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Howard, Ben Christopher; Baker, Ian; Kettridge, Nicholas; Ullah, Sami; Krause, Stefan

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# Wood Increases Greenhouse Gas Emissions and Nitrate Removal Rates in River Sediments: Risks and Opportunities for Instream Wood Restoration

Ben Christopher Howard,\* Ian Baker, Nicholas Kettridge, Sami Ullah, and Stefan Krause



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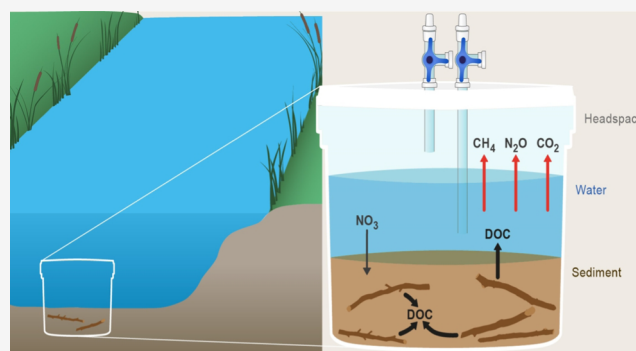
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**ABSTRACT:** The (re)introduction of wood into rivers is becoming increasingly popular, but its impact on streambed biogeochemical cycling is still poorly understood. It could affect fundamental ecosystem processes through multiple, potentially interacting mechanisms and lead to cascading effects for ecosystem function and the delivery of associated (dis)services. Here, a microcosm study explored the impacts of instream wood on key biogeochemical functions for different streambed sediment types throughout a typical temperate climate temperature range. The effects on a suite of physiochemical characteristics and microbial metabolic activity (MMA) were measured, as well as on associated ecosystem services (nitrate removal rate) and dis-services (greenhouse gas [GHG] emissions). Streambed wood significantly increased MMA, a key ecosystem process that underpins stream biogeochemical cycling. This likely explained an associated increase in the removal rate of nitrate and the emission of some GHGs. This study demonstrates that instream wood is a fundamental driver of stream biogeochemical activity. Omitting streambed wood from mechanistic studies of streambed biogeochemical activity could reduce the representativeness of results to real systems, with consequences, for instance, for global GHG emission estimates. If such ulterior impacts of (re)introducing instream wood are not considered, decision-makers may fail to identify risks and opportunities of restoration programs.

**KEYWORDS:** streambed biogeochemical cycling, instream wood, large woody debris, greenhouse gas emissions, nutrient removal, wood restoration, ecosystem (dis)services, microbial metabolic activity



## 1. INTRODUCTION

**1.1. Switching the Paradigm—from Removal to Reintroduction.** Streams and rivers can deliver societally important ecosystem services, but their capacity to do so has been reduced by centuries of mismanagement, like channelization and simplification, e.g., the removal of instream wood.<sup>1</sup> The realization of the potential of the river corridor to provide nature-based solutions to some of society's most wicked challenges, and the amenability of these systems to restoration, has contributed to the rapid increase in the number and scale of river restoration projects globally.<sup>2,3</sup> One of the most widely encouraged means in which to achieve restoration is the (re)introduction of instream wood,<sup>3–5</sup> which for centuries had been removed for navigation and to reduce localized flooding.<sup>6</sup> This technique is now recommended in policy in the European Union Water Framework Directive<sup>7</sup> and in national guidelines in the United States.<sup>8</sup> Furthermore, the rate of indirect introduction of wood to river corridors (e.g., from falling trees and branches) is likely to increase as riparian zones are rewooded,<sup>9</sup> as landscapes are rewilded,<sup>10</sup> and, in the U.K., as beavers (*Castor fiber*) are reintroduced.<sup>11</sup>

**1.2. Neglected Ulterior Impacts.** Instream wood restoration has been adopted to deliver a range of objectives, such as habitat improvement,<sup>12</sup> natural flood management,<sup>13,14</sup> increased hydrological connectivity,<sup>15–18</sup> and water quality objectives.<sup>19,20</sup> However, notwithstanding management objectives, changes to the abundance and distribution of instream wood are likely to also result in ulterior impacts that are not yet fully understood and the consequences of which cannot yet be reliably predicted.<sup>5,21,22</sup> For example, instream wood is probably an important driver and control of biogeochemical activity, such as cycles of carbon (C) and nitrogen (N). It affects the production, transport, and bioavailability of dissolved organic carbon (DOC),<sup>23,24</sup> which is a key control

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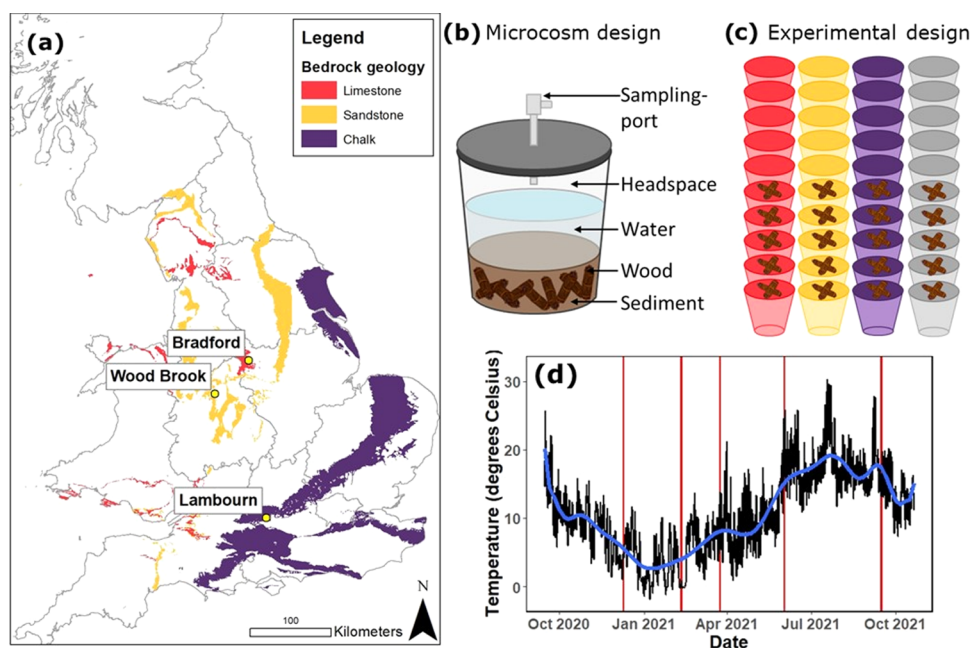


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**Figure 1.** (a) River sediment sampling locations with underlying bedrock geology. (b) Schematic microcosm design with wood buried by sediment at the bottom and water column and headspace above, with sampling tube and stopcock for gas sampling. (c) Microcosm treatments where pink = limestone sediment, yellow = sandstone sediment, purple = chalk sediment, and gray = no sediment; wood was added to half the treatments, and each treatment was replicated 5 times. The location of treatments was randomized. (d) Water temperature ( $^{\circ}\text{C}$ ) before and during the experimental period, where the black line represents temperature at 12 noon, the blue line represents the LOESS (locally weighted smoothing), and the vertical red lines indicate measurement dates.

of biogeochemical reactions, for example, it can limit nitrate ( $\text{NO}_3^-$ ) turnover.<sup>25</sup> Similarly, its decomposition can introduce nutrients and modify stoichiometric ratios of C/N/Phosphorus(P), which control respiration rates.<sup>26,27</sup> It could also increase streambed hydraulic conductivity, create preferential flow paths, and encourage bioturbating aquatic invertebrates, all of which could modify surface water–groundwater interactions, which control reaction rates and fluxes.<sup>28–30</sup> Other potential mechanisms include providing a surface for microbes and biofilms and creating heterogeneity in the structure of the streambed, which can drive hyporheic exchange and facilitate a matrix of redox conditions that are necessary for many step-wise biogeochemical reactions.<sup>19,31,32</sup>

