity is expected to be similar or slightly lower after sediment disturbance such as sieving compared to that in situ measurements.^{73–75} The sieving sizes were chosen to preserve as much of the size characteristics present in the field, while larger stones and debris that would otherwise occupy a disproportionately large volume inside the mesocosms were removed. The storage temperature was monitored using a Tinytag Aquatic 2 temperature logger (Gemini Data Loggers Ltd., Chichester, U.K.).

Organic matter content was determined by loss on ignition (LOI). Samples were prepared in triplicate for each sediment type (low, medium, and high for both chalk and sandstone). Sediment was milled, sieved (2 mm), homogenized, and dried at 105 °C for at least 12 h. LOI was performed using an oven at 550 °C for 6 h.⁷⁶ Carbonate content was then determined using the

Table 1. OM Content, Carbonate Content, and Specific UV Absorption at 254 and 280 nm (SUVA) As Measured from the Bulk Sediment^a

geological background	sediment origin	sediment name	% OM	% carbonate	SUVA (1 mg ⁻¹ m ⁻¹)
chalk	under vegetation	Chalk _{High} (CH)	3.625 ± 0.069	11.620 ± 0.499	2.098 ± 0.180
	straight section, no vegetation	Chalk _{Medium} (CM)	1.847 ± 0.063	18.668 ± 1.423	1.277 ± 0.109
	gravel section	Chalk _{Low} (CL)	1.409 ± 0.047	17.170 ± 2.259	1.498 ± 0.092
sandstone	under vegetation	Sandstone _{High} (SH)	3.616 ± 0.116	0.357 ± 0.017	2.642 ± 0.511
	gravel section	Sandstone _{Medium} (SM)	0.838 ± 0.031	0.234 ± 0.016	1.813 ± 0.252
	straight section, no vegetation	Sandstone _{Low} (SL)	0.667 ± 0.014	0.253 ± 0.028	3.209 ± 0.263

^aThe new sediment name has been chosen to reflect actual OM content and varies between chalk and sandstone sediments. Sediment names are abbreviated in figures (abbreviations in brackets). Mean values listed with SD and $n = 3$.

same oven at a temperature of 950 °C for 6 h.⁷⁶ Weight loss relative to original dry sample weight was measured immediately to prevent ambient moisture uptake and then used as the fraction of OM or carbonate.

OM quality was measured by taking sieved sediments (2 mm) in triplicate and extracting DOC from the sediments using 2 M KCl.⁷⁷ DOC content of sediment extracts was determined using a Shimadzu TOC-L analyzer (Shimadzu Corporation, Japan). The sediment extracts were measured for specific ultraviolet absorption (SUVA) at 254 and 280 nm wavelengths.^{78,79} Absorption was measured using a Varian Cary Eclipse UV–vis spectrophotometer (Agilent Technologies, Santa Clara, U.S.A.). A 10 mm path length quartz cuvette was used, and the absorbance between 250 and 280 nm at full wavelength scanning was recorded with 18.2 MΩ ultrapure water for blank correction.

Microcosm Setup. Microcosms were 1 L amber jars with 53/400 size septa-capped lids (Fisher Scientific Ltd., U.K.). The glassware was acid-rinsed with 10% HCl before use and triple washed with 18.2 MΩ ultrapure water. Incubation of each sediment type was done in triplicate. Each of the six sediment types were measured by wet volume using a 300 mL glass beaker and transferred into the microcosms. To control for background concentrations, 500 mL of ultrapure water was added to the wet sediment. The headspace volume of each jar was calculated after the experiment, based on total water and sediment volume. The sediments were then gently, laterally shaken for 30 s to promote mixing of the wet sediment sample and the water column. Three control treatments were prepared as three jars with 500 mL of ultrapure water only. The microcosms were left for 48 h in the dark at the incubation temperature of 15.35 ± 0.15 °C to settle the sediment and minimize turbidity. The refrigerated incubator used was a generic type with forced air circulation and no internal lights.

Microbial Metabolic Activity. Aerobic microbial metabolic activity (MMA) can be successfully measured using the “smart” tracer resazurin. It was first used in ecophysiological applications by Haggerty et al.⁸⁰ It proved useful in many different setups, such as reach-scale field experiments^{81–85} and flume-scale⁸⁶ to microcosm setups.^{87,88} The resazurin (raz)/resorufin (rru) tracer system⁸⁰ was used to quantify MMA in the microcosms to be able to objectively compare activity across the different sediment types. In this process, by acting as a terminal electron acceptor, raz is irreversibly reduced to the strongly fluorescent rru, which can be measured using a fluorometer. Two Albillia GGUN-FL30 fluorometers (Albillia SARL, Switzerland) were used to measure rru production.⁸⁹ The fluorometers were calibrated to either water extracts of the sandstone or chalk at the start of the experiment to ensure best accuracy and to control for variations in background fluorescence. Water extracts were sampled from the incubation

