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Key Points:
- Methane spatial variability is high
- Variability is related to stream geomorphology
- Mapping stream biogeochemistry is vital for good study design

Supporting Information:
- Supporting Information S1
- Data Set S1
- Data Set S2
- Data Set S3
- Data Set S4

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Spatial heterogeneity of within-stream methane concentrations

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Abstract Streams, rivers, and other freshwater features may be significant sources of CH_4 to the atmosphere. However, high spatial and temporal variabilities hinder our ability to understand the underlying processes of CH_4 production and delivery to streams and also challenge the use of scaling approaches across large areas. We studied a stream having high geomorphic variability to assess the underlying scale of CH_4 spatial variability and to examine whether the physical structure of a stream can explain the variation in surface CH_4. A combination of high-resolution CH_4 mapping, a survey of groundwater CH_4 concentrations, quantitative analysis of methanogen DNA, and sediment CH_4 production potentials illustrates the spatial and geomorphic controls on CH_4 emissions to the atmosphere. We observed significant spatial clustering with high CH_4 concentrations in organic-rich stream reaches and lake transitions. These sites were also enriched in the methane-producing *mcrA* gene and had highest CH_4 production rates in the laboratory. In contrast, mineral-rich reaches had significantly lower concentrations and had lesser abundances of *mcrA*. Strong relationships between CH_4 and the physical structure of this aquatic system, along with high spatial variability, suggest that future investigations will benefit from viewing streams as landscapes, as opposed to ecosystems simply embedded in larger terrestrial mosaics. In light of such high spatial variability, we recommend that future workers evaluate stream networks first by using similar spatial tools in order to build effective sampling programs.

1. Introduction

Streams and rivers are biogeochemical integrators in landscapes [*Bormann and Likens*, 1967; *Fisher and Welter*, 2005], acting as both receivers and processors of biologically active elements such as nitrogen, phosphorus, and carbon. And despite their diminutive spatial extent, these ecosystems often make an unexpectedly large contribution to the biogeochemical cycling of the landscapes in which they are embedded. This has been particularly well demonstrated by recent continental- and global-scale assessments of stream and river carbon dioxide and methane (CO_2 and CH_4) emissions to the atmosphere [*Raymond et al.*, 2013; *Borges et al.*, 2015, *Stanley et al.*, 2016]. However, high spatial and temporal variabilities in the concentrations and fluxes of these gases at small spatial scales pose a major challenge for generating larger scale budgets and deriving an ecological understanding of the underlying processes shaping their distribution. This issue appears to be particularly acute in the case of CH_4, as a common theme among studies with detailed sampling regimes is the high degree of variability—often reaching or exceeding 3 orders of magnitude over subkilometer distances [e.g., *Lilley et al.*, 1996; *Jones and Mulholland*, 1998a; *Crawford et al.*, 2014a, 2014b]. In this context we ask: what are patterns and controls of spatial variability in stream CH_4 concentrations?

Two perspectives have emerged from studies that have addressed questions of lotic CH_4 patterns and controls. One line of inquiry has emphasized the role of terrestrial-aquatic connections and the fate of terrestrially derived CH_4 that is exported to and subsequently outgassed from receiving waters [*Richey et al.*, 1988; *Huotari et al.*, 2013; *Leith et al.*, 2014]. In pioneering work on stream CH_4 processes, *Jones and Mulholland* [1998b] concluded that terrestrial geomorphology controls stream CH_4 concentrations by constraining groundwater flow paths and thus dictating whether or not these pathways pass through anoxic, CH_4-producing soils before discharging into the channel. Similarly, others have also argued that lateral CH_4 seepage from peatlands, wetlands, and other terrestrial components dictates aquatic concentrations and emissions [*Hope et al.*, 2001; *Borges et al.*, 2015]—that is, terrestrial processes are simply reflected in streams as a redox "signal."

The expectation that CH_4 in streams is externally derived is partly justified, given ubiquitous oxygenated conditions which should prevent methanogenesis [*Stanley et al.*, 2016]. However, investigations of hyporheic elemental cycling in streams lend support to the second perspective that focuses on the potential for in situ CH_4 production [*Baker et al.*, 1994, *Clilverd et al.*, 2008]—although here the definition of the stream boundary is important. Several authors have provided evidence that streams can produce CH_4 in patches of anoxic sediments [*Schindler and Krabbenhoft*, 1998; *Crawford and Stanley*, 2016]. If such internal generation is a major contributor to the overall CH_4 balance of a stream, then spatial patterns may overlay physical attributes such as areas of fine sediment deposition or debris accumulations where methanogens could persist.

Arguments (or hypotheses) that CH_4 in streams are dependent on external inputs versus internal generation are by no means mutually exclusive. Yet few studies have simultaneously evaluated both perspectives or considered where methanogens or methanogenesis occur with respect to larger scale distributions of CH_4 concentrations in these environments. Here we use several approaches to determine spatial patterns of CH_4, as well as investigate where this gas is generated both to understand these observed patterns and to improve the capacity to reliably upscale from local measurements. The first goal was to determine if spatial patterns in CH_4 could be identified, and if so, to consider how any such patterns related to the physical structure of the aquatic environment. The strategy of generating detailed maps of CH_4 is particularly well suited for identifying source or sink areas and thus inferring controls on CH_4 concentrations in lotic systems. The extremely low solubility of CH_4 means that any deviation from atmospheric equilibrium in the water column should reflect points of local production/input (maxima) or consumption/rapid efflux (minima) [*Teodoru et al.*, 2009; *Crawford et al.*, 2014a], as opposed to an integration of processes further upstream. Our second goal was to seek out the sites supporting CH_4 delivery or production and connect these to patterns of CH_4 distribution in an effort to distinguish between import of CH_4 versus internal generation. This involved a broad survey of potential groundwater sources of CH_4, determining patterns of markers for methanogenic Archaea, and quantifying methanogenesis potential for distinct sediment types across this geomorphically diverse stream section. Collectively, by using new and diverse tools in a highly variable geomorphic setting including wetlands, lakes, and more typical stream reaches, we were able to uncover geographic patterns of CH_4 biogeochemistry in a temperate landscape.