These impacts on biogeochemical activity could lead to changes to the delivery of some of the important ecosystem (dis)services that rivers and streams provide, such as controlling the flux of C and N from freshwater and terrestrial environments to marine environments and the atmosphere.<sup>24,33</sup> Streams and rivers provide an ecosystem service by retaining and removing  $27 \text{ Tg N yr}^{-1}$ , >40% of the inputs they receive, although they still contribute  $37 \text{ Tg N yr}^{-1}$  to coastal waters.<sup>34</sup> Some of this removal is attributable to transformations along the river corridor, which can transform polluting aqueous species, such as  $\text{NO}_3^-$ , to environmentally benign dinitrogen ( $\text{N}_2$ ) gas or the harmful greenhouse gas (GHG) nitrous oxide ( $\text{N}_2\text{O}$ ).<sup>35,36</sup> Furthermore, the mineralization of DOC in aerobic respiration and anaerobic respiration leads to the production of carbon dioxide ( $\text{CO}_2$ ) and methane ( $\text{CH}_4$ ), respectively, also harmful GHGs.<sup>37</sup> Streams and rivers provide an ecosystem dis-service, emitting an estimated  $1.8 \text{ Pg CO}_2\text{--C yr}^{-1}$ ,<sup>38</sup>  $26.8 \text{ Tg CH}_4\text{--C yr}^{-1}$ ,<sup>39</sup> and  $0.68 \text{ Tg N}_2\text{O--N yr}^{-1}$ ,<sup>40</sup> and these emissions are likely to increase as the temperature of surface waters increase as a result of climate change.<sup>41,42</sup> Despite the potential con-

sequences, the effects of instream wood restoration on these ecosystem (dis)services are yet to be demonstrated by a controlled and systematic investigation.

**1.3. Study Motivations and Aims.** The aim of this work is to determine the importance of instream wood for biogeochemical activity in streams and rivers, which contribute disproportionately to global biogeochemical cycles for their relatively small surface area.<sup>19,24,42,43</sup> We aim to evaluate the consequences of excluding instream wood in mechanistic studies which explore streambed biogeochemistry, in which it is usually ignored or explicitly omitted despite often composing a significant proportion area of streambed sediments (e.g., refs 37 and 42). In addition, we aim to frame the results of this study saliently to a management context, highlighting the ulterior impacts of instream wood restoration, which are often neglected, and encouraging decision-makers to consider the opportunities, risks, and conflicts that arise from the co-delivery of ecosystem (dis)services.<sup>44</sup>

These aims have been pursued using a microcosm experiment, in which  $\text{NO}_3^-$  removal rate and total GHG flux are used as examples of potentially associated ecosystem services and dis-services, respectively. The microbial metabolic activity (MMA) of the heterotrophic bacteria that facilitate these reactions is used as an indicator of system metabolism, a fundamental control of biogeochemical activity more widely.<sup>45,46</sup> Well-documented key controls on streambed biogeochemical activity are considered by using treatments of different river sediments—which differ in geology, (C) content, and grain size distribution—and by conducting the experiments throughout a range of temperatures representing a temperate climate.<sup>37,42</sup> The comparison of incubation experiments with in situ measurements remains a challenge and therefore these experiments do not provide absolute and scalable estimates to allow extrapolation to in situ conditions.<sup>42</sup>

They do, however, allow for the systematic analysis of the effect of streambed wood on MMA,  $\text{NO}_3^-$  removal rate, and GHG fluxes.

## 2. METHODS

**2.1. Microcosm Setup.** Microcosm chambers were constructed from 1750 mL buckets with gas-tight lids made of polypropylene, which blocked out most of the ultraviolet light, and fitted with sampling ports with a three-way stopcock (Figure 1b). Each of the 8 treatments was replicated 5 times, resulting in 40 microcosms in total (Figure 1c). Microcosms were stored outside at EcoLaboratory (University of Birmingham, U.K.), allowing the temperature to fluctuate with environmental conditions (Figure 1d). Locations were randomized, and no significant difference was observed in temperature between treatments (Supporting Information (SI), Figure S1). From assembly in May 2019 until December 2020, microcosms were undisturbed to allow for microbial communities to stabilize and wood to begin to decompose.<sup>42</sup> Lids remained closed, but sampling ports remained open to allow gas exchange.

**2.2. Sediment Treatments.** In order to capture variability in streambed sediment characteristics, which are a key control of streambed biogeochemistry,<sup>37</sup> 4 sediment treatments were investigated, including no sediment, and sediments extracted from streams overlying sandstone, limestone, or chalk geologies (Figure 1c). Sandstone sediment was collected from Wood Brook in Staffordshire, U.K. (lat 52.80, long  $-2.30$ ), from under total tree cover (Figure 1a). Chalk sediment was collected from the river Lambourn in Berkshire, U.K. (lat 51.44, long  $-1.38$ ), from under partial tree cover. Limestone sediment was collected from the river Bradford in Derbyshire, U.K. (lat 53.17, long  $-1.68$ ), from under no tree cover. Sediments were collected from the top 10 cm and in the thalweg in February 2019 and stored in a cold room (at approx. 4 °C) until microcosm assembly in May 2019, at which point 600 mL (510 mL in cases where wood was also added) of wet sediment was added to relevant microcosms. Large stones and wood ( $>2$  cm) were removed before assembly, which would have occupied a disproportionately large volume of the total sediment.<sup>37</sup> After the decommissioning of the microcosms, grain size analysis and loss on ignition (LOI) (for organic matter and carbonate) were conducted, Figure S2a,b, respectively.<sup>47</sup>

**2.3. Microcosm Composition and Construction.** Common hazel (*Corylus avellana*) typically surrounds water courses in Northern Europe, and its wood is often used in river restoration, for example, in fascines.<sup>48</sup> It was introduced to half of the microcosms (those of with wood treatment), which received 5 mm  $\times$  50 mm long sections of large (20 mm), medium (10 mm), and small (5 mm) diameter wood pieces. Wood was added before sediment and so remained buried for the extent of the experiment to simulate buried streambed wood, except in the treatments without sediment. Each microcosm received 500 mL of artificial stream water—a nutrient mixture of 10 mg/L  $\text{NO}_3^-$ , 0.1 mg/L  $\text{NH}_4^+$ , and 0.1 mg/L  $\text{PO}_4^{3-}$  prepared in ultrapure water (18.2 M $\Omega$ ·cm)—which probably resulted in slightly varying nutrient concentrations when mixed with porewater. Minor differences in starting nutrient concentrations are likely to have a limited impact on experimental results following the period of stabilization, after which equilibration of concentrations is