jars before the start of the experiment. For calibration, we used stock solutions of raz and rru made up in tap water, which were diluted into final solutions of 108.67 and 95.33 ng l⁻¹ for raz and rru, using water extracts of sandstone or chalk sediments. The appropriate calibration curve was applied in data analysis. The conversion of raz to rru is reported as μg rru produced per μg of added raz, to normalize to slightly varying initial raz concentrations. The pH of the microcosms was monitored using a hand-held Hanna HI-98129 pH meter (Hanna Instruments, Woonsocket, U.S.A.) to ensure pH was near 8 for accurate fluorometric detection of rru.⁸⁹ Detection limits, calculated precision, and accuracy of this type of fluorometers are listed in Table S2. The target concentration of raz for the starting conditions inside the microcosms was 150 ng l⁻¹.

Gas Sample Analysis. Headspace gas samples were analyzed on an Agilent 7890A gas chromatograph (GC) equipped with a 1 mL sample loop, a platinum catalyst for CO₂-to-CH₄ conversion, and an FID for CH₄ detection (Agilent Technologies, Santa Clara, U.S.A.). Samples were eluted from the column at CH₄ = 3.5 min and CO₂ = 5.7 min, with a total run time of 7 min. The GC was setup in splitless mode with an oven temperature of 60 °C. The FID was set at 250 °C, H₂ flow at 48 mL min⁻¹, air flow at 500 mL min⁻¹, and N₂ makeup flow at 2 mL min⁻¹. Samples were manually injected into the sample loop using a gastight syringe with valve (SGE Analytical Science, Australia). Detection limits, accuracy, and precision for CO₂ and CH₄ were calculated by dilution of a standard gas mixture of known concentration and listed in Table S3. A randomly chosen set of samples were measured twice on the GC to monitor device performance as well as sampling precision.

Sampling Procedure. Microcosms were sampled at $T = 0, 5, 10, 24,$ and 29 h of the incubations to calculate mean hourly rates of MMA, CO₂, and CH₄ production. Headspace gas concentrations were measured before and after each incubation time step to calculate the difference in concentration during each time step. A 15 mL gas sample was taken using a 20 mL syringe with a hypodermic needle and stopcock to pierce the septum. The syringe was purged three times with ultrapure helium 5.0 (BOC Industrial gases, Manchester, U.K.) before each sampling. The 15 mL gas sample was transferred to a 12 mL pre-evacuated Exetainer (Labco Limited, Lampeter, U.K.), causing samples to be slightly overpressurized. The Exetainers were stored at room temperature until analysis. After headspace gas sampling, the jar was opened for MMA measurement.

Samples for fluorometric analysis of rru were extracted from the microcosms using a syringe and filtered to control for turbidity using a 0.45 μm nylon syringe filter (ThamesRestek, High Wycombe, U.K.). The filtered sample was injected directly into the measurement chamber of the fluorometer and measured for at least 3 min at a 10 s measurement interval, which was

