

BROWN, ROBERT S., M.S. Potential Effects of the Invasive Bivalve, *Corbicula fluminea*, on Methane Cycle Processes in an Urban Stream. (2018)
Directed by Dr. Anne E. Hershey. 62 pp.

Methanogenesis, subsequent methane oxidation and flux from sediments are subject to local biogeochemical conditions in streams. The invasive bivalve, *Corbicula fluminea*, has been shown to affect stream biogeochemistry and may have a density dependent effect on methane cycle processes via bioturbation and respiration. The response of methanogenesis rate, potential methane oxidation rate and net methane flux to *Corbicula* density was tested using laboratory microcosms. Potential methane oxidation decreased and net methane flux increased with increasing *Corbicula* density. This suggests that as *Corbicula* populations become denser, they have potential to increase methane flux from stream sediments by reducing methane oxidation. To test the response of sediment pore water methane concentration to *Corbicula* density, cage enclosures containing assigned *Corbicula* densities were installed in 3 blocks along a 50m stream reach. *Corbicula* had a marginally significant positive effect on pore water methane concentration in the downstream reach, but no significant effect in middle or upstream reaches. Sediment organic matter was highest downstream, providing potential substrate for methanogenesis. Active channel width was highest in the middle block, due to the presence of a sandbar. Increased organic matter availability and changes in hyporheic flow beneath the sandbar may have impacted *Corbicula*'s relationship with *in situ* methane concentrations. Considering the heterogeneity of urban streams, these relationships are likely site specific and probably vary within North Buffalo Creek.

POTENTIAL EFFECTS OF THE INVASIVE BIVALVE, *CORBICULA FLUMINEA*,
ON METHANE CYCLE PROCESSES IN AN URBAN STREAM

by

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A Thesis Submitted to
the Faculty of The Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Greensboro
2018

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To my mentors, friends and family who have guided and supported me.

APPROVAL PAGE

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ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Anne Hershey, for her expertise, guidance and support throughout the development of my research. Thank you, Dr. Steve Whalen, one of my committee members, for providing support for methane analysis at UNC Chapel Hill. Thank you to my other two committee members, Dr. Olav Rueppell and Dr. Haimeng Zhang, for your guidance and support throughout my graduate career.

I am grateful for the support and friendship of my fellow graduate students who have helped me grow personally and professionally. I would like to thank Peter Blum, Josh Brigham, and Austin Gray specifically. I would not have been able to complete my research without each of you.

I would like to thank my sources of funding and support. The UNCG Biology Department, the Julia Taylor Morton Endowment and the John O'Brien memorial award. I would also like to thank RiverLink and AmeriCorps Project Conserve for supporting me as I work to finish my degree.

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CHAPTER I

INTRODUCTION

Global Methane Cycles

Flowing waters are known to play a significant role in organic matter processing (Cole *et al.*, 2007; Aufendkampe *et al.*, 2011). Organic matter is metabolized as it moves through the watershed (Stanley *et al.*, 2012; Trimmer *et al.*; 2012; Shelley *et al.*, 2015; Trimmer *et al.*; 2015, Bernhardt *et al.*, 2017a), leading to the production of CO₂ and CH₄, which may be emitted to the atmosphere (Cole *et al.*, 2007; Bastviken *et al.*, 2011; Stanley *et al.*, 2016). Carbon dioxide and methane are the two most significant greenhouse gases in the atmosphere and both are increasing due to human activity (Myhre *et al.*, 2013). Although CO₂ is more abundant, atmospheric methane concentrations have increased by 250% since 1750 (Myhre *et al.*, 2013) and CH₄ is more potent over long time scales (Bastviken *et al.*, 2011), with a global warming potential 20-35 times greater on a per molecule basis than CO₂ (Shindell *et al.*, 2009).