2. Methods

2.1. Site Description

Allequash Creek is a groundwater-dominated headwater stream located in the Northern Highlands Lake District (NHLD) near Boulder Junction, WI, USA. The stream originates from a groundwater-fed spring pond impounded by a large beaver dam (Figure 1). The stream then flows through a transition zone characterized by wetland vegetation and numerous small beaver dams before entering the main study area (middle site), which is part of the U.S. Geological Survey (USGS) Water, Energy and Biogeochemical Budgets (WEBB) program. The bulk of the middle site is a peat-filled Holocene lake basin bisected by the stream channel. This wetland reach is a zone of groundwater discharge [*Lowry et al.*, 2007*; Lowry et al.*, 2009; *Pint et al.*, 2003] and is characterized by distinct sediment types (organic-rich peats, muck, and sand) separated by a sharp transition in texture and organic matter content [*Crawford et al.*, 2014b]. Past the first USGS stream gage (USGS site number 05357206), the stream becomes much narrower with sandy substrate and is surrounded by a dense forest above the upper basin of Allequash Lake. The upper lake basin transitions to a much shallower lower basin having both open water (pelagic) and dense macrophyte habitats. The stream reforms downstream of lower Allequash Lake as a wide (15 m) wetland-like reach and then rapidly transitions back to a mineral-rich geomorphology surrounded by forest before terminating in Trout Lake.

We used published geomorphic data [*Crawford et al.*, 2014b, *Watters and Stanley*, 2007], observations of valley floor width, riparian cover, and aerial imagery to construct an a priori geomorphic classification map of Allequash Creek shown in Figure 1 by using geographic information system. These 10 site types represent distinct geomorphic patches as well as intersecting lake environments including as follows: lake open water, lake macrophytes, lake transition, spring ponds, stream sand/gravel forested, stream organic-rich wetlands, stream sand forested (closed canopy), stream sand forested (open canopy), stream sand wetland, and

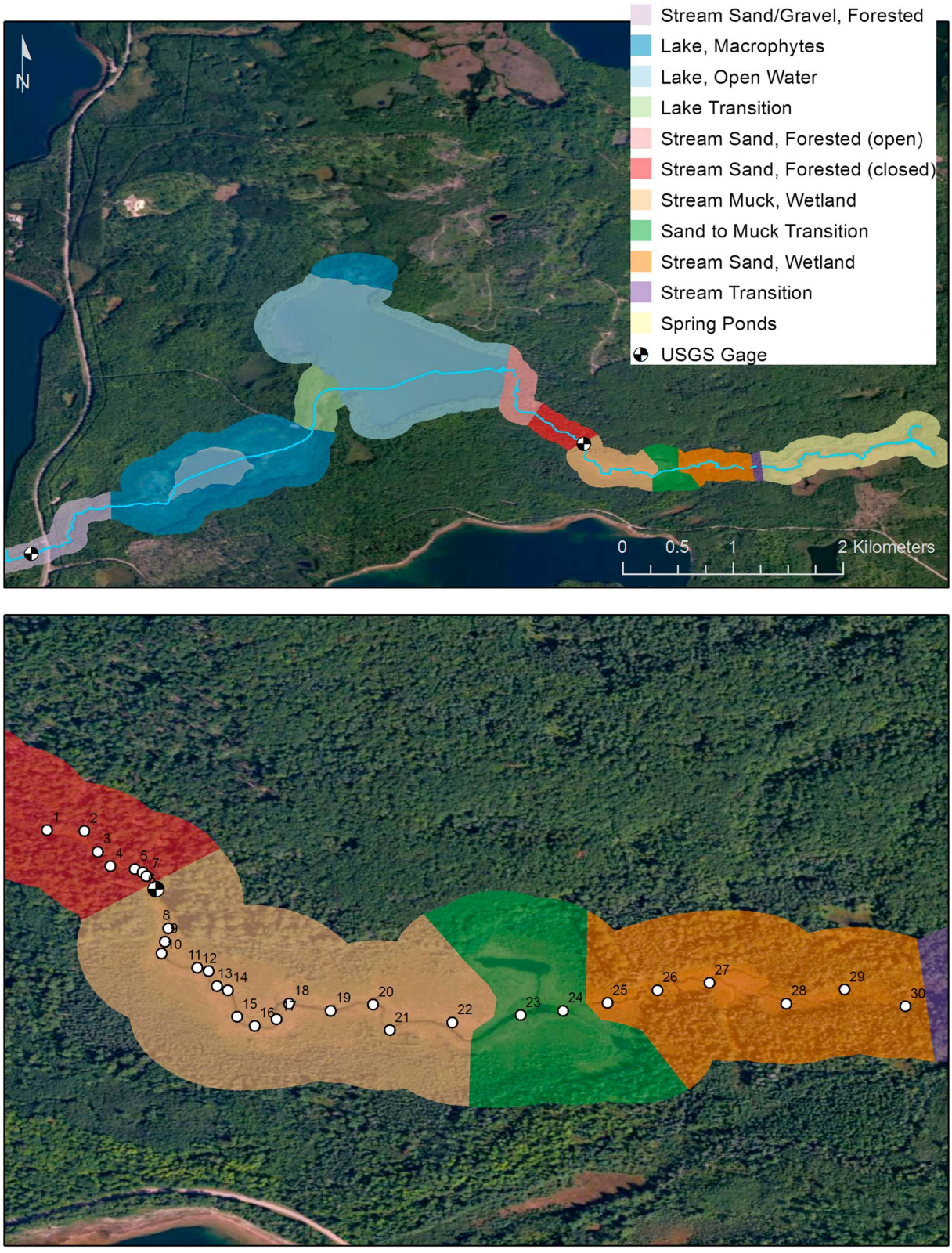

Figure 1. Geomorphic map of Allequash Creek with (top) blue line denoting the sampling path of the biogeochemical mapping platform and the (bottom) focus area around the middle site indicating transect locations; "muck" refers to organic-rich sediments; the extent of colored areas (top and bottom) conveys the general extent of potential in-stream, groundwater, and riparian zones of influence and does not represent stark ecosystem boundaries.

stream transition. This map was used to understand spatial patterns in CH_4 concentrations in surface waters and to search for concordance between CH_4 concentrations and geomorphic structure.