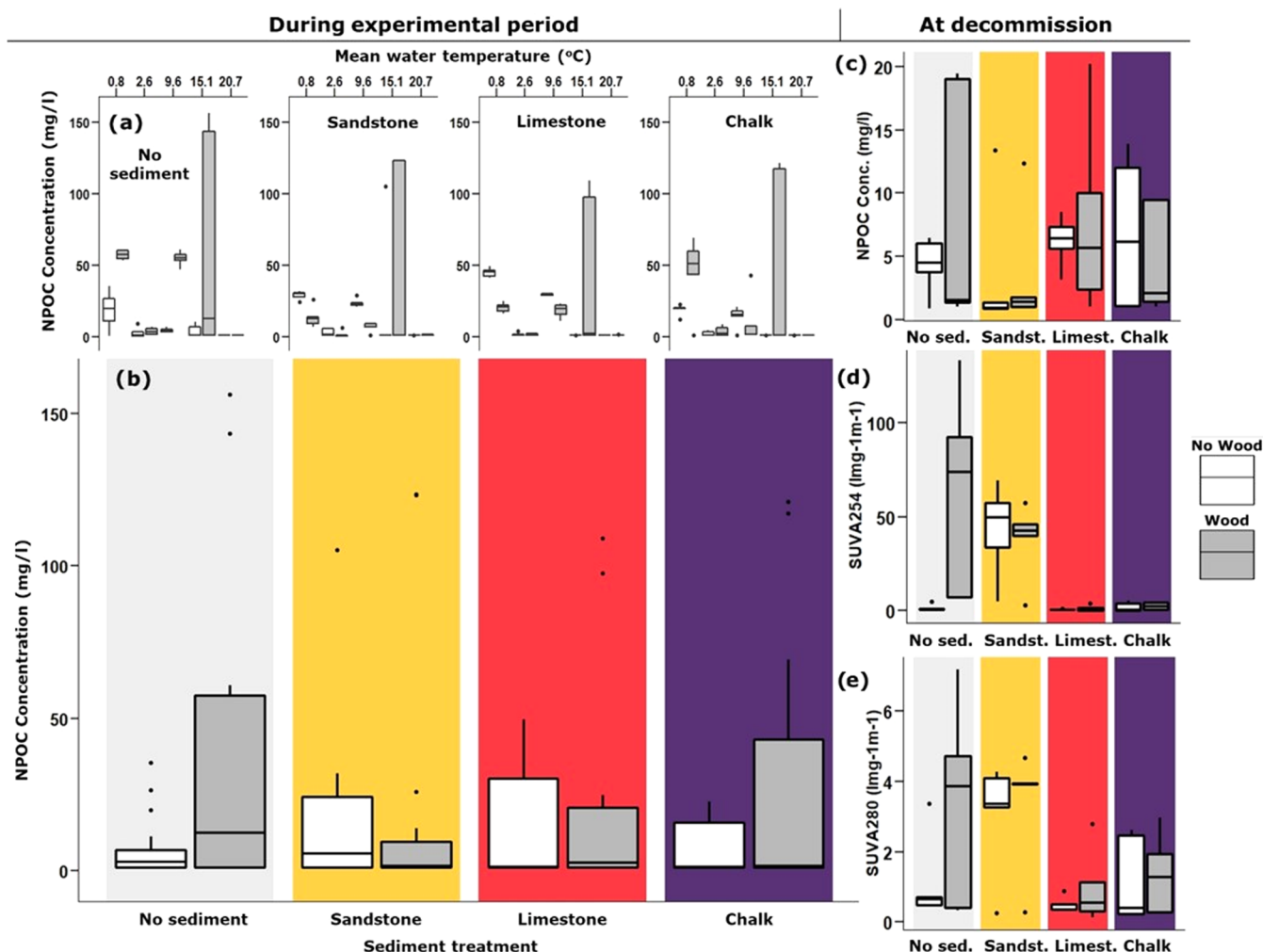
likely. The total wet volume of each microcosm was 1100 mL, leaving 650 mL headspace.

**2.4. Spiking Procedure.** Five spiking events were conducted throughout the experimental period (December 2020 to October 2021), scheduled to capture annual temperature variability (Figure 1d). Microcosms were spiked with  $\text{NaNO}_3$  and resazurin (raz) (Apollo Scientific Ltd., lot: AS456027) prepared in 12 mL volume of ultrapure water 12 h previously. Raz is a smart tracer, which is transformed to its daughter compound, resorufin (rru), in mildly reducing conditions, thus providing a proxy for MMA.<sup>46,49</sup> Concentrations of  $\text{NO}_3^-$  and raz were measured 2 days prior to the preparation of spikes to enable target concentrations to be more accurately achieved. Concentrations of  $\text{NO}_3^-$  in the water column were elevated to 50 mg/L, the maximum concentration in safe drinking water.<sup>50</sup> Concentrations of raz were elevated to 0.15 mg/L, a typical target concentration that allows high detection but would decay sufficiently between spiking events.<sup>46</sup>

**2.5. Water Sampling and Analysis.** 12 mL of water was sampled at 0 and 26 h (after 15 s of mixing the column water), after pH (Figure S3) and temperature (Figure S1) were measured with a Hanna probe (HI-98129), and the concentration of dissolved oxygen (DO) was measured with a FireSting Fibreoptic DO probe. The water was replaced with ultrapure water after each measurement period, not after sampling at hour 0 in order to maintain a consistent water volume. Water samples were immediately filtered with 0.45  $\mu\text{m}$  glass fiber syringe filters (Cole-Parmer) and stored for no more than 48 h before analysis.<sup>46,51</sup> Concentrations of  $\text{NO}_3^-$  were analyzed on a continuous flow analyzer (San++, Skalar, Breda, The Netherlands), with a limit of detection and precision of  $0.001 \pm 1\%$  mg/L  $\text{NO}_3^-$ -N, respectively. Concentrations of nonpurgeable organic carbon (NPOC) were analyzed on a Shimadzu TOC-L analyzer (Shimadzu Corporation, Japan) with a limit of detection of 4  $\mu\text{g}$  C/L. NPOC refers to the (C) remaining after the sample has been purged of inorganic (C); because these samples were filtered to remove materials larger than 0.45  $\mu\text{m}$  (e.g., particulate organic matter), NPOC here can be considered DOC. Specific ultraviolet absorbance (SUVA) was measured at wavelengths of 254 and 280 nm on a ultraviolet visible light spectrophotometer (Jenway 6850) and normalized for concentrations of DOC.<sup>52</sup> Concentrations of raz and rru were measured on a Varian Cary Eclipse benchtop fluorescence spectrophotometer (Agilent Technologies, Inc.). Emission/excitation wavelength pairs of 600/617.97 nm for raz and 570/586 nm for rru were used, which are within the ranges suggested by Haggerty, Argerich, and Martí.<sup>49</sup> Signal overlap was corrected using the MATLAB code provided by Knapp et al.<sup>46</sup>

**2.6. Gas Sampling and Analysis.** 15 mL of gas was sampled at 2, 6, and 24 h using a gas-tight syringe (SGE Analytical Science). Gas was sampled at 0 h for every fifth microcosm (i.e., 8 times). The gas samples were immediately transferred to pre-evacuated 12 mL gas vials (Exetainer vial, Labco Ltd.) for storage under positive pressure and at room temperature for no more than 3 months.<sup>53,54</sup> Gas samples were analyzed on an Agilent 7890A Gas Chromatograph using a 1 mL sample loop and an oven temperature of 60 °C. A flame ionization detector measured  $\text{CH}_4$  and  $\text{CO}_2$  (which is methanized with a catalyst before analysis), and an electron capture detector measured  $\text{N}_2\text{O}$ . The instrument operated with a hydrogen flow of 48 mL  $\text{min}^{-1}$ , air flow of 500 mL  $\text{min}^{-1}$ ,