Methane is produced by methanogenic archaea under anaerobic conditions (Lui and Whitman, 2008). Methanogenesis is common in lentic sediments such as wetlands (Segers, 1998; Whalen; 2005) and lakes (Bastviken, 2004; Tranvik *et al.*, 2009), which are well known sources of CH₄ to the atmosphere. Lotic sediments are generally oxic, but methanogenesis can occur where anoxic microsites exist (Conrad, 2009; Smith,

2013). Methane may then be consumed by methane oxidizing bacteria in oxic sediment or in the water column (Conrad, 2009), creating a potential energy source for higher trophic levels (Trimmer *et al.*, 2009; Jones and Grey, 2011). Alternatively, methane released from sediments may be emitted to the atmosphere (Bastviken *et al.*, 2011). Emissions to the atmosphere are often the primary concern when predicting ecosystem contributions to GHG fluxes (Myhre *et al.*, 2013). These emissions estimates are valuable for building global climate models (Battin *et al.*, 2009; Hamdan *et al.*, 2016). However, climate change modeling scenarios depend on scaled-up estimates of GHG emissions from site-scale measurements within ecosystems (Battin *et al.*, 2009), often leading to misrepresented contributions of individual ecosystems outside those directly measured (Aufendkampe *et al.*, 2011). Many studies have called for closer attention to the contributions of inland waters to GHG emissions (Cole *et al.*, 2007; Battin *et al.*, 2009; Tranvik *et al.*, 2009; Aufendkampe *et al.*, 2011; Bastviken *et al.*, 2011; Hamdan *et al.*, 2016). Focus on methane cycle processes within streams has recently gained momentum (Shelley *et al.*, 2015; Trimmer *et al.*, 2015; Crawford and Stanley, 2016; Liang *et al.*, 2016; Schade *et al.*; 2016; Crawford *et al.*, 2017), facilitated by pioneering work (Jones and Mulholland, 1998a; 1998b) and catalyzed by recent reviews (Trimmer *et al.*, 2012; Stanley *et al.*, 2016). Further, there is a growing need to understand the ecology of methane in streams (Stanley *et al.*, 2016) in the context of anthropogenic influences (Crawford and Stanley, 2016) and watershed scale variables (Crawford *et al.*, 2017). While human activities have direct effects on global CH₄ emissions (Myhre *et al.*, 2013), natural systems account for approximately 35-50% of methane emissions (Hamdan *et al.*,

2016), warranting fine scale study of stream methane cycle processes (Stanley *et al.*, 2016).

Controls on Methane Cycle Processes

Teasing apart natural and anthropogenic impacts on methane emissions becomes more complicated when considering how humans have altered natural systems, such as streams and rivers, impacting the way these systems metabolize organic matter and the products of its decomposition (Bodmer *et al.*, 2016; Crawford and Stanley, 2016). Within streams, methanogenesis, methane oxidation and methane flux may be affected by distal factors (Stanley *et al.*, 2016), such as geomorphology and hydrology adjacent to the stream (Jones and Mulholland, 1998b; Crawford *et al.*, 2017), which can affect hyporheic flow and impact local redox conditions within the stream channel, potentially affecting habitat availability for methanogenic archaea and methanotrophic bacteria (Crawford *et al.*, 2017).

Rates of methanogenesis, methane oxidation and methane flux in stream sediment are also influenced by proximal factors such as temperature, oxygen availability, organic matter content and competition with other microbes for substrate (Stanley *et al.*, 2016). Methane processing rates may also be related to nutrient availability, though specific mechanisms of association in streams are understudied (Bodmer *et al.*, 2016; Stanley *et al.*, 2016; Crawford and Stanley, 2016). Nutrient cycles in streams are dynamic and there is strong evidence that these cycles are driven by benthic consumers (Atkinson *et al.*, 2017), and the distribution of primary producers and consumers has been altered by human activity (Vitousek, 1990; Strayer, 2012).

Human actions have altered lotic ecosystem structure and function in a variety of ways (Meyer *et al.*, 2005; Walsh *et al.*, 2005; Bernhardt *et al.*, 2008; Roy *et al.*, 2016; Smith and Kushal, 2016). The urban stream syndrome (Walsh *et al.*, 2005) highlights structural changes in streams including altered flow regimes, geomorphology, and higher nutrient concentrations. Altered structural characteristics may affect lotic microbial communities (Hosen *et al.*, 2017) and associated ecosystem functions (Smith *et al.*, 2017), especially in urban headwaters (Hosen *et al.*, 2017). Structural changes to urban streams may generate spatial and temporal heterogeneity (Pickett *et al.*, 2017), which control stream biogeochemical processes (Bernhardt *et al.* 2017b) and are hypothesized to confound predictions of methane cycle processes in streams (Stanley *et al.*, 2016), although there is evidence for higher CH₄ emissions in human dominated landscapes (Crawford and Stanley, 2016).