2.2. Patterns of Surface Water Methane

We determined stream CH_4 concentrations at a large spatial extent (10 km) and fine grain (35,000 total measurements) by using a biogeochemical mapping platform on a small boat [*Crawford et al.*, 2015]. Water was pumped on board where gases were stripped from the water by using a sprayer-type equilibrator and analyzed with a Los Gatos Research ultraportable greenhouse gas analyzer (using a cavity-enhanced absorption technique). Survey speeds were very slow (<3 kph) to enable the detection of small-scale changes in CH_4 concentrations over short distances. CH_4 concentrations were corrected for hydraulic and equilibrator lags by using first-order step-change experiments detailed in *Crawford et al.* [2015] following the outline provided in *Fozdar et al.* [1985]. Lag-corrected CH_4 values were georeferenced by using concurrent Global Positioning System readings with the Wide Area Augmentation System capability enabled. The high-resolution transect was sampled on 24 and 25 July 2014 (two morning to afternoon segments were combined into one data set). Both days were similar in terms of weather and in-stream conditions. Maximum daily air temperatures were 22.4°C and 20.9°C. Mean daily air temperatures were 18.5° and 19.1°C. At the middle site, daily mean discharges were 3.3 and 3.7×10^{-2} m^3 (July's mean Q is 3.7×10^{-2} m^3). Mean water temperatures were 18.1°C and 17.8°C (July's mean temperature is 19.6).

Using the high-resolution spatial CH_4 data sets, we assessed the degree of spatial autocorrelation by using semivariograms (spherical model, using the function autofitVariogram in the R package *automap*). We focused on the semivariogram range parameter which describes the average scale of autocorrelation (i.e., the average patch size). We also assessed the structure of spatial autocorrelation by using the global Moran's I statistic. The Moran's I statistic evaluates whether a series of geospatial observations are randomly distributed in space (the null model), clustered, or dispersed. Statistically significant positive values indicate spatial clustering, whereas negative values indicate dispersed patterns. We used the Anselin Local Moran's I statistic for spatial cluster analysis of high-resolution CH_4 data [*Anselin*, 1995]. Statistically significant values of Local Moran's I identify regions (clusters) of high or low values relative to the global data set, in addition to outliers (e.g., low outliers surrounded by high values). The analysis was executed by using the Spatial Statistics toolbox in ArcMap 10.2.

2.3. Groundwater Methane Sources

We analyzed groundwater CH_4 from a series of wells near the middle and lower sites and from wells at the head of the drainage near the spring ponds (upper site) during the time frame of this study by using a headspace equilibration method [*Striegl et al.*, 2001]. Wells were developed by using a peristaltic pump, and a minimum of two well volumes were purged before sample collection. The goal was to evaluate additional (external) lateral and vertical sources of CH_4 beyond the hyporheic zone. Despite a relatively homogenous sand aquifer, groundwater flow paths and residence times are complex in this catchment [*Pint et al.*, 2003; *Walker et al.*, 2003]. A combination of flow paths including deep groundwater derived from meteoric recharge, deep groundwater derived from lakes, and meteoric riparian water all contribute to surface flow in the catchment. These water sources and flow paths have been studied for over a decade as part of the USGS WEBB program [*Pint et al.*, 2003; *Walker et al.*, 2003]. Differences in substrate, organic matter availability, oxygen conditions, and other metrics of redox state were previously shown to relate to the concentrations of dissolved gases in wells at the middle site based on historical data [*Crawford et al.*, 2014b]. Here we expand the survey to correspond to the timing of surface water mapping and to determine whether patterns previously observed at the middle site held for the catchment in general.

2.4. Sediment Methanogen Distribution and Abundance

CH_4 production potential within stream sediments was first determined by extracting DNA from stream sediment cores and quantifying the abundance of methanogenic Archaea. We collected 14 cores approximately 22 cm long in sand and organic-rich wetland locations near the middle site (locations correspond to odd numbered transect locations in Figure 1; also corresponding to CH_4 bubble trap locations described in *Crawford et al.* [2014b]). Sediment cores were collected by using a 2.5 cm diameter, 30 cm length, stainless steel corer with an internal polycarbonate tube attached to a one-way flow valve and a PVC extension. Intact

cores were transported to the laboratory within 2 h and immediately frozen. Sediment cores were split into 2 cm segments followed by DNA extraction by using a PowerSoil DNA isolation kit (MoBio Laboratories Inc., Carlsbad, CA). We used quantitative polymerase chain reaction (qPCR) targeting the gene encoding the alpha subunit of methyl coenzyme-M reductase (*mcrA*) to quantify both longitudinal and vertical distributions of methanogens. The *mcrA* gene encodes a component of the terminal enzyme complex in the methane generation pathway and is thought to be unique to methanogens and well conserved [*Thauer*, 1998]. Many previous studies have used *mcrA* as a genetic marker to determine methanogen abundance and community composition [*Luton et al.*, 2002; *Earl et al.*, 2003; *Freitag et al.*, 2010; *West et al.*, 2012].

Each extracted sample containing *mcrA* was amplified in a 20 μL qPCR reaction in an ep gradient s realplex^2 master cycler (Eppendorf), using SYBR Green as the reporter dye. Each reaction contained 1 μL of 1/10 diluted sample DNA template, 1× iQ SYBR Green Supermix (Biorad), and 0.25 μM of each primer targeting *mcrA*: mcrAqF (5′-AYGGTATGGARCAGTACGA-3′) and mcrAqR (5′-TGVAGRTCGTABCCGWAGAA-3′) [*West et al.*, 2012]. Thermocycling conditions for the *mcrA* qPCR were as follows: an initial denaturation at 94°C for 1 min, followed by 40 cycles of 94°C denaturation for 40 s, 54°C annealing for 30 s, 72°C elongation for 30 s, and a fluorescent detection at 85°C for 20 s. Melting curves were run to ensure absence of nonspecific amplification. Amplification, fluorescence data collection, and initial data analysis were all performed by using the Eppendorf realplex^2 software (Eppendorf, Hauppauge, NY, USA).