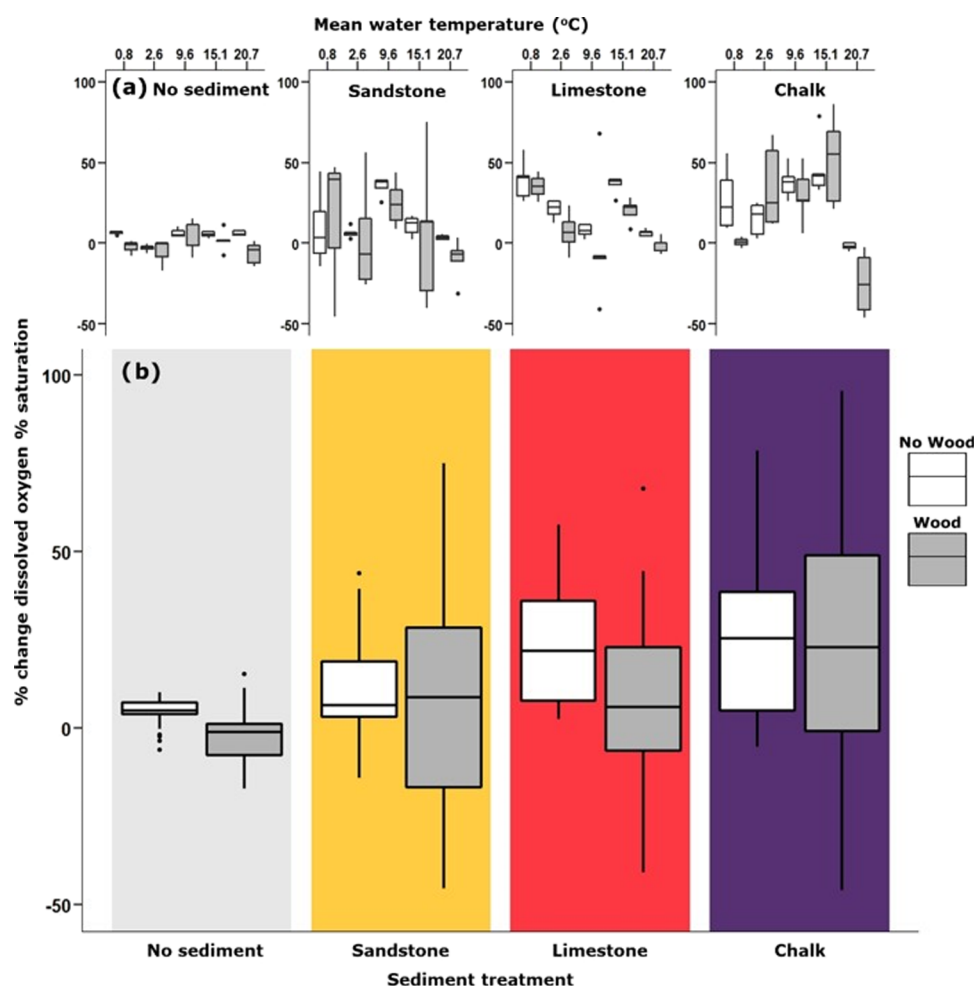


**Figure 2.** Measures of carbon quality and quantity in microcosms containing different treatments of sediment (none = gray, sandstone = yellow, limestone = pink, chalk = purple) and wood (no wood = white infill, wood = gray infill). Panels (a, b) represent the concentration of nonpurgeable organic carbon (NPOC) separately at five measurement periods (represented by mean water temperatures) and amalgamated, respectively. Panel (c) represents the concentration of DOC at the end of the study, where triplicates of sampling and analysis were conducted to ensure the accuracy of results. Panels (d, e) represent the specific ultraviolet absorbance at 254 and 280 nm, respectively, both at decommission.

and a makeup pure nitrogen flow of  $2 \text{ mL min}^{-1}$ . Final gas fluxes are presented rather than production because, in some cases, microcosms represented a sink of GHGs. Fluxes were calculated using the methodology by Pitombo et al.,<sup>55</sup> with adjustments to account for the changing volume and pressure due to the sampling regime (i.e., gas was not replaced after sampling, reducing volume and leading to negative pressure). Fluxes were calculated between 2 and 24 h, discounting 0–2 h because samples at hour 0 were not extracted from individual microcosms. Linearity was not always observed throughout this period for every treatment, but treating flux rates as linear represented the most appropriate method to apply to all treatments and, thus, allowed a comparison, which was a primary aim of this study (see Figure S4). Fluxes are reported as per 24 h and normalized for sediment (and wood) mass.<sup>55</sup> Gas flux values of microcosms not containing sediments (i.e., controls) are only reported in the text (i.e., not used in statistical tests or figures) and are reported as flux per microcosm per day.

**2.7. Statistical Analysis.** Some gas samples were missing due to challenges with sampling or analysis, resulting in  $n = 141$ . Q–Q plots and histograms were used to confirm the

normality of the response variable and/or residuals (within treatment groups), as per the assumptions of the statistical test performed. Linear mixed-effects models (LMEM) were applied with a restricted maximum likelihood estimation using the lme4 package<sup>56</sup> in RStudio.<sup>57</sup> These tests were first performed on amalgamated data (i.e., every sediment treatment and temperature period together), excluding the no sediment treatment (i.e., sandstone, limestone, and chalk only). This isolated the effect of the inclusion of streambed wood from the confounding effects of sediment and temperature. In these models, wood treatment (wood or no wood), sediment type (sandstone, limestone, and chalk), and temperature (0.8, 2.6, 9.6, 15.1, 20.7 °C) were all included as fixed effects. The microcosm number (representing subsamples within one sediment treatment and repeated measures at different temperatures) was included as a random effect to avoid pseudoreplication in treating subsampled observations and repeated measures as independent.<sup>58</sup> Following the execution of two LMEMs—one including the fixed term, wood treatment, and one “null model” not including this term—the model outputs were compared using a likelihood ratio test.<sup>56</sup> The respective  $P$  values are reported in text, and model



**Figure 3.** Percentage change in dissolved oxygen percent saturation over a 26 h measurement period in microcosms containing different treatments of sediment (none = gray, sandstone = yellow, limestone = pink, chalk = purple) and wood (no wood = white infill, wood = gray infill), presented separately at five measurement periods (represented by mean water temperatures) (a) and amalgamated (b).

outputs of the likelihood ratio test are reported in [Tables S1–S7](#). The same series of models and tests were performed on subsets of data, which included only one sediment type (no sediment, sandstone, limestone, and chalk) per model set, where wood treatment and temperature are included as fixed effects and microcosm number is included as a random effect.