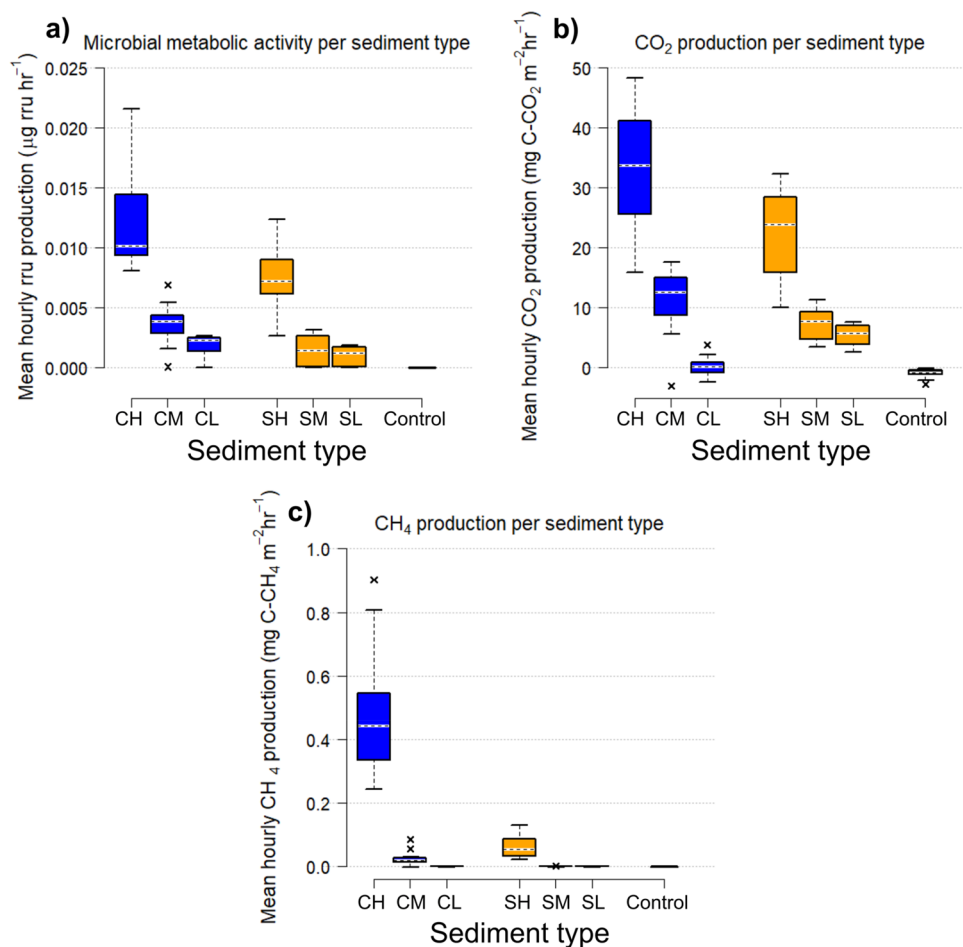


Figure 2. Mean hourly production per sediment type for (a) microbial metabolic activity expressed as rru production normalized for initial raz concentration, (b) carbon dioxide production, and (c) methane production. All values are mean hourly production for each incubation time step. Error bars indicate standard deviation within each group ($n = 12$) of three replicates.

averaged to calculate concentrations. Approximately half of a randomly chosen set of samples were measured again on the second fluorometer to control and correct for drift and instrument errors. Samples were transferred back into the microcosm after measurement on the fluorometer to keep the volume constant, and the fluorometers were rinsed with ultrapure water. After fluorometric analysis, the headspace of the microcosm was equilibrated with ambient air, the lid was closed, and a headspace gas sample was taken before the microcosm was returned to the incubator. A detailed description of the experimental procedure can be found in [Supporting Information text S1](#).

Statistical Analysis. We used descriptive statistics to compare results between sediment types. All mean values listed are noted as mean \pm standard deviation. Error bars in figures represent one standard deviation, unless stated otherwise. For bivariate analysis comparing two sediment types, data were first tested for normal distribution using a Shapiro–Wilks test. A Welch’s t test was used to compare two groups of normally distributed samples, and results were reported as t (degrees of freedom), p , and sample size n . A Mann–Whitney–Wilcoxon test was used where the distribution followed a nonparametric distribution with results reported as U , p , and sample size n . A Kruskal–Wallis test was used to compare whole geology groups, including all different sediment subgroups (see [Supporting Information](#)). Simple linear regression was applied

in some cases with results reported as F (degrees of freedom), p , R^2 , and sample size n .

Experimental Limitations. There are many variables involved in GHG production from sediment respiration. We control for temperature in this experiment at 15.35 ± 0.15 °C, a temperature typical of these types of streams in the UK, and hence isolate impacts of OM and geological background. Although further temperature effects are out of the scope of this study, OM and geological background have also been shown to affect the temperature sensitivity of streambed sediments and associated GHG emissions, further underlying their importance as controls.²⁹ In many natural streambed environments there will be advective processes as drivers for hyporheic exchange and MMA,^{90–93} which we were unable to replicate in the current setup. However, we acknowledge the importance of advection-driven exchange between groundwater and surface water,^{90,91,94,95} considering that recent studies have pointed out the important role of hydraulic forcing as a driver for nutrient turnover in the streambed.^{67,69} Normally, aerobic respiration starts when water enters the streambed and continues flowing consistently along a flow path until dissolved oxygen is depleted or when water enters back into the stream again.^{67,68} Therefore, the microcosm setup in this experiment best simulates a stream under low flow conditions where exchange between the water column and the sediment is diffusion-dominated, rather than forcing by moving water. This