Despite collecting >20 cores, we were not able to perform cluster analysis similar to that for CH_4 concentrations on the genetic data because the sample size was too low. Instead, we elected to compare organic-rich versus sand sediment *mcrA* gene abundance by using a *t* test. To determine if methanogen abundance was correlated with CH_4 production, we fit a linear model (log transformed) of *mcrA* abundance versus average CH_4 ebullition documented in the same year [see *Crawford et al.*, 2014b] with the R statistical programming language [*R Core Team*, 2014]. We contend that the comparison between microbial communities and integrated CH4 bubble flux over time is a stronger comparison than that of point measurements of CH4 concentration.

2.5. Sediment Methane Production Potential

We collected surface sediments from Allequash Creek and placed them in sealed jars the morning of the start of laboratory experiments. These sediments were presumed to be mostly anoxic per the oxygen profile study by *Crawford et al.* [2014b]. In the lab, ~75 mL of water saturated surface sediments was transferred into a 150 mL glass container, flushed with N_2, sealed with a gas-tight lid equipped with a butyl rubber septum for headspace gas sampling, and placed on a shaker table in the dark at room temperature (~22°C). Gas samples were collected after 24 h for CH_4 determination by using a Shimadzu GC-2014 gas chromatograph. Headspace volume of each sample was determined after gas sampling, then sediments were transferred to a preweighted aluminum pan for drying (72 h at 50°C) and ashing (4 h at 500°C). CH_4 production potential was determined as headspace CH_4 accumulation per gram of dry sediment and per gram of ash-free dry mass (AFDM) per hour. Production rates were based on two-point measurements of CH4 concentration and are thus presumed to be linear over time. Because gas production rates could not be transformed to meet assumptions of normality, significant differences among treatments were assessed by using a Kruskal-Wallis test followed by Wilcoxon rank tests for pairwise comparisons with Bonferroni-adjusted *P* value using R.

3. Results

3.1. Patterns of Surface Water Methane

High-resolution CH_4 data from Allequash Creek illustrated high spatial variability, spanning 3 orders of magnitude (0.0033 to 2.84 μmol L^{-1} CH_4; Figure 2). Across the entire 10 km study section, CH_4 was significantly spatially autocorrelated to a distance of 1100 m (Figure 3). Furthermore, the data showed a statistically significant clustering pattern (Moran's index = 0.81, $p < 0.01$). Least concentrations were found in the sandy wetland reach above the middle site (~0.22 μmol L^{-1} CH_4), the upper basin of Allequash Lake (~0.14 μmol L^{-1} CH_4), and beyond the stream outlet in Trout Lake (~0.05 μmol L^{-1} CH_4), whereas sites affected by beaver dams above the middle site, the middle section of the wetland, and the lake-stream transition downstream of Allequash Lake showed greatest concentrations (>2 μM). The spatial CH_4 pattern qualitatively reflected broad-scale landscape patterns within Allequash Creek and mapped onto the a

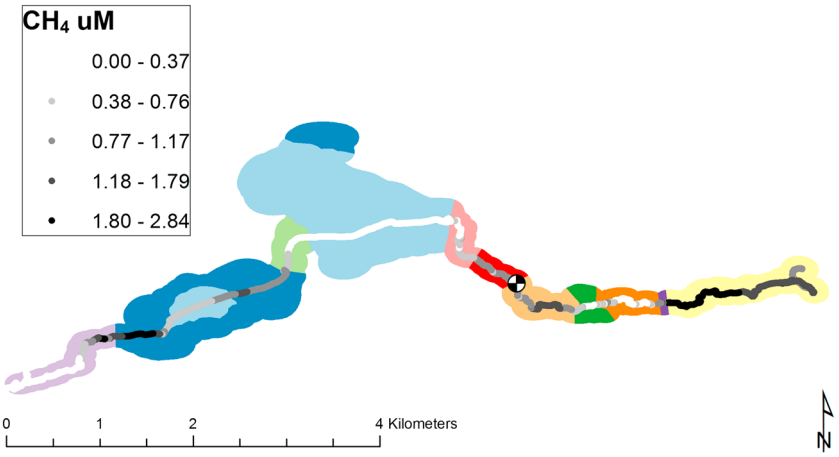

Figure 2. Map of Allequash Creek watercourse CH_4 documented with a biogeochemical mapping platform.

priori geomorphic classification (Figure 2). The clearest examples of CH_4 concentration changes occurred at lake-stream interfaces such as the outlet of Allequash Lake, but similarly strong transitions also occurred across geomorphic boundaries within the stream itself. Most notable was the beaver dam-to-sand CH_4 transition in the upper portion of the transect, the slightly longer sand-to-organic transition above the middle site stream gage, and the transition from a wide, macrophyte-rich section below Allequash Lake (lower basin) to a sand/gravel forested section above the lower site stream gage.

Spatial cluster analysis (Anselin Local Moran's I statistic) showed distinct areas where not only high values were surrounded by high values (sometimes referred to as hot spots in the spatial analysis literature) but also low values were surrounded by low values along the stream (Figure 4). High- and low-CH_4 clusters ($p < 0.05$) were typically present near the centers of geomorphic patches with some evidence of clustering across the a priori map boundaries (especially stream/sand-lake transition). Furthermore, the high-concentration clusters were associated with organic-rich sediment zones (e.g., wetland and spring ponds), whereas the low clusters were associated with mineral-rich reaches. The high-resolution spatial data support the hypothesis that CH_4 concentrations in surface waters correspond to the geomorphic structure of this heterogeneous stream.