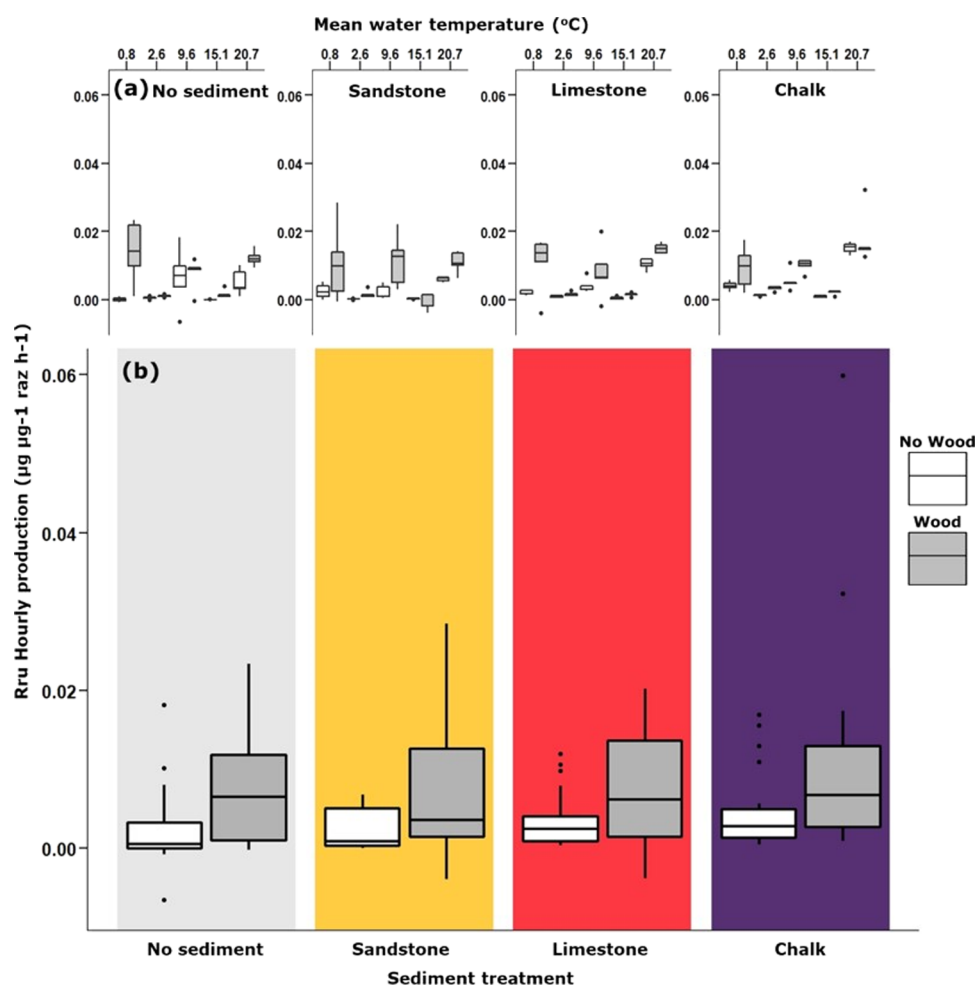
### 3. RESULTS

**3.1. Carbon Quantity and Quality.** Over the entire temperature regime, DOC was not significantly different in microcosms containing wood compared to those without ([Figure 2b,c](#)). However, the concentration of DOC was significantly higher in microcosms containing wood for sediment treatments of no sediment ( $P < 0.05$ ) and chalk ( $P < 0.05$ ). This difference was most profound for microcosms containing no sediment, where an average difference of 475% was observed over the entire temperature regime. For those microcosms containing sediment and no wood, the concentration of DOC was very similar in sandstone ( $15.69 \text{ mg/L} \pm 21.97$ ) and limestone ( $15.65 \text{ mg/L} \pm 18.43$ ) and lower in chalk ( $7.41 \text{ mg/L} \pm 8.23$ ). Higher concentrations of DOC than expected were observed in the microcosms containing no wood or sediment ( $6.23 \text{ mg/L} \pm 8.65$ ), though still less than half those observed in sandstone and limestone treatments,

suggesting contamination (e.g., by insects) or autochthonous production (e.g., primary production).

At the final ( $15.1^\circ\text{C}$ ) measurement period (October 2021) ([Figure 2a](#)), the average concentration of DOC in treatments containing wood was notably higher than at other periods and highly variable within sediment treatment groups (no sediment =  $62.79 \text{ mg/L} \pm 71.29$ ; sandstone =  $49.97 \text{ mg/L} \pm 28.19$ ; limestone =  $42.17 \text{ mg/L} \pm 50.14$ ; chalk =  $48.24 \text{ mg/L} \pm 57.96$ ). These spikes in DOC concentration were not observed in microcosms not containing wood, leading to large percentage differences between microcosms containing wood and those not, of 1460% in no sediment treatments, 129% in sandstone, 4587% in limestone, and 4839% in chalk. Patterns of similar magnitude were not observed when DOC was rigorously measured with repeated sampling and analysis at decommission ([Figure 2c](#)).

There was little apparent difference in the  $\text{SUVA}_{254}$  between microcosms containing wood and those not, except for the microcosms containing no sediment, where those containing wood exhibited higher absorbances, indicating a higher aromaticity and, therefore, a lower bioavailability ([Figure 2d](#)).<sup>52</sup> Similar patterns were observed for  $\text{SUVA}_{280}$  in the no sediment, sandstone, and limestone, but in the chalk treatments, microcosms containing wood had a higher (by 45%) absorbance than those without, indicating a higher



**Figure 4.** Production rate of rru per hour normalized for the starting concentration of raz in microcosms containing different treatments of sediment (none = gray, sandstone = yellow, limestone = pink, chalk = purple) and wood (no wood = white infill, wood = gray infill), presented separately at five measurement periods (represented by mean water temperatures) (a) and amalgamated (b).

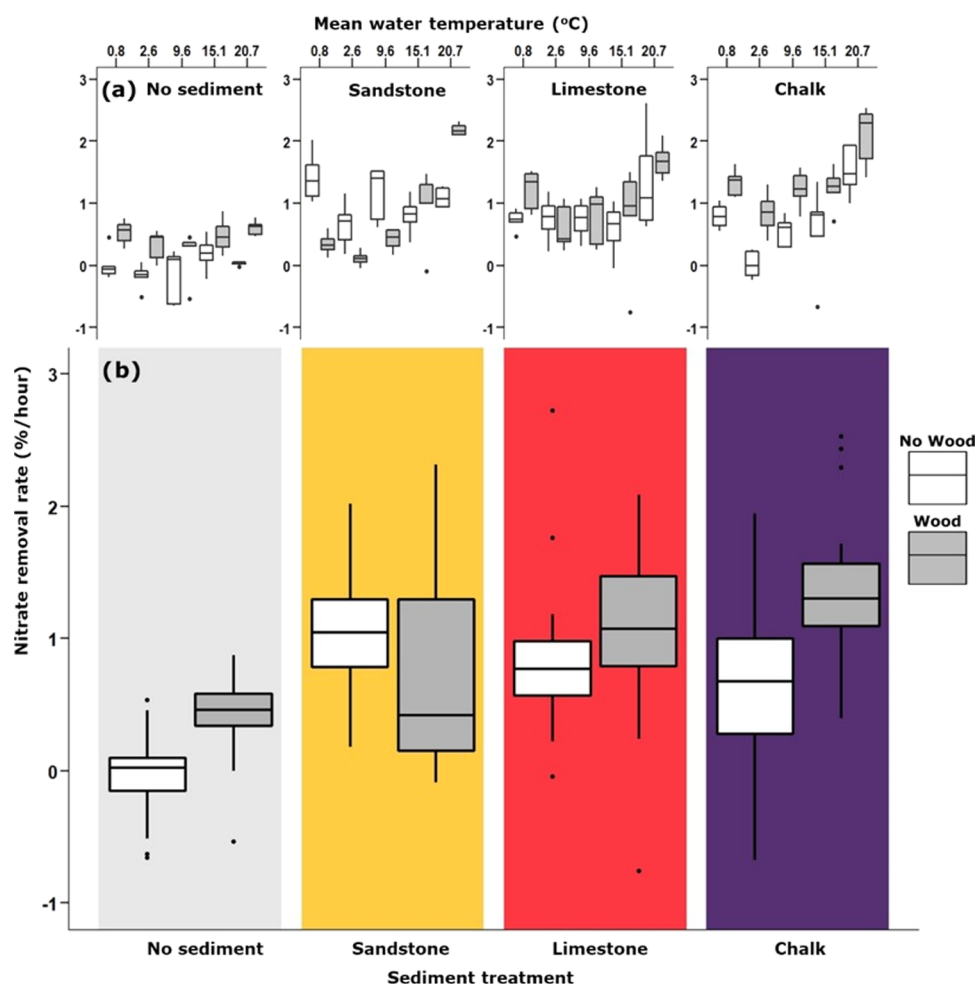
molecular weight of DOC, which is associated with lower volatility and bioavailability (Figure 2e).<sup>59</sup> Both  $SUVA_{254}$  and  $SUVA_{280}$  indicate that sandstone sediments provided the lowest quality (i.e., the least bioavailable) DOC, while limestone and chalk provided DOC of similar quality.

**3.2. Dissolved Oxygen.** DO data is presented as the percentage change in the DO percent saturation (DO%) over the 26 h measurement period to account for differences in starting concentrations. Over the entire temperature regime, the percentage change in the DO% was not significantly different in microcosms containing wood compared to those without (Figure 3b). Only in microcosms of no sediment was a significant difference observed ( $P < 0.05$ ), with an average  $-1.7\%$  change in microcosms containing wood and an average  $4.33\%$  change in those not (Figure 3b). Patterns were somewhat temperature-related, especially for microcosms of chalk sediment, but the relationships are seemingly complex, probably related to other temporally dependent factors (Figure 3a). Microcosms containing wood exhibited a higher variability within sediment treatments compared to those not, as well as in microcosms of sandstone and chalk sediments compared to limestone or no sediment (Figure 3a).

**3.3. Microbial Metabolic Activity.** MMA results are presented as hourly production of rru ( $\mu\text{g}$ ) normalized for the starting concentration of raz ( $\mu\text{g}$ ) at hour 0 (as in ref 42).