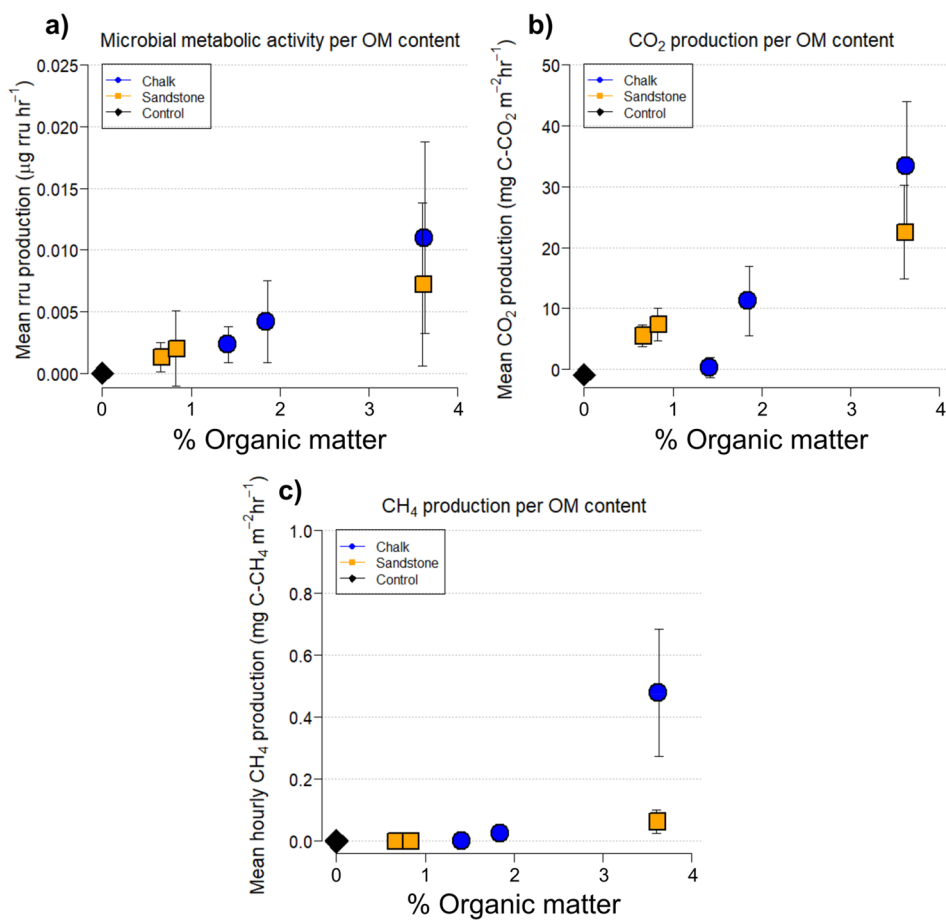


Figure 3. Mean hourly production per organic matter content for (a) microbial metabolic activity expressed as rru production normalized for initial raz concentration, (b) carbon dioxide production, and (c) methane production. Error bars indicate standard deviation within each group ($n = 12$) of three replicates.

simplification of exchange processes complicates comparison to natural streambed environments but is necessary to isolate microbial activity inside the sediments itself. As we sampled undisturbed headspaces of the incubated sediments, the concentration change of gases, particularly CH_4 , represents a net flux inclusive of methanogenesis and methanotrophy and is assumed to include ebullitive events. Even under anaerobic conditions, CH_4 oxidation can occur^{17,96} and thus our measurements represent net fluxes of CH_4 where positive fluxes show net emission and negative fluxes show net consumption.

RESULTS AND DISCUSSION

Sediment types are referred to by OM content (Table 1). All means are given with one standard deviation, and the mean incubation temperature was 15.35 ± 0.15 °C (mean \pm SD). MMA was clearly detected (Figure 2a), indicated by the conversion of raz to rru (interpreted as $\mu\text{g rru}$ produced per hour, normalized for initial raz concentration). CO_2 and CH_4 production were observed in all but the control microcosms. Incubation time had no significant effect on the reported rates of MMA (Figure S1), CO_2 (Figure S2), and CH_4 (Figure S3). In general, sediments with higher OM content produced more rru, CO_2 , and CH_4 and were substantially influenced by the aromaticity of the OM.

OM quality by aromaticity of sediment extractions varied between 1.207 and $3.469 \text{ l mg}^{-1} \text{ m}^{-1}$ as SUVA (Table 1). Mean aromaticity was significantly higher ($t(16) = 3.5546$, $p =$

0.00264 , $n = 18$) in sandstone sediments ($2.554 \pm 0.684 \text{ l mg}^{-1} \text{ m}^{-1}$) compared to chalk sediments ($1.624 \pm 0.385 \text{ l mg}^{-1} \text{ m}^{-1}$). Aromaticity and OM content were not correlated by simple linear regression ($F(16) = 0.01309$, $p = 0.91$, $R^2 = 0.0008$, $n = 18$). This suggests that OM in sandstone sediments is more recalcitrant and thus more difficult to metabolize by microbial communities,⁹⁷ while it is not simply a consequence of total OM content.