Benthic Consumer Effects (*Corbicula fluminea*)

Prolific alterations to stream ecosystems by anthropogenic activity arguably solidifies humanity's place as the most important engineer in these systems (Romero *et al.* 2015). However, ecosystem effects of anthropogenic activity have been amplified by the introduction of non-native invasive species (Vitousek, 1990; Sousa *et al.*, 2011; Strayer, 2012). Exotic freshwater mollusks have spread across the globe because of human activity (Strayer, 1999). Specifically, *Corbicula fluminea*, commonly known as the Asian clam, is among the most successful aquatic invaders in North America (Strayer, 1999; Sousa *et al.*, 2014). *Corbicula* is widespread throughout the southeastern United States (Crespo *et al.*, 2015) and adaptations that make it a successful invader enable it to

act as an ecosystem engineer in streams (Sousa *et al.*, 2008b). *Corbicula* distributions appear to be related to dispersal ability, rather than intolerance for degraded stream conditions (Pereira *et al.*, 2017). Tolerance for impaired stream conditions enable it to persist in heterogeneous urban streams (Bullard and Hershey, 2013), where it is often a dominant benthic consumer in terms of biomass (Turek and Hoellein, 2015).

Hypothesized proximal controls of methane in streams (Stanley *et al.*, 2016) include many factors on which *Corbicula fluminea* has a measurable effect. *Corbicula* impacts benthic organic matter dynamics (Hakenkamp and Palmer, 1999), microbial community structure (Vaughn and Hakenkamp, 2001; Novias *et al.*, 2016) and nitrogen and oxygen availability (Zhang *et al.*, 2011; Zhang *et al.*, 2013; Pigneur *et al.*, 2014; Turek and Hoellein, 2015), effects that suggest this invasive bivalve may also have significant influence on methane production, consumption, and flux from stream sediments.

Corbicula is native to Southeast Asia and was first documented in the western United States in 1920s (Sousa *et al.*, 2008a; Crespo *et al.*, 2015). Due to human activity, *Corbicula* has continued to spread during the 20th century. Its range now includes much of Europe, and North and South America (Crespo *et al.*, 2015). The invasion is widespread from 30-50° latitude and appears to be constrained only by lack of upstream dispersal mechanisms (Pereira *et al.*, 2017) and extreme temperatures at high altitudes and latitudes (Crespo *et al.*, 2015). On a local scale, *Corbicula*'s spread is attributed to its high growth and reproduction rates. Hermaphroditic reproduction typically occurs twice a year and individuals mature in 3-6 months, though growth is continuous throughout life

(Sousa *et al.*, 2008a). Life span can vary from 1-5 years, depending on available resources and habitat (Sousa *et al.*, 2008b).

In contrast with native bivalves, *Corbicula* prefers sandy habitats where it benefits from multiple feeding modes (Vaughn and Hakenkamp, 2001). While many bivalves pedal feed from sediment during early life, *Corbicula* can pedal and filter feed throughout life (Vaughn and Hakenkamp, 2001; Sousa *et al.*, 2008a), allowing it to grow more quickly than when limited to one food source (Hakenkamp and Palmer, 1999).

Corbicula's wide trophic niche has also been demonstrated stoichiometrically (Atkinson *et al.*, 2010). *Corbicula* was shown to assimilate a wider range of food sources and feed at a faster rate than a native unionid mussel (Atkinson *et al.*, 2010), potentially allowing it to outcompete native unionid mussels for resources (Ferreira-Rodriguez *et al.*, 2016).

Corbicula feeding rate and range are strong contributors to its success in North America (Vaughn and Hakenkamp, 2001) and are hypothesized to drive its impact on biogeochemical cycling (Sousa *et al.*, 2008b; Sousa *et al.*, 2009; Sousa *et al.*, 2011, Bullard and Hershey, 2013, Pigneur *et al.*, 2014).