Similar MMA was observed in other studies of similar design (e.g.,<sup>37</sup>). Over the entire temperature regime, MMA was significantly higher in microcosms containing wood compared to those without ( $P < 0.05$ ) (Figure 4b). Within sediment treatments too, MMA was significantly higher in microcosms containing wood for no sediment (209.18% higher,  $P < 0.05$ ), sandstone (188.16% higher,  $P < 0.05$ ), limestone (103.37% higher,  $P < 0.05$ ), and chalk (129.86% higher,  $P < 0.05$ ) (Figure 4b). MMA in the microcosms of no sediment and no wood was lower than in other sediment treatments containing no wood but still substantial ( $0.0023 \mu\text{g} \mu\text{g}^{-1} \text{raz h}^{-1} \pm 0.0047$ ). The variability in MMA was higher within groups containing wood compared to those without, and the patterns in MMA were positively associated with temperature, though other factors are influential, for example, at the  $0.8^\circ\text{C}$  measurement period (December 2020), where a higher than expected MMA was observed (Figure 4a).

**3.4. Nitrate Removal Rate.** The rate of  $\text{NO}_3^-$  removal is presented as the percentage reduction in the concentration of  $\text{NO}_3^-$  per hour during the measurement period, to account for the small differences in starting concentration at hour 0 (i.e., a positive value indicates removal). Over the entire temperature regime, the  $\text{NO}_3^-$  removal rate was significantly higher in microcosms containing wood compared to those without ( $P < 0.05$ ) (Figure 5b). However, within sediment treatments,



**Figure 5.** Rate of nitrate removal per hour in microcosms containing different treatments of sediment (none = gray, sandstone = yellow, limestone = pink, chalk = purple) and wood (no wood = white infill, wood = gray infill), presented separately at five measurement periods (represented by mean water temperatures) (a) and amalgamated (b).

$\text{NO}_3^-$  removal rate was only significantly higher in microcosms containing wood compared to those not for microcosms of no sediment ( $P < 0.05$ ) or chalk sediment ( $P < 0.05$ ) (Figure 5b). The  $\text{NO}_3^-$  removal rate was consistently lower in microcosms of no sediment compared to those containing sediment, and only small rates were observed in microcosms of no sediment and not containing wood ( $-0.024\% \pm 0.28$ ). Strong temperature-related trends were observed for all treatments, though as with MMA, other factors (as discussed in Section 4.2) are evident, again at the 0.8 °C measurement period (December 2020).

**3.5. Greenhouse Gas Fluxes.** Over the entire temperature regime, in microcosms containing wood, the  $\text{CO}_2$  flux and the  $\text{CH}_4$  flux were significantly higher ( $P < 0.05$ ) (Figure 6a,c), but the  $\text{N}_2\text{O}$  flux was not ( $P > 0.05$ ) (Figure 5e). Within sediment treatments, the  $\text{CO}_2$  flux was only significantly higher in microcosms containing wood for limestone ( $P < 0.05$ ) (Figure 6b). While the  $\text{CH}_4$  and  $\text{N}_2\text{O}$  fluxes appeared to be higher in microcosms containing wood, the difference was not significant for any sediment treatments ( $P > 0.05$ ) (Figure 6c,f). The total flux of all GHG was negligible for microcosms containing no sediment and not containing wood (mean  $\text{CO}_2 = 91.4 \pm 27.69 \mu\text{g}^{-1} \text{ day}^{-1}$ ; mean  $\text{CH}_4 = -0.88 \mu\text{g}^{-1} \text{ day}^{-1} \pm 3.49$ ;  $\text{N}_2\text{O} = -7.33 \mu\text{g}^{-1} \text{ day}^{-1} \pm 23.22$ ) and of microcosms containing no sediment and wood, only  $\text{CO}_2$  was produced at

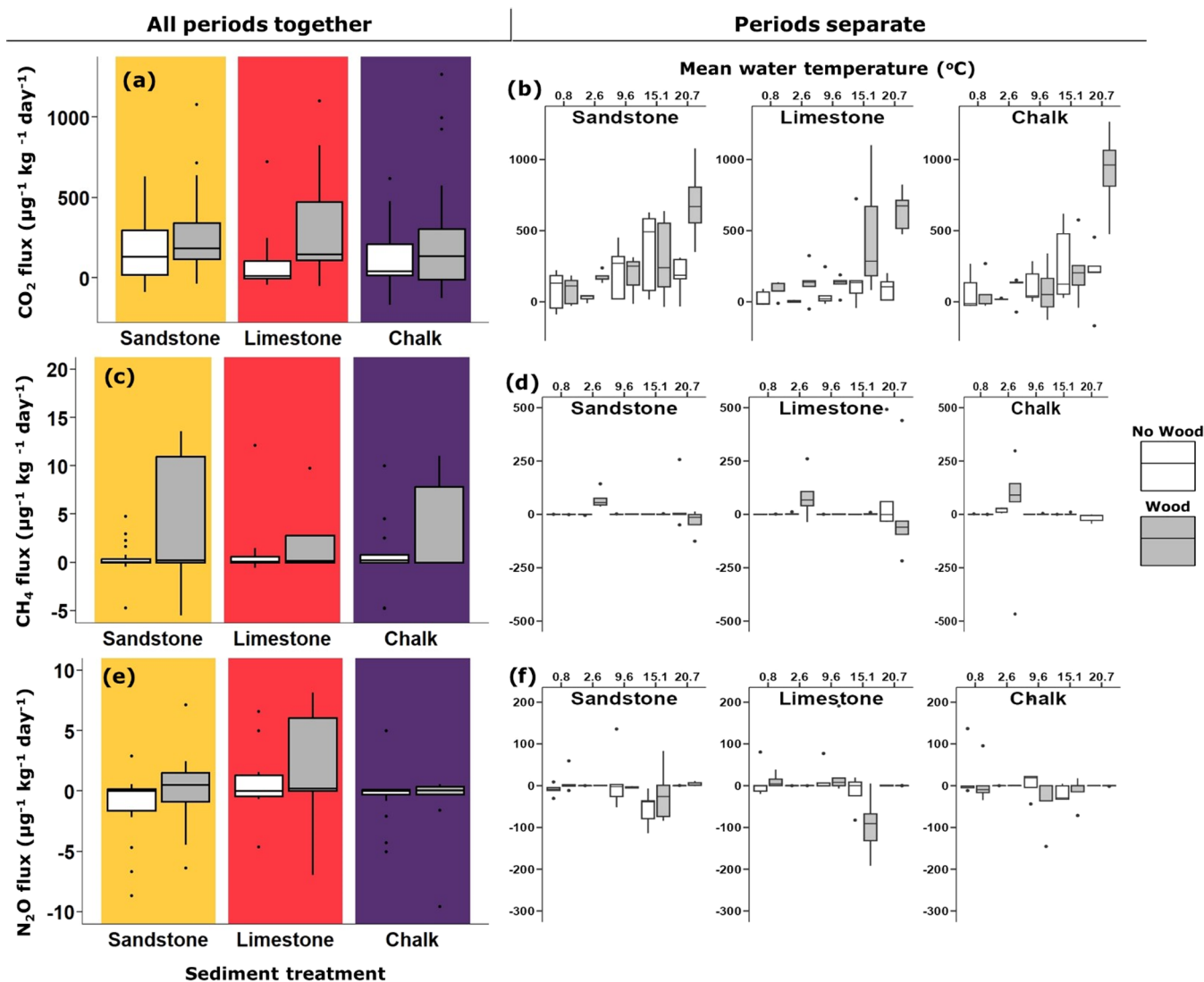
notable rates. Temperature trends were evident for  $\text{CO}_2$  fluxes only (Figure 6b,d,f).