MMA varied for different sediment types (Figure 2a). Sediments with the highest OM content of 3.6% were responsible for 65% of the total MMA in streambed sediments of both geological backgrounds (Chalk_{High} and Sandstone_{High}). Overall, chalk sediments produced on average 67% more rru per hour than the sandstone sediments. A generally increasing trend in MMA with increasing OM content was observed (Figure 3a). Mean hourly MMA in chalk sediments was $0.00584 \pm 0.00612 \mu\text{g rru hr}^{-1}$ and in sandstone was $0.00349 \pm 0.00492 \mu\text{g rru hr}^{-1}$. The highest MMA was found in Chalk_{High} sediments, with a mean production rate of $0.011 \pm 0.0078 \mu\text{g rru hr}^{-1}$. Chalk_{High} saw 53% higher MMA than the second highest sediment Sandstone_{High}, which experienced production rates of $0.00721 \pm 0.0066 \mu\text{g rru hr}^{-1}$. Chalk_{High} produced 163% and 370% more rru than lower OM content sediments Chalk_{Medium} and Chalk_{Low}, respectively. Sandstone_{High} produced 262% and 463% more than Sandstone_{Medium} and Sandstone_{Low}, respectively.

CO_2 production followed similar patterns as observed for MMA (Figures 2a,b and 3a,b). Mean hourly CO_2 production in

chalk sediments was $14.97 \pm 15.54 \text{ mg C-CO}_2 \text{ m}^{-2} \text{ hr}^{-1}$ and for sandstone was $11.83 \pm 9.03 \text{ mg C-CO}_2 \text{ m}^{-2} \text{ hr}^{-1}$, which was 27% higher in chalk compared to sandstone. Like MMA, the highest CO₂ production was found in Chalk_{High} sediments, with a mean of $33.40 \pm 10.55 \text{ mg C-CO}_2 \text{ m}^{-2} \text{ hr}^{-1}$. Chalk_{High} produced 48% more CO₂ than the second highest sediment Sandstone_{High}, which produced $22.53 \pm 7.72 \text{ mg C-CO}_2 \text{ m}^{-2} \text{ hr}^{-1}$. Chalk_{High} produced 197% more CO₂ than the lower OM content sediment Chalk_{Medium} and Chalk_{Low} produced at rates comparable to the controls (Figure 2b). Sandstone_{High} produced 203% and 308% more CO₂ than Sandstone_{Medium} and Sandstone_{Low}, respectively. Sediments with the highest OM content (Chalk_{High} and Sandstone_{High}) were responsible for 62% of total CO₂ production in both chalk and sandstone (Figure 3b).

Patterns of methane production from different sediment types differed from those observed for MMA and CO₂ (Figures 2c and 3c). Overall, mean chalk sediment production was $0.1685 \pm 0.2503 \text{ mg C-CH}_4 \text{ m}^{-2} \text{ hr}^{-1}$ compared to only $0.0213 \pm 0.0365 \text{ mg C-CH}_4 \text{ m}^{-2} \text{ hr}^{-1}$ in sandstone and there was 692% more CH₄ in chalk compared to sandstone. Again, Chalk_{High} sediment was characterized by the highest CH₄ production with a mean of $0.4778 \pm 0.2042 \text{ mg C-CH}_4 \text{ m}^{-2} \text{ hr}^{-1}$. Chalk_{High} produced 656% more than the second highest producer Sandstone_{High}, which produced only $0.0632 \pm 0.0369 \text{ mg C-CH}_4 \text{ m}^{-2} \text{ hr}^{-1}$. Compared to lower OM content sediments, Chalk_{High} produced 1689% more than Chalk_{Medium}, whereas CH₄ production in Chalk_{Low} was similar to that of the controls. In Sandstone_{Medium} and Sandstone_{Low}, no difference in methane production was observed compared to the controls. Sediments with the highest OM content produced 95% and 100% of all methane in chalk and sandstone, respectively (Figure 3c). Methane production accounted for 1.1% of total C losses in chalk sediments, compared to only 0.2% in sandstone.

Our results highlight the substantial potential for streambed sediments from agricultural lowland rivers to produce significant amounts of CO₂ and CH₄. We sampled only the top of the streambed (see Methods), although respiration can also occur at greater depths in the streambed, depending on availability of OM and oxic/anoxic zonation.^{2,34,98,99} Especially the presence of dissolved oxygen is normally driven by exchanging streamwater in natural streambeds (see Experimental Limitations) and is an important control on MMA. Substantial MMA and CO₂ and CH₄ production was observed that was in a similar range to in situ forest stream sediments⁹ and ponds¹⁰⁰ and lower than CO₂ and CH₄ production from Mediterranean stream sediment incubations.⁶⁶

MMA increased with higher OM content. Rru production was found to be linearly correlated by simple linear regression with CO₂ production and proved a good measure for respiration (Figure 4; $F(70) = 32.91, p \ll 0.001, R^2 = 0.320, n = 72$). Higher MMA was observed in chalk sediments compared to sandstone, and finer sediments in both geologies represented 62% of MMA. OM quantity alone does not explain the discrepancy in MMA, and we suggest this difference arises from OM quality, expressed as aromaticity. Sandstone sediments had significantly higher aromaticity than chalk sediments. Higher aromaticity is typically associated with lower carbon quality and higher recalcitrance^{78,79} and can, therefore, explain our observations of generally lower MMA and lower CO₂ and CH₄ production in the sandstone sediments.