3.2. Groundwater Methane

As was observed for surface waters, groundwater CH_4 concentrations showed pronounced spatial variability, spanning a range from subsaturation with respect to the atmosphere to extreme supersaturation (range = 0.014 to 251.3 μmol L^{-1} CH_4; Figure 5). In general, greatest CH_4 concentrations were observed near the stream channel in riparian pore waters (median = 162 μmol L^{-1} CH_4), whereas deeper groundwaters [see *Walker et al.*, 2003; *Pint et al.*, 2003] had lesser concentrations (median = 0.478 μmol L^{-1} CH_4) similar to the pattern documented for the middle site by *Crawford et al.* [2014b]. The lake recharge samples taken from deep wells approximately 50 m away from the stream had slightly greater CH_4 concentrations than other hillslope groundwaters in the catchment. The least groundwater CH_4 concentration was observed in the

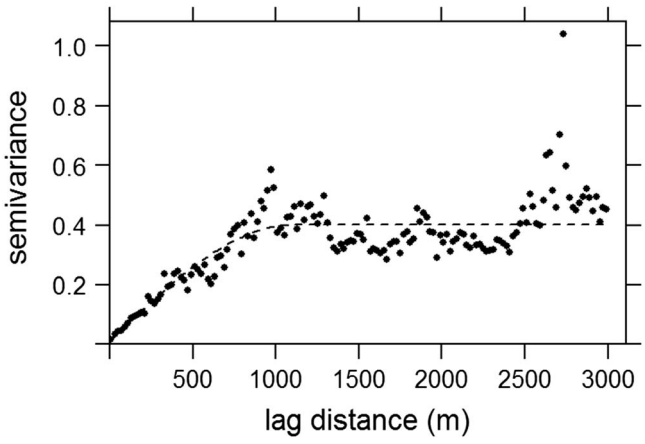

Figure 3. Empirical (points) and modeled (line) semivariogram of Allequash Creek CH_4.

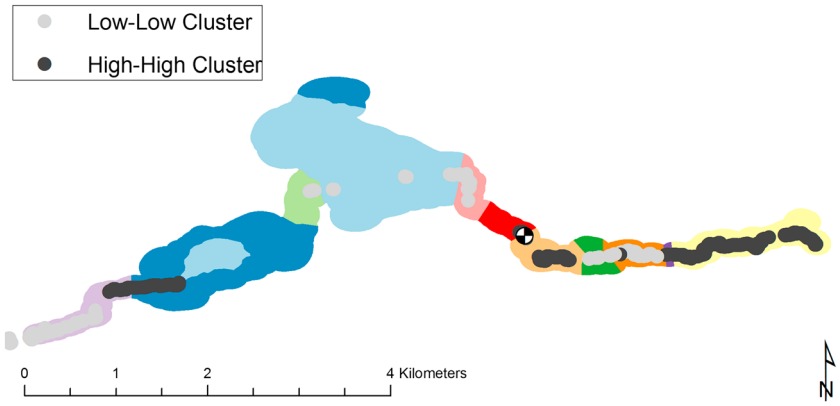

Figure 4. Map of CH_4 cluster analysis results using Anselin Local Moran's I statistic showing areas of high- and low-CH_4 concentrations associated with geomorphic type; statistically nonsignificant values ($p > 0.05$) are omitted for clarity.

deepest well to the north of the spring ponds (0.014 μmol L^{-1} CH_4), but replicate samples at this site were similar to equivalent wells at the middle and lower sites (0.067 to 0.503 μmol L^{-1} CH_4).

3.3. Sediment Methanogen Distribution and Abundance

The *mcrA* gene was present in sediments at every location that was sampled along Allequash Creek. The depth-integrated density of *mcrA* copies ranged from 1.29×10^3 to 5.40×10^4 copies g^{-1} sediment and showed relatively strong depth dependence (Figure 6) with a generalized peak between 2 and 4 cm below the sediment-water interface. Copies of *mcrA* were detected to depths of 22 cm below the sediment-water interface, but densities at deep locations were less than densities at shallower depths (0 to 8 cm). There was a strong longitudinal pattern as greatest depth-integrated *mcrA* densities were found in the organic-rich wetland reach of Allequash Creek, with lesser densities in the sand sediments (*t* test, $p < 0.01$; Figure 6). Furthermore, depth-integrated *mcrA* density was positively correlated with average CH_4 bubble production ($p < 0.05$, $R^2 = 0.37$; Figure 7) documented at the same locations in 2013 by *Crawford et al.* [2014b].

3.4. Sediment Methane Production Potential

Clear visual differences in each of the three sediment classes were consistent with significant differences in the organic matter content of muck, peat, and sand samples (Kruskal-Wallis $\chi^2 = 18.74$, $p < 0.001$). As expected, the poorly decomposed fibric peat sediments were predominantly composed of organic material, the more amorphous muck samples were roughly 50% organic, and sands had extremely low organic content (mean = 1.5%). Although CH_4 production was undetectable for a few ($n = 4$) individual replicates, mean rates were positive for all sediment types. Average rates ranged from 0.008 to 3.34 μmol CH_4 g^{-1} d^{-1} among sediment types, and muck sediments were significantly more productive than peat or sand when standardized by either dry weight (Kruskal-Wallis $\chi^2 = 15.73$, $P < 0.001$) or AFDM (Kruskal-Wallis $\chi^2 = 8.23$, $P < 0.05$) (Figure 8). Differences between peat and sand were marginally significant ($P < 0.1$) when normalized by sediment dry weight and nonsignificant when expressed in terms of CH_4 produced per gram AFDM.

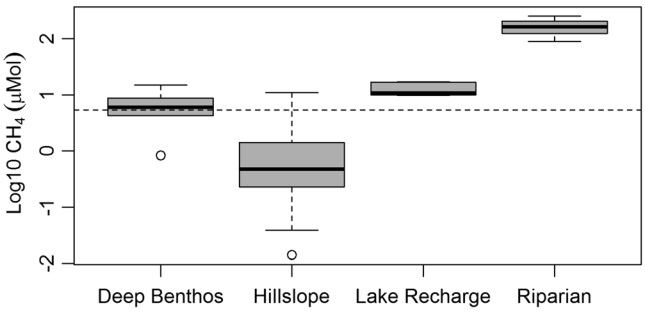

Figure 5. Spatial pattern of CH_4 in groundwater sources in the Allequash Creek catchment; the dashed line is the median CH_4 concentration documented in the main channel at the middle site.