Corbicula's bioturbation activity may be driving increased oxygen uptake in stream sediments (Zhang *et al.*, 2011; Zhang *et al.*, 2013; Pigneur *et al.*, 2014; Turek and Hoellein, 2015), enhancing oxidation potential. *Corbicula*'s effect on sediment redox state may vary with density and population structure, where dense populations consume more oxygen through respiration. Additionally, large individuals are known to burrow deeper into sediments, while smaller individuals maximize total sediment redistribution (Majdi *et al.*, 2014). *Corbicula* density and biomass, along with variation in feeding

mode and associated changes in bioturbation and respiration are likely strong drivers of benthic biogeochemistry (Vaughn and Hakenkamp, 2001; Pigneur *et al.*, 2014) and may be mechanisms by which *Corbicula* influences methanogenesis, methane oxidation, and flux from stream sediment.

Current Research

The present study employed laboratory experiments to investigate the relationship between *Corbicula* biomass and methane flux from urban stream sediments (Figure 1), where *Corbicula* may have a density dependent effect on methanogenesis or CH₄ oxidation (Figure 2). Field experiments were employed to measure the *in situ* effects of *Corbicula* biomass on pore water methane concentration, where the response of pore water methane concentration may be density dependent (Figure 3). Specific goals of this study are: 1) measure the response of net methane flux, methanogenesis and potential CH₄ oxidation rates to a gradient of *Corbicula* density in laboratory microcosms; and 2) measure *in situ* response of sediment pore water methane concentration to a gradient of *Corbicula* density in North Buffalo Creek. Investigating the relationship between this invasive bivalve and lotic CH₄ cycle processes tested new methods for evaluating how benthic consumers affect the relative contributions of methanogenesis and CH₄ oxidation to CH₄ flux from sediments and methane concentration within sediments. Results will generate data useful in hypothesizing relationships of benthic consumer effects on anaerobic processes in largely aerobic ecosystems.

Objectives and Hypotheses

Hypotheses for the present study were informed by results of a preliminary experiment conducted in December 2016.

Objective 1: Measure the effects of *Corbicula* density on net CH₄ flux, potential methane oxidation and methanogenesis rates in laboratory microcosms.

Hypothesis 1a: Low densities of *Corbicula* reduce net CH₄ flux by oxygenating sediments via bioturbation, increasing oxidation rates relative to methanogenesis.

Hypothesis 1b: High densities of *Corbicula* increase methanogenesis rates relative to oxidation by consuming oxygen through respiration, increasing net CH₄ flux from sediments.

Objective 2: Measure the effects of *Corbicula* density on methane concentration in sediment pore water within an urban stream.

Hypothesis 2a: Low densities of *Corbicula* decrease pore water methane concentration by increasing oxygen delivery to sediments via bioturbation.

Hypothesis 2b: High densities of *Corbicula* increase pore water methane concentration by facilitating formation of anoxic microsites through increased oxygen consumption via respiration.

Hypothesis 2c: Alternatively, high densities of *Corbicula* further increase CH₄ oxidation rates *in situ* by increasing oxygen delivery to sediments from surface water via bioturbation.

CHAPTER II

METHODS

Study System

North Buffalo Creek (NBC) is a 4th order stream draining the city of Greensboro and located in the headwaters of the Cape Fear River Basin, NC, USA. During this study, NBC received waste water effluent downstream of the city, where land use transitions from urban to a mix of forest, neighborhoods and agriculture. A survey of four sites along NBC in July 2016 found methane concentrations of 18.89 ± 3.64 mg/L (mean \pm se) in stream water. *Corbicula* density, estimated for four 20m reaches at six plots per reach using a 0.092m² surber sampler, was 146 ± 33 m⁻² (mean \pm se). Maximum and minimum densities were 550m⁻² and 0m⁻², respectively. Pore water was collected at each plot immediately before *Corbicula* were surveyed. Methane concentrations in pore water were 1041.17 ± 469.51 mg/L (mean \pm se).

Materials for laboratory experiments conducted in March 2017 were collected from Rankin Mill Road (RMR), one of the four sites surveyed in July 2016. RMR is located downstream of a then active waste water treatment plant (WWTP), just outside the city of Greensboro. RMR was surrounded by forest until recently. The construction of I-840 resulted in deforestation on the left bank, leaving only a narrow riparian buffer (1-5m).