## 4. DISCUSSION

**4.1. Wood in Streambeds—A Fundamental Component of Whole Stream Metabolism and Biogeochemical Cycling.** Streambed sediments containing wood generally exhibited higher biogeochemical activity (as indicated by elevated MMA in Figure 4) in a range of sediment types and throughout temperatures typical of a temperate climate. This demonstrates the importance of wood as a driver of fundamental ecosystem processes. Changes to the abundance and distribution of streambed wood, for example, by its (re)introduction in river restoration, are therefore likely to lead to changes in biogeochemical activity, with associated and potentially cascading effects for ecological and biogeochemical cycles, well beyond those measured here.<sup>45,60</sup> This contributes to the growing body of evidence that suggests wood is a fundamental component of river ecosystems, which is vital for their proper function and health.<sup>3,5</sup>

**4.2. Effects on Turnover Mechanisms.** Several mechanisms by which instream wood could accelerate biogeochemical activity have been identified, for example, by increasing surface water–groundwater interactions and providing a surface for microbes and biofilms.<sup>28,32</sup> In this study, only the





**Figure 6.** Flux of  $\text{CO}_2$  (a, b),  $\text{CH}_4$  (c, d), and  $\text{N}_2\text{O}$  (e, f) per day in microcosms containing different treatments of sediment (sandstone = yellow, limestone = pink, chalk = purple) and wood (no wood = white infill, wood = gray infill), presented separately at five measurement periods (represented by mean water temperatures) (b, d, f) and together (a–c).  $\text{Kg}^{-1}$  refers to per kg of sediment. Graphs of  $\text{CH}_4$  (c) and  $\text{N}_2\text{O}$  (e) are zoomed in to focus on the interquartile range of the boxplots, excluding some outliers; graphs including all data are presented in Figure S5.

effect of streambed wood on DOC was explicitly measured, which was overall found to be insignificant for both quantity (i.e., concentration) and quality (i.e., bioavailability). Differences were observed in the no sediment and chalk treatments, however, which in the absence of wood provided the lowest concentrations of DOC to the water column. This suggests that where sediments contribute little DOC to the water column, the introduction of wood could significantly increase the concentration of DOC, although the quality of this DOC may be initially lower than that which is aquatically derived (as indicated by the elevated SUVA in Figure 2). This increase in DOC could have a particularly substantial effect on biogeochemical reaction rates in already DOC-limited environments, despite the lower quality.<sup>25</sup> Consequently, instream wood restoration may be especially impactful in systems where the availability of DOC limits reaction rates (i.e., rather than transport alone), such as where stoichiometric ratios of C to oxidizing agents (e.g.,  $\text{NO}_3^-$ ) are especially low.<sup>61</sup>

The absence of an increase in DOC concentration in microcosms containing wood is likely explained by the prompt

consumption of DOC by microbes. It may also be explained by the slow rate of wood decomposition in the microcosm environment, in which decomposition was effectively limited to chemical and biological processes (e.g., enzymatic decomposition), excluding the physical processes that are the primary drivers of decomposition in *in situ* conditions (e.g., by causing physical fragmentation).<sup>62</sup> Furthermore, smaller pieces of wood decompose more quickly,<sup>62</sup> and wood can be immobilized in the streambed, where it can decompose over long durations (i.e., longer than investigated here), especially in lowland environmental settings.<sup>19</sup> Therefore, the rate of wood decomposition may be expected to be higher in *in situ* conditions compared to in the microcosm environment, which could result in a higher contribution of wood to DOC concentrations. On the contrary, DOC could be quickly transported out of sediments in river environments.<sup>19</sup>

Freshwater carbon dynamics are complex, controlled by a series of biotic and abiotic processes like decomposition, production, mineralization, and transport.<sup>24,62</sup> The relative prevalence of the processes active in the microcosm environ-

ment is largely controlled by physiochemical conditions, like temperature and the availability of oxidizing agents (e.g., oxygen and  $\text{NO}_3^-$ ).<sup>63</sup> Therefore, the measured concentration and bioavailability of DOC in microcosms at each measurement period were determined by recent (hours to days in these systems) reaction histories (i.e., production and consumption) and antecedent environmental conditions.<sup>63</sup> The snapshot of the carbon dynamics afforded by the infrequent sampling limits inference but still allows us to formulate hypotheses. High DOC concentrations were observed in all treatments at the first measurement period (0.8 °C), conducted in December 2020, where microcosms had been left to stabilize for approximately 1 year. Similarly, high DOC concentrations were observed at the 15.1 °C measurement period (September 2021), where a longer time had elapsed since the previous spiking (~3 months) than for other measurement periods (~1 month). These longer periods without the introduction of  $\text{NO}_3^-$  could have allowed a large DOC stock to accumulate while DOC consumption (e.g., by aerobic respiration) was limited by nutrient availability.<sup>64</sup> Subsequently, this could have resulted in a faster consumption of carbon and transformation of  $\text{NO}_3^-$  immediately following spiking, which has been termed “luxury uptake” by other authors.<sup>65,66</sup> This hypothesis could also explain the higher than expected  $\text{NO}_3^-$  removal rates and  $\text{CO}_2$  fluxes, which were observed at the 0.8 and 15.1 °C periods.<sup>67</sup>

MMA and the change in the concentration of DO were expected to exhibit similar patterns because they are both measures of aerobic respiration. Such a response was not evident, however, and instead, the depletion in DO during measurement periods was not significantly increased in microcosms containing wood while MMA was. This may be explained by oxygen production as a product of photosynthesis as well as by an artifact of the experimental procedure, where the DO concentration was measured at hour 0 before mixing (the last disturbance of the water having been ~1 week previous), whereas measurement at hour 26 was after more recent mixing (at hour 0), which could introduce oxygen to the water column. High MMA in microcosms containing neither wood nor sediment may be explained by respiration of autochthonous carbon, which can be extremely bioavailable.<sup>23</sup>

An increase in the concentration and bioavailability of DOC probably explained some of the increases in MMA, the rate of  $\text{NO}_3^-$  removal, and the production of GHGs. However, it did not explain these responses entirely, and it is evident that other mechanisms are also responsible, such as those outlined in the introduction. Further research is necessary to identify and quantify the mechanisms by which streambed wood leads to a response in biogeochemical activities (as outlined in Section 1.2), for example, its capacity to host microbial communities and biofilms and its effect on the structural properties of sediments (e.g., hydraulic conductivity and preferential flow paths).

**4.3. River Restoration Using Instream Wood—Effects on Ecosystem (Dis)services.** Managing elevated concentrations of N in freshwaters is one of the biggest challenges facing environmental engineers in the 21st century.<sup>70</sup> Here, we demonstrate that the (re)introduction of instream wood may be a viable technique by which to accelerate the removal of N in the river corridor, offering a nature-based solution to this persistent challenge. It could be especially effective in streams where the availability of C limits reaction rates and where

wood and DOC are not quickly transported downstream, for example, some lowland environmental settings.<sup>19,25</sup>

While the observed reduction in  $\text{NO}_3^-$  concentrations could represent an ecosystem service, it could also be explained by a number of N transformation pathways that lead to both temporary removal, such as dissimilatory nitrate reduction to ammonia (DNRA), and permanent removal from water, such as complete denitrification.<sup>36,68</sup> It was outside the scope of this study to quantify the relative removal pathway prevalence, but it is likely that denitrification was responsible for at least some removal (leading to permanent removal), as evidenced by the accompanied (visual) increase in  $\text{N}_2\text{O}$  emissions in microcosms containing wood.<sup>36</sup> The transformation of  $\text{NO}_3^-$  in denitrification is positive if complete transformation occurs (i.e., to  $\text{N}_2$ ), but incomplete transformation (i.e., to  $\text{N}_2\text{O}$ ) may merely represent pollution swapping, which is not overall a suitable mitigation solution.<sup>69</sup> A strong relationship between  $\text{NO}_3^-$  removal rate and temperature was evident, as has been reported by other authors.<sup>70</sup> This suggests that  $\text{NO}_3^-$  removal rate in rivers and streams could increase as river water temperature increases.<sup>41</sup>

The contribution of streams and rivers to global GHG emissions is of global significance.<sup>38–40</sup> Some of the key controls of GHG production in riverbeds are well understood, for example, organic matter content and sediment grain size distribution.<sup>36,37,41</sup> These controls were evident here, where sandstone sediments were the finest (smallest grain size distribution) (Figure S6) and had the highest (C) content (Figure S2a), resulting in the highest  $\text{CO}_2$  emission. This response is also evident in the measured oxidative redox potential, which was lower in microcosms containing sandstone sediments compared to other sediment types, suggesting reducing conditions (Figure S7). However, this work demonstrates that previously well-documented controls do not represent all of the processes that determine GHG flux from riverbeds. For example, the presence of wood in the streambed is also an important driver of GHG production. Notably, the presence of wood resulted in larger differences in GHG emissions than differences between the different sediment types themselves.