4. Discussion

We observed remarkable spatial variability in stream CH_4 concentrations across a geomorphically variable stream flow path (Figure 2). Although such variability may first be viewed as a challenge to understanding single streams or larger regional dynamics, when placed in a landscape view,

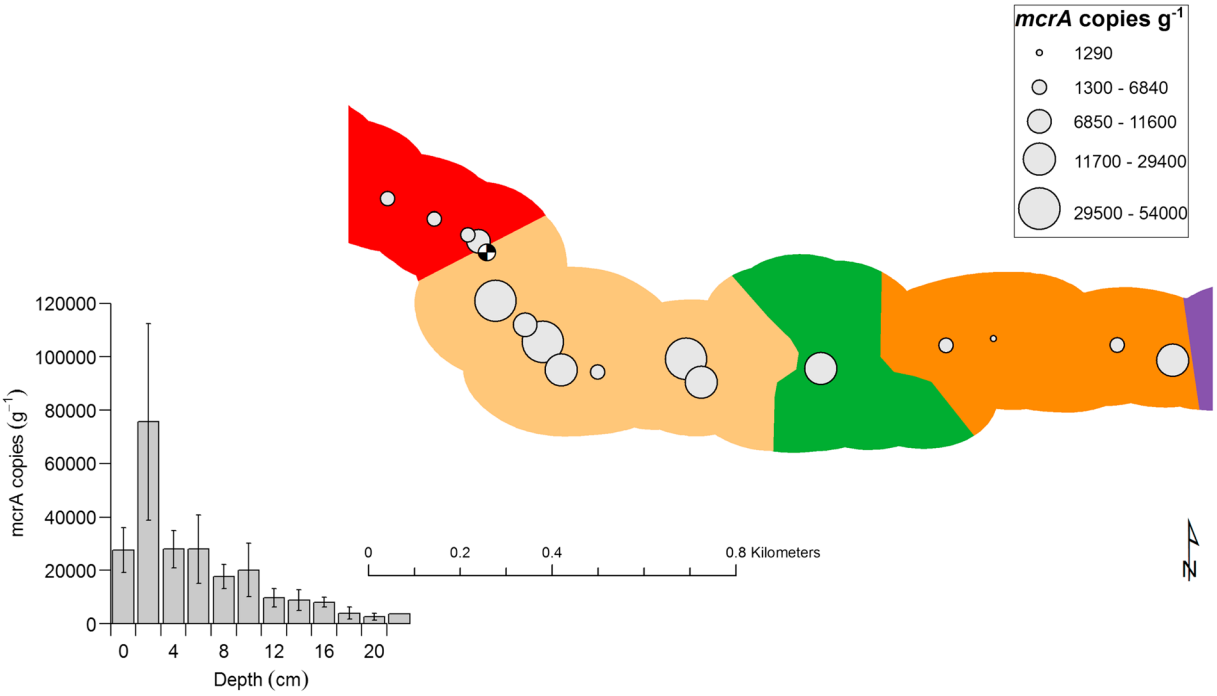

Figure 6. (left) Composite depth profile of *mcrA* gene abundance from sediment cores collected along Allequash Creek and (right) map of depth-integrated *mcrA* gene abundance along Allequash Creek.

predictable patterns emerge. Patterns of longitudinal, vertical, and lateral connectivities of stream and other aquatic patches are expressed in the dissolved CH_4 pool. We suggest that viewing streams *as* landscapes themselves and using tools and concepts from landscape ecology can lead to an improved understanding of aquatic greenhouse gas (GHG) processes. This will require relating spatial CH_4 patterns with improved maps of geomorphic structure in streams [*Carbonneau et al.*, 2012], lakes, and wetlands. Such an approach may also help reduce uncertainty with respect to larger scale GHG budgets, as previous approaches considering streams as embedded elements *in* landscapes have not yielded strong predictive power based on terrestrial metrics [e.g., *Wallin et al.*, 2014; *Campeau and del Giorgio*, 2014]. We suggest that patterns observed in Allequash Creek will be stronger relative to less variable geomorphic settings. The large gradients between muck, sand, lakes, transitions, and ponds may not be representative of streams in general, but this approach is worthy of exploration for other stream, river, and lake networks. The biogeochemical mapping approach demonstrated here may be especially useful before the establishment of larger studies, as the broader perspective and finer resolution data may inform sampling designs and study locations for CH_4 and other biogeochemical studies.

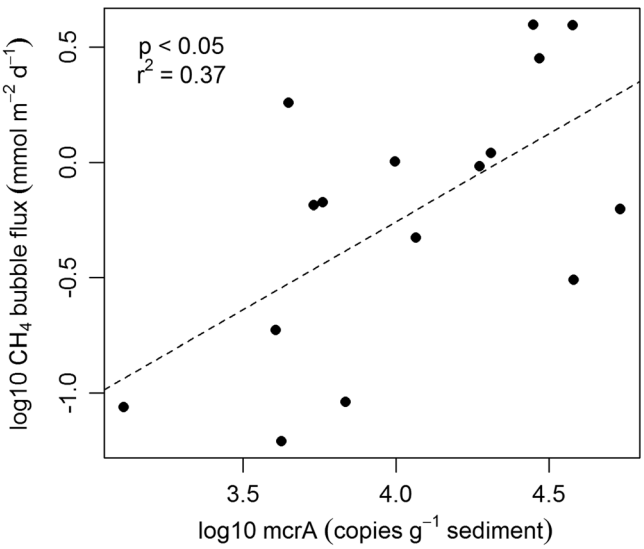

Figure 7. Relationship between depth averaged *mcrA* gene abundance and average CH_4 ebullition rate along Allequash Creek; ebullition data are from *Crawford et al.* [2014b].