Latham Park (LP) was not one of the four survey sites but was selected for field experiments due to shallow water depth, mostly sandy substrate and ease of access. LP is located within a city park upstream of the WWTP and has a narrow riparian buffer (1-5m) on each side of the stream composed of mostly shrubs, grasses and a few trees. Banks are incised, and sandbars are abundant in the stream channel. A beaver dam was observed in LP in December 2016, but was removed by the city. No other impoundments were observed prior to the experiment in August 2017.

Laboratory Experiments

Intact sediment cores (9-12.5 cm depth), *Corbicula* and water were collected from RMR in March 2017, transported to the lab, and used to construct microcosms to examine the effect of *Corbicula* density on three methane cycle processes: methanogenesis rate, potential methane oxidation rate and net methane flux from sediment. Microcosms were constructed from plastic cylinders with 4.5 cm internal diameter and 25cm length, sealed on the bottom with rubber stoppers and on the top with beveled polycarbonate caps with sampling ports. Microcosms were filled with water, leaving no headspace, so that methanogenesis rate, potential oxidation rate, and net methane flux could be calculated based on change over time in surface water methane concentration in respective microcosms, as described in Hershey *et al.*, 2015.

Microcosm cores were assigned to three treatment groups to measure methanogenesis rate, net CH₄ flux, and rate of potential CH₄ oxidation. Methanogenesis rates were estimated by amending four replicate microcosms at each of four *Corbicula* densities with methyl fluoride (CH₃F) to achieve approximately 3.5% saturation of CH₃F

in stream water (Table 1). CH_3F inhibits methane oxidation at low concentrations without inhibiting methanogenesis (Frenzel and Bosse 1996). 3.5% amendments were found to achieve this outcome in NBC (Bullard, 2010). By inhibiting CH_4 oxidation, change over time in surface water methane concentration at differing *Corbicula* densities provided an estimate of methanogenesis rate as a function of *Corbicula* density.

Potential CH_4 oxidation rates were estimated by artificially increasing CH_4 concentrations in microcosms. To amend microcosms with CH_4 , two 125ml vials were filled with deionized water and sealed with rubber butyl stoppers. 10ml high purity CH_4 (purchased from Matheson Gas) was added to each vial using a needle and syringe. A second syringe and needle allowed water to leave the vial as CH_4 was added. After 10ml CH_4 was added, water from the second syringe was forced back into the vial to retain added CH_4 . To avoid over pressuring the vial, 2ml water was removed for each 10ml CH_4 added. This process was repeated five times to yield a pressurized solution super saturated in CH_4 .

Each of four replicate microcosms at each of four *Corbicula* densities (Table 1) were amended with 4.75ml super saturated CH_4 solution. This ratio of CH_4 to water was found to achieve a substrate saturated response of CH_4 oxidation (Lofton 2012). Change in surface water methane concentration in microcosms amended with CH_4 was designed to assess potential CH_4 oxidation as a function of *Corbicula* density. Net CH_4 flux was measured as change in surface water methane concentration over time in four replicate microcosms not receiving methyl fluoride or methane additions at each of four *Corbicula* densities (Table 1).

Corbicula were added to achieve densities of 0, 1, 2, or 3 individuals per microcosm, equivalent to approximately 0, 510, 1020 or 1530 *Corbicula* m⁻², respectively. Results refer to these densities as zero, low, medium and high, respectively. *Corbicula* shell length was 18.82 ± 0.13mm (mean ± se). *Corbicula* were occasionally found in microcosms assigned to the zero-density treatment. These *Corbicula* were counted, measured and included in analysis.

Each microcosm was fitted with a suspended magnetic stir bar to prevent water column stratification during the experiment. All microcosms were incubated at 22°C and stirred at 2 rpm for the duration of the experiment. At the end of the experiment, dissolved oxygen (final DO) was measured with a Sonde and sediment organic matter (%C) was estimated by ash-free dry mass for each microcosm. *Corbicula* were removed from microcosms, counted and measured to account for additional individuals present in intact sediment cores. One microcosm in the net flux, low-density treatment was visibly leaking water at the beginning of the experiment and discarded.