Our results suggest that the observed headspace CO₂ concentrations did not originate from inorganic carbonate

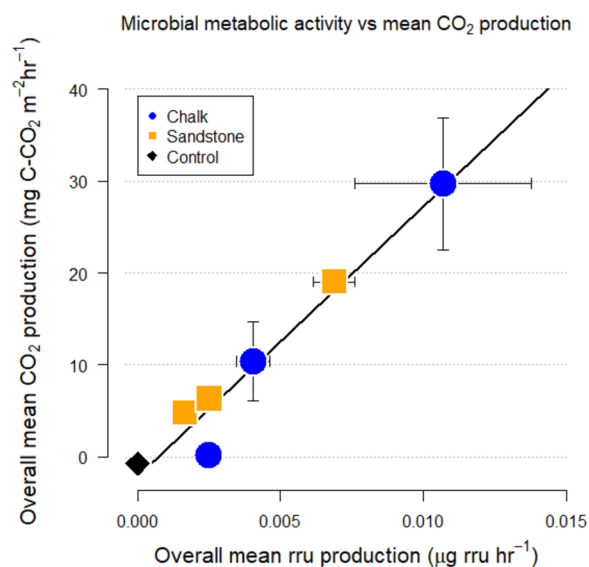


Figure 4. Mean CO₂ production increased with mean microbial metabolic activity. All values are based on overall means of all time steps. Error bars indicate standard deviation ($n = 12$). A trend line has been fitted through the combined chalk and sandstone data points to illustrate the general rru-to-CO₂ relation, independent of sediment type. This trend line represents the simple linear regression model that has been fitted. Although control treatments are excluded from the linear regression model, they are plotted for reference.

weathering.^{101,102} First, although average hourly CO₂ production in Chalk_{Low} sediment with 17.2% carbonate content was slightly but significantly different from the control treatment ($U = 113, p = 0.017, n = 12$), the means were both negative and very close to zero (0.266 ± 1.674 and $-0.878 \pm 0.796 \text{ mg C m}^{-2} \text{ hr}^{-1}$ for Chalk_{Low} and control, respectively). We interpret a negative production in this context as a net CO₂ uptake from the headspace into the water column. Second, sediments with highest and lowest carbonate contents within our experiment (Chalk_{Medium}: 18.67%, Sandstone_{Medium}: 0.23%; Table 1) did not show a significantly different hourly CO₂ production from each other ($t(16) = 2.0912, p = 0.053, n = 12$). This suggests that it is unlikely that inorganic carbonate weathering alone was an important source of CO₂ measured in the headspace of the microcosms.

We could measure substantial CO₂ and CH₄ production and MMA throughout the experiment. In natural streambed environments, aerobic respiration takes place along flow paths inside the sediment and increases with flow velocity.⁶⁷ Considering the absence of any downwelling fluxes into the sediment, we can assume that the observed GHG production measured in the microcosms can be underestimations of an in situ measurement. The microcosms did not show signs of carbon depletion, as CO₂ production and MMA were not significantly different throughout the duration of the experiment (Figures S1 and S2). Therefore, although we are unable to test this hypothesis, microbial activity inside a streambed could be higher still than what was measured in this experiment, because flowing water would introduce oxygen and dissolved carbon deeper into the streambed sediments. The rate of oxygen uptake by biological activity, and thus CO₂ production, would then be a function of downwelling velocity of the streamwater into the streambed, especially in agricultural streams where carbon and nutrients are not often limited.^{67,69} The absence of downwelling water could, however, lead to an overestimation of CH₄

production, since depleted oxygen is not replenished and methanogenesis by facultative or obligatory anaerobes can become a more favorable pathway for carbon turnover.^{98,103,104}