4.1. Surface Water CH_4

The high-resolution CH_4 survey using the biogeochemical mapping platform lends strong support to the idea that

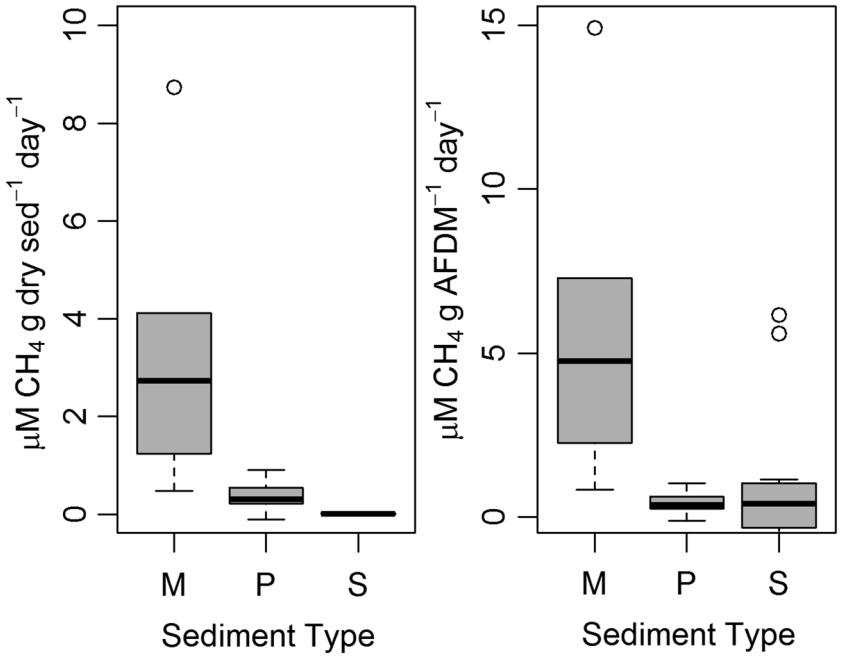

Figure 8. CH_4 production potentials from muck (M), peat (P), and sand (S) sediments standardized by (left) dry sediment weight and (right) ash-free dry mass (AFDM) from Allequash Creek.

geomorphic patches control the spatial variation of CH_4 concentrations in streams. Surface water CH_4 patterns mapped closely onto the a priori geomorphic classification map, illustrated often-sharp transitions across most of the geomorphic boundaries (Figure 2), and showed statistically significant clustering near the centers of geomorphic patches (Figure 4). High-concentration clusters were found in organic-rich geomorphic patches (wetland reach, spring ponds, and lake outlet), whereas low-concentration clusters were observed in organic-poor reaches such as downstream of the spring ponds, and where Allequash Creek approaches Allequash Lake. Our data support the notion that CH_4 concentrations are statistically patchy within streams. Spatial autocorrelation range (1100 m; Figure 3) was indicative of the nonconservative behavior of CH_4 due to rapid outgassing. Variable downstream CH_4 sources lead to statistically significant spatial clustering (Moran's index = 0.81, $p < 0.01$) as opposed to random or dispersed patterning. Given the expectation that groundwater delivery and hyporheic and riparian production lead to excess CH_4 in the channel, discontinuities should lead to changes in CH_4 concentration.

4.2. Groundwater CH_4

Looking beyond the stream channel, CH_4 concentrations from diverse groundwater sources indicate that CH_4 is produced at highest rates in riparian environments (Figure 5). Unsurprisingly, these locations are generally anoxic [*Crawford et al.*, 2014b], which should support local methanogenesis. Allequash Creek experiences high rates of groundwater discharge in the upper to middle reaches, transitioning to weak recharge in the lower most portions of the catchment [*Walker et al.*, 2003; *Pint et al.*, 2003]. If discharging groundwaters contain high CH_4, then we can assume a significant contribution from terrestrial landscapes to the surface water budget. Yet only groundwater environments adjacent to the stream had concentrations that were sufficient to support the intense CH_4 supersaturation observed in the channel. High CH_4 concentrations in organic-rich, anoxic areas contrast strongly with the carbon-poor sand aquifer that is open to the atmosphere and is thus sufficiently well oxygenated to prevent high methanogenesis. Processes in deeper groundwater and on hillslopes are not directly expressed in the channel. Instead, groundwater chemistry is substantially transformed as multiple flow paths converge on riparian and hyporheic environments. Because conditions immediately adjacent to the stream are at least partially driving channel concentrations, looking to upland land cover conditions may provide little explanatory power for surface water CH_4 concentrations.

4.3. Sediment Methanogens

The ubiquitous presence of methanogenic Archaea in stream sediments extending to 22 cm below the sediment-water interface indicates that CH_4 is likely produced within the stream channel. Greatest densities of the *mcrA* gene found in the organic-rich wetland sediments above the middle site stream gage corresponded to the high CH_4 concentration cluster, whereas lower abundances corresponded to one of the low-concentration clusters. Methanogen abundances were also significantly correlated with documented CH_4 ebullition rates from stream sediments (Figure 7), which at least suggests that the spatial patterns in CH_4 production integrated over time are related to the abundance of these Archaea. While the detection of the *mcrA* gene only suggests the presence of active methanogens within sediments, the *mcrA* depth distributions corresponded well with hyporheic gas measurements made by *Schindler and Krabbenhoft* [1998] in sand sediments at the middle and lower sites in Allequash Creek (maximum hyporheic CH_4 concentrations between 2 and 15 cm depth). To our knowledge, the presence and distribution of methanogenic Archaea in river sediments have only been established for a river of the Czech Republic. In the River Sitka, *Buriánková et al.* [2012] and *Buriánková et al.* [2013] showed spatial variability in methanogen abundances and the diversity of methanogenic Archaea in hyporheic sediments.

4.4. Sediment Methane Production Potential

Laboratory incubations were consistent with *mcrA* occurrence in confirming the potential for CH_4 production within the stream channel. The vast majority of all replicates exhibited positive CH_4 production over 24 h regardless of sediment type. Incubations only represent potential production and are not likely to represent in situ rates. However, significant production rates over short periods indicate that there are viable communities of methanogens present within the sediments. Sediment type had a strong effect on this potential, with CH_4 production by muck sediments far outpacing that of sand and peat sediments, both in terms of total sediment dry weight and when normalized to the organic matter content of the sediments. The limited difference between these latter two categories was unexpected and demonstrates that poorly decomposed peat provides a poor habitat for this process and may even be associated with materials that inhibit methane generation [*Minderlein and Blodeau*, 2010].