The increase in GHG emissions should be expected as a result of the (re)introduction of wood in river restoration, which may offset the benefits of  $\text{NO}_3^-$  removal, essentially representing a movement of pollutants from one system to another. However, the  $\text{N}_2\text{O}$  production is not directly attributable to the wood additions, instead being a consequence of the initial introduction of reactive N (e.g., by fertilizer addition), as described by the nitrogen cascade concept.<sup>71</sup> Similarly, GHG emissions from wood decomposition may occur whether the wood decomposes in the stream environment or terrestrially. Despite these mitigations, we posit that instream wood (re)introduction may still represent a risk because it can serve to accelerate the rate of GHG emission, which could contribute to climate change. On the contrary, ecosystem restoration should seek to reduce and delay the emissions of GHG to mitigate climate change. It remains unclear how different the volumes and species of GHGs emitted from wood decomposition are in different environments, and at what rates they are emitted; further research is required to provide insight into these important dynamics. The many benefits of wood-based restoration are likely to outweigh the risks, and therefore river managers should not forgo wood-based restoration due to concerns of

increased GHG production alone. However, it is important that information on risks and benefits is made available to allow careful analysis and consideration by researchers, policymakers, and practitioners.

**4.4. Consequences for Estimates of Global Carbon and Nitrogen Cycles.** Wood can constitute a substantial portion of the total streambed volume, but it has typically been ignored or explicitly omitted from studies exploring streambed function, and especially biogeochemical function, to more easily achieve homogeneity or to focus on geological origin (e.g.,<sup>37</sup>). In this study, we demonstrate that excluding wood from mechanistic experiments may reduce the representation of microcosm results to real systems and lead to misestimations of fundamental ecosystem processes (e.g., MMA) and associated (dis)services (e.g.,  $\text{NO}_3^-$  removal rate and GHG emissions). Results from these studies (e.g.,<sup>36,39,40</sup>) have directly informed global models and estimates of the flux of elements between streams and rivers and other systems, for example, GHG emissions to the atmosphere and the flux of N compounds to marine environments. The accuracy of mechanistic studies and, as such, of global models and flux estimates, may be improved by considering streambed wood in future studies, for example, as an independent or confounding variable.

**4.5. Study Limitations and Uncertainties.** Further limitations of this study are highlighted to prevent improper inference or application of results. Different sediment types have been used here to better understand and represent the range of the potential effects of wood on streambed biogeochemistry. However, sediment treatment labels (i.e., sandstone, limestone, chalk, and no sediment) are not necessarily representative of those geologies. Furthermore, several mechanisms that control the effect of wood in rivers are absent in the microcosm environment, while some mechanisms may be exaggerated. For example, the development of microbial assemblages could explain some of the variance observed between microcosms of the same treatment, especially toward the end of the experimental period. The transport of solutes and gases in the microcosm environment is limited to diffusive transport, which could have resulted in the induction of anaerobic conditions more quickly and at shallower depths than in in situ conditions, where hyporheic exchange may accelerate transport and mixing.<sup>19</sup> Consequently, the relative production rates of anaerobically produced GHGs ( $\text{CH}_4$  and  $\text{N}_2\text{O}$ ) may be overestimated relative to aerobically produced  $\text{CO}_2$ . Furthermore, transformation of gases in the water column or headspace of the microcosm could modify the relative concentrations of GHGs actually emitted from sediments before sampling, for example, oxidation of  $\text{CH}_4$  to  $\text{CO}_2$ .<sup>72</sup> Other potential limitations include the irregular spiking to high  $\text{NO}_3^-$  concentrations (atypical of most systems in reality), which could also affect microbial assemblage dynamics, though this could represent storm-driven high nutrient loading.<sup>66</sup> Furthermore, high concentrations of DOC and associated metrics in treatments without sediment are not at all representative of instream wood in the surface water column of streams because, in these circumstances, DOC would be quickly transported.<sup>19</sup> While these artifacts of the experimental design may prevent reliable inference and scaling of metrics to absolute values, the broad patterns observed here are likely to be somewhat representative of in situ observations.

## 5. CONCLUSIONS

This research contributes to the significant body of evidence that suggests instream wood is a fundamental driver and control of key ecosystem processes. Changes to the abundance and distribution of instream wood should be expected to lead to significant changes in most facets of ecosystem function, as well as the delivery of both services and dis-services, which often occur exclusively simultaneously and are, therefore, in conflict. Wood-based restoration could offer suitable nature-based solutions to persistent challenges, like high nutrient concentrations, which work with natural processes and deliver multiple co-benefits, as is now widely advocated for in policy.<sup>73</sup> Conversely, it could also lead to significant dis-services, such as increased GHG emissions, which managers and planners have a serious responsibility to consider and minimize; also soon to be supported in policy (e.g., in the U.K.<sup>73</sup>).

There are always trade-offs in environmental management, which must be carefully considered by decision-makers using appropriate frameworks and sufficient evidence. This work demonstrates that while reductionism can be useful in environmental research, holism is necessary when applying this research to policy and practice. Considering processes and objectives in isolation could generate unintended, ulterior, and cascading consequences. We advocate a more holistic perspective in environmental research and practice—one which considers the intrinsic interconnectedness of ecosystem processes that determine ecosystem function and the delivery of ecosystem (dis)services.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsestwater.3c00014>.

Additional physiochemical parameters of the water (temperature, pH, oxidative redox potential); physical properties of sediments (loss on ignition, grain size distribution); details of greenhouse gas flux calculations; and details of statistical tests and results (PDF)

## ■ AUTHOR INFORMATION

### Corresponding Author

**Ben Christopher Howard** – School of Geography, Earth & Environmental Sciences, University of Birmingham, Birmingham B15 2TT, U.K.; Birmingham Institute of Forest Research, University of Birmingham, Birmingham B15 2TT, U.K.; Present Address: Civil and Environmental Engineering, Imperial College London, London SW72BX, U.K.; [orcid.org/0000-0003-4010-8131](https://orcid.org/0000-0003-4010-8131); Email: [ben.howard@imperial.ac.uk](mailto:ben.howard@imperial.ac.uk)

### Authors

**Ian Baker** – Small Woods Association, Telford TF8 7DR, U.K.  
**Nicholas Kettridge** – School of Geography, Earth & Environmental Sciences, University of Birmingham, Birmingham B15 2TT, U.K.; Birmingham Institute of Forest Research, University of Birmingham, Birmingham B15 2TT, U.K.

**Sami Ullah** – School of Geography, Earth & Environmental Sciences, University of Birmingham, Birmingham B15 2TT, U.K.; Birmingham Institute of Forest Research, University of Birmingham, Birmingham B15 2TT, U.K.; [orcid.org/0000-0002-9153-8847](https://orcid.org/0000-0002-9153-8847)



Stefan Krause — School of Geography, Earth & Environmental Sciences, University of Birmingham, Birmingham B15 2TT, U.K.; Birmingham Institute of Forest Research, University of Birmingham, Birmingham B15 2TT, U.K.

Complete contact information is available at:

<https://pubs.acs.org/10.1021/acsestwater.3c00014>

### Author Contributions

B.C.H. is now at Imperial College London, U.K. B.C.H. led all aspects of the conceptualization, sampling and laboratory analysis, data analysis, interpretation, and writing. I.B., N.K., S.U., and S.K. contributed to the conceptualization of the research, planning, and organization of laboratory analysis, interpretation of data, and funding acquisition. All authors read and provided feedback on the manuscript.

### Notes

The authors declare no competing financial interest.

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