Our findings extend previous knowledge from forest streams to agricultural streams. We expected higher GHG production in agricultural streambed sediments than in forest streambed sediments, since soil OM from surrounding land surfaces can be eroded and transported into streams.^{47,51,52} Hedin⁹ found mean hourly CO₂ production of 5.8 mg C m⁻² hr⁻¹, between 1.1 and 14.2 mg C m⁻² hr⁻¹ (mean temperature 14.6 °C), in an in situ streambed incubation where water exchange between stream and streambed was blocked during the 4 h incubation periods. The streambed sediments from our agricultural sites produced between 0.27 and 33.40 mg C m⁻² hr⁻¹, with a mean value of 13.40 mg C m⁻² hr⁻¹, which suggests higher production rates were observed in the presented study under laboratory conditions. It should be noted that such a comparison is not straightforward given the large range. However, a similar pattern is observed when comparing our results with observed ranges of 13–106 mg C m⁻² hr⁻¹ in an in situ incubation experiment in an agricultural lowland stream in the Czech Republic,¹⁰⁵ supporting the hypothesis that land use has a strong influence on microbial respiration and associated GHG production in streambeds. We acknowledge that this study ignores any seasonal variations or the absence of water exchange between stream and streambed that was discussed in the section [Experimental Limitations](#), which would likely be found under in situ conditions. Nevertheless, the information presented above highlights the influence of land use: streambeds in agricultural streams can produce at least twice as much CO₂ compared to those in forest streams due to higher C and nutrient loading from the surrounding catchment due to land use.^{14,16,106}

The streambed sediments produce a substantial proportion of total CO₂ that is emitted from streams. Previous studies have often suggested that the source of CO₂ emitted from streams is mainly inorganic CO₂ laterally transported into the stream by groundwater.^{20,22,30,107} When taking the limitations in consideration as discussed in the section [Experimental Limitations](#), the results from this study suggest that a substantial proportion of total CO₂ production in streams may be ascribed to local microbial respiration in the streambed. The mean CO₂ production rate for all sediments of 13.40 ± 12.72 mg C–CO₂ m⁻² hr⁻¹ equals 117.36 g C m⁻² yr⁻¹. When applying this average value to the U.K. and considering a U.K. land surface cover by streams and rivers of 0.86%,³⁰ we estimate the potential CO₂ production from streambed sediments to be between 0.02 and 2.52 t C km⁻² yr⁻¹, with a mean of 1.01 t C km⁻² yr⁻¹. Previous research estimates between 1.4 and 2.9 t C km⁻² yr⁻¹ of total excess CO₂ evasion from U.K. streams and rivers, depending on the role of photosynthesis;¹⁰⁸ therefore, our results suggest that the tops of the streambed and riverbed alone may be responsible for between 1.4% and 86% of total CO₂ fluxes from U.K. streams and rivers, with a mean contribution of 35% under optimal conditions. The true contributions are likely to be on the lower end of the range of estimation, depending on spatial and seasonal variability and we take caution extrapolating to field conditions given that our estimates are based on laboratory incubations under optimal conditions. These findings are crucial for estimates of global C budgets but also have significant consequences for the design of excess nutrient attenuation strategies.

CH₄ production varied strongly between chalk and sandstone sediments, which varied in OM quality. Our study could not

differentiate between CH₄ production and consumption; however, our measures of net fluxes are representative of both processes, given that methanotrophy can occur under anaerobic conditions^{77,96} and that methanogenesis can occur in anoxic microsites in otherwise predominantly oxic sedimentary environments.¹⁰⁹ Chalk_{High} was the single highest methane producer. We believe this can be explained by differences in OM quality between sediments or microbial communities, such as more productive microbial communities in the chalk sediments. Similar median emission rates (by ebullitive transport) of 0.375 mg C–CH₄ m⁻² hr⁻¹ were found in an extensive global study,³³ and Sanders et al.⁴⁷ found similar mean emission rates from the water column in chalk streams of 0.168 mg C–CH₄ m⁻² hr⁻¹. The average CH₄ production rate for all sediments was 0.09 ± 0.19 mg C–CH₄ m⁻² hr⁻¹ and ranged between 0.0 and 0.48 mg C–CH₄ m⁻² hr⁻¹, which equals a mean annual flux of 0.83 g C–CH₄ m⁻² yr⁻¹. Our results are comparable to the lower end of the range of 0–1.5 mg C–CH₄ m⁻² hr⁻¹ found in an agricultural lowland stream in the Czech Republic.¹⁰⁵ When comparing the mean flux observed here (from Chalk_{High} sediments) with the best estimate of CH₄ emissions from chalk streams in the U.K. of 3.2 × 10⁻⁶ Tg yr⁻¹,⁴⁷ we find that our estimated total of 3.0 × 10⁻⁵ Tg yr⁻¹ are approximately one magnitude higher than estimates based on field observations. A possible explanation for the higher estimates can be found in the absence of macrophytes and associated rhizosphere in the sediments, which are known to promote methane oxidation.^{47,110}