Low rates of CH_4 production by sand correspond to observations of low stream water concentrations in sandy reaches, while areas with CH_4-rich surface waters were dominated by organic sediments. Concordance between larger scale geomorphic structure and sediment composition is not unusual in streams, and in this case, their cooccurrences reenforce the idea that the physical structure of a stream is a strong determinant of CH_4 distribution. However, the strong differences between peat and muck sediments demonstrate that organic content alone is not necessarily a sufficient predictor of CH_4 enrichment.

4.5. Production Versus Loss Controls

Clearly absent from the previous analysis is the role of loss pathways for dissolved CH_4. Our main premise is that the geomorphology, local groundwater conditions, and methanogen communities are the primary drivers shaping spatial patterns of surface water CH_4 concentrations. However, these patterns are likely also modified by gas exchange with the atmosphere and methane oxidation, both of which should reduce CH_4 concentrations at different rates across the watershed. At present, we do not have information regarding the spatial patterns of these two processes which prevents significant discussion with respect to this landscape. However, we predict that variations in gas exchange are quite low in this sheltered, low-relief stream system, but with some higher variability in the lake system due to wind variability, bathymetry, and macrophyte coverage which could influence gas exchange rates. Methane oxidation may have the potential to change spatial patterns in the stream and lake CH_4 concentrations, as previous work has demonstrated high (and variable) oxidation rates in streams, which has also been connected to geomorphology and hydraulic exchanges [*Trimmer et al.*, 2010; *Shelley et al.*, 2014a, 2014b]. There is a clear need for rigorous studies that include all CH_4 production and loss pathways in order to understand landscape-scale patterns of CH_4 concentrations in stream and lake systems.

4.6. Streams in the Landscape Mosaic and Legacies

NHLD streams are embedded within a mosaic of lakes that may influence their biogeochemistry in addition to terrestrial and riparian environments. Distinct and rapid changes in CH_4 along the stream-lake

discontinuum were observed at pelagic-macrophyte and lake-stream boundaries including both inlet and outlet features. However, previous work does not support the contention that lakes affect dissolved gases in outlet streams [*Crawford et al.*, 2014a]. Even if lakes are supersaturated with CH_4, short residence times of dissolved gases should prevent an extensive downstream effect. In fact, significant lake biogeochemical signatures in downstream reaches have not been observed for carbon of any form in NHLD streams [*Lottig et al.*, 2011, 2013]. In this context, any apparent lake effect is simply a blurring of the boundary (spatial autocorrelation effect) as lake-derived gases are removed and stream processes are expressed in the water column. However, lakes likely control downstream CH_4 processes indirectly due to their influence on the geomorphic structure of inlet and outlet streams [e.g., *Arp et al.*, 2007]. Whereas many stream or lake ecologists might tend to focus on their ecosystem of choice [*Jones*, 2010], the view of a stream-lake discontinuum leads to a broader understanding of the patchiness and connectivity of freshwater biogeochemistry.

Although the focus of this work is almost exclusively on spatial patterns, we should consider the questions of what generated these geomorphic patches and at what time scales might they change? Allequash Creek may illustrate idiosyncratic patterns. Therefore, we must also consider how to best use this example to understand CH_4 cycling within the larger catchment or the NHLD. Legacies of landscape formation, disturbance, and hydrology all play a role in the creation and maintenance of aquatic patches. The middle site of Allequash Creek (organic-rich wetland reach) is a former glacial lake basin that was filled in with peat relatively soon after glacial retreat ~12,000 B.P. [*Attig*, 1985; *Watters and Stanley*, 2007]. At some point during landscape evolution, hydraulic gradients led to channel formation, connecting the spring ponds to the east with Allequash Lake to the west. Over time, the interactions between the channel and vegetation led to distinctive channel forms and patterns of sediment structure [*Watters and Stanley*, 2007]. On a very different time scale, North American beavers (*Castor canadensis*) have altered—and continue to alter—the structure of stream reaches in Allequash Creek via dam formation. This biotic driver of patchiness is directly related to changing CH_4 patterns in this and many other landscapes [*Ford and Naiman*, 1988; *Whitfield et al.*, 2015]. Hydrologic disturbance can also lead to changes in stream patch structure. Relative to many streams, however, disturbances from floods are infrequent in Allequash Creek due to the high infiltration capacity of the catchment and substantial groundwater buffering [*Watters and Stanley*, 2007]. Therefore, many of the geomorphic patches in Allequash Creek are relatively stable over a multiyear time frame. Other headwater streams of the NHLD share similar features with Allequash Creek including high groundwater discharge, peatland drainage, beaver impoundments, lake discontinuities, and direct flow over sandy glacial till. We argue that although Allequash Creek may exhibit a unique configuration of patch elements, with a unique pattern of CH_4 production and emission, the basic relationships between CH_4 and geomorphology are likely to apply to other streams in the region. Quantifying the distributions and configurations of stream patches in space and the deviations from a more linear geomorphic perspective [e.g., *Carbonneau et al.*, 2012] and their broader relationships with CH_4 production and other biogeochemical cycles will lead to improved large-scale assessments and will enhance our understanding of aquatic elemental cycling. Additional insight may come from repeated spatial measurements to differentiate between patches that vary significantly over time from those that remain stable.

5. Conclusions

The nonconservative nature of CH_4 and other gases due to rapid air-water exchange has typically been treated as a barrier to robust characterizations of broad patterns. Spatial variability hinders regional to global extrapolation techniques (upscaling) based on limited point measurements. However, spatial variability of an ecosystem response variable such as CH_4 may not be random in space and time but can be understood by using the concepts of patches and boundaries [*Naiman et al.*, 1988] as demonstrated for Allequash Creek. We argue that GHG spatial variability should be viewed as an opportunity for aquatic scientists as opposed to a statistical and logistical challenges (in the sense of *Palmer et al.* [1997]). Additional high-resolution spatial chemistry data sets paired with geomorphic information from stream ecosystems will help improve our understanding of the global freshwater CH_4 budget. Importantly, repeated measurements over time will be necessary to elucidate how spatial patterns in aquatic systems change. The challenge will be to match our scales of observations to the scales of the drivers of gas production, consumption, and emission.

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