Both the Chalk_{High} and Sandstone_{High} sediments were collected from underneath macrophytes, yet only Chalk_{High} produced CH₄ at rates similar to those observed in the field.⁴⁷ Observed ratios of CH₄/CO₂ ranged from 0 to 0.011 in Chalk_{High}, which is low compared to earlier findings of 0.22 underneath macrophytes in a U.K. chalk river¹⁷ but similar to a difference of 3 orders of magnitude reported in a survey of a small lowland agricultural stream.¹⁰⁵ However, comparing CH₄/CO₂ ratios across different landscapes and methods is precarious as little is known about this anaerobic scaling.³³ Fine sediments trapped by vegetated patches are associated with a higher diversity of methanogenic archaea in the streambed.⁴⁵ Average OM contents were higher in chalk sediments, and sediments with higher OM content produced more CH₄ than sediments with lower OM contents. Streambed OM content has previously been shown to control CH₄ production, although this was only significant at OM contents in excess of 8%.¹⁰³ This supports our observations that although there is evidence that OM controls GHG production, the magnitude of production shows substantial differences across different substrates.

We found evidence that OM quality (measured by aromaticity) is a main driver of streambed sediment respiration that varied significantly between sediment types of the two different background geologies. Where Hedin⁹ only hypothesized the influence OM quality differences, this study was able to show that OM quality is an important control on streambed sediment respiration. Higher MMA in chalk sediments and a possible difference in carbon quality are reflected in CO₂ production, where sediments with similar OM content (3.6%) produce more CO₂ in different geologic environments. When available substrates vary along a river and thus with streambed sediment, local microbial communities are expected to adapt and specialize to that reach.^{11,42} This can be partially explained by the fact that reactivity of OM usually decreases with molecule size as OM is further broken down.¹¹¹ Fresher OM availability drove higher respiration rates in our high OM sediments,

Chalk_{High} and Sandstone_{High}. Additionally, OM quality likely varied between the Chalk_{High} and Sandstone_{High} collection site due to differences in macrophyte communities^{11,12} and associated differences in microbial communities that may exist.^{11,13} We may, therefore, expect differences in streambed microbial communities between chalk and sandstone sediments, despite other physical river characteristics being similar.

In summary, this study provides new conceptual system-level mechanistic understanding of the potential of streambed sediments in agricultural streams to produce substantial amounts of CO₂ and CH₄ across a gradient of OM contents, where highest OM sediments showed the highest production rates and lower OM sediments showed lower production rates. MMA and associated GHG production are related to higher OM content as an important driver, which is conditioned by different geological backgrounds. These differences in OM between geological backgrounds are reflected in significant differences in OM quality (aromaticity) that can support different microbial communities that mineralize OM and C at different rates. We provide evidence that mineralization of OM can produce substantial CO₂ emissions from streambed sediments under controlled conditions and the streambed itself, when assuming similar conditions. When compared with excess CO₂ fluxes from streams, we find that streambed CO₂ production can represent 35% (ranging between 1.4 and 86%) of total stream CO₂ evasion under optimal conditions. Similar numbers for CO₂ evasion have been estimated before,²² and we now provide new evidence that a large portion of the internal CO₂ production of agricultural streams can be attributed to the top of the streambed. Methane production varies even stronger between sediment from different geologic backgrounds, suggesting a microbial community-driven variation between streambed sediments. Stream water exchange processes were outside the scope of this study but may lead to even higher in situ MMA and associated GHG production, which may be addressed by future experimental designs. Our findings lead us to the conclusion that both OM quantity and quality are important controls on streambed OM mineralization and improve our understanding of the potential impacts of human-induced land use change that may lead to increased OM and nutrient loading in lowland streams.

■ ASSOCIATED CONTENT

📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.est.8b04243](https://doi.org/10.1021/acs.est.8b04243).

Figures S1–S3, containing MMA, CO₂, and CH₄ production per time step; Text S1, describing the experimental procedure in detail; Tables S1–S6, listing river characteristics of the sampled rivers Lambourn and Tern, fluorometer and GC instrument parameters, and additional statistical results (PDF)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Author Contributions

‡P.R. and S.C.W. contributed equally and declare a shared first-authorship. P.R. and S.C.W., together with S.K., contributed equally to designing and conceptualising the study, performing the experiments, and collecting and analyzing data. P.R. wrote the manuscript with support of S.C.W. S.U. provided guidance on application of the GHG analysis method and support with data analysis and manuscript revisions. D.M.H. provided support with manuscript revisions. S.K. instigated the study and advised on the experimental design and the interpretation and the conceptualisation of the results and supported the data analysis, interpretation, and manuscript revisions.

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Notes

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