Water samples (5ml) were collected from each microcosm with replacement at approximately 0, 5, 11 and 16 hours after all microcosms were sealed. Replacement solutions corresponded to initial CH₃F or CH₄ concentrations for each microcosm. Samples were stored upside down in 27 ml glass vials which had been evacuated, filled with N₂ gas at 1 atm, and sealed with rubber butyl stoppers and aluminum crimps. Net CH₄ flux, methanogenesis rates and potential CH₄ oxidation rates were calculated from 0 to 5 hours because methane concentration inconsistently increased and decreased in microcosms estimating methanogenesis and net CH₄ flux from 0 to 16 hours. For

microcosms used to estimate potential methane oxidation, the 0-5h period represented the highest rate of potential methane oxidation.

Field Experiments

Corbicula densities were manipulated in NBC at LP to measure response of pore water methane concentration to a *Corbicula* density gradient. Experimental units consisted of benthic baskets made from plastic food containers, plastic hardware mesh and duct tape, sewn together with fishing line. Baskets were approximately 1L in volume with a 5.5cm² opening cut into each side. Openings cut into the bottom of the container and lids were 7cm² and 7.5cm², respectively. Plastic hardware mesh (mesh size 1mm) was duct taped over each opening and sewn into place using fishing line.

A randomized block design was used, in which 10 baskets were installed in three blocks (upstream, middle, downstream) within LP over a 50m reach (n = 30). The study reach was characterized by water depth less than 20cm, mostly sandy substrate with some gravel and a sand bar near the right bank. *Corbicula* were collected immediately downstream of the study reach and densities were assigned to baskets in each block using a random number generator. Each basket was assigned one of 5 *Corbicula* densities (0, 4, 8, 12, or 16 individuals). This approximated a gradient of 0 to 1120 *Corbicula* m⁻². *Corbicula* shell length was 17.76 ± 0.13mm (mean ± se). Each block contained 2 replicates of each density, randomly assigned to a location within each block. *Corbicula* density deviated from assigned densities in some baskets. All *Corbicula* in each basket were counted and measured at the end of the experiment and included in analysis.

Rebar was placed in the streambed in 2 x 5 rows for each block. Sediment was removed immediately downstream of each piece of rebar and used to fill each basket. Baskets were buried at the sediment-water interface and attached to rebar with zipties. Sediment depth to refusal (DTR) and water depth were measured for each basket when rebar was placed into the stream bed and baskets were attached. DTR approximates the depth of fine grain sediments and generally has a positive correlation with sediment organic matter (Crawford and Stanley, 2016). Sediment organic matter content (%C) was estimated for each basket at the end of the experiment by ash-free dry mass, where sediment from each basket was combusted at 550°C. Active channel width was measured for each block when baskets were installed. Pore water was sampled at shallow and deep locations within each basket (approximately 2 and 7 cm below the sediment-water interface, respectively) by zip-tying 2 Rhizon® pore water samplers to wire fixed inside each basket. Rhizon® pore water samplers were purchased from Rhizosphere Research Products and consisted of a 5cm porous membrane and 30cm of tubing connected to a sampling port that was zip-tied to rebar above the water surface.

Baskets equilibrated for 24 hours after installation before the first samples were taken. Pore water was sampled from each Rhizon® every 24 hours after baskets were installed. Surface water was collected immediately downstream of each of the three blocks each time pore water was collected. All samples were stored upside down in 27ml vials until analysis. Sampling was terminated and baskets were removed after 72 hours, resulting in 6 pore water samples (3 shallow, 3 deep) for each basket and 3 surface water samples for each block (Table 1). The sampling port for one Rhizon® (downstream,

shallow, 16 *Corbicula*) went missing within the first 24 hours of the experiment.

Therefore, a total of 177 pore water samples were collected over the course of the experiment.

Sample Analysis

Each vial was analyzed for methane concentration in the headspace using a Shimadzu GC-8A gas chromatograph with flame ionization detection (GC-FID) at the University of North Carolina at Chapel Hill. Each vial was shaken vigorously for 5 seconds to allow equilibration of water with headspace before sample analysis. A 3ml syringe and needle was used to remove 3ml of gas from each vial. To prevent any water from entering the column, 0.5ml of gas was purged and the needle was wiped off before 2ml gas was injected into the column. The remaining 0.5ml was purged and the syringe was wiped off before moving to the next sample. Methane concentration was recorded at approximately one-minute retention time. All methane readings were corrected for methane dissolved in water, and microcosm samples were corrected for sediment volume in each microcosm. Methane cycle process rates derived from microcosms were expressed in $\mu\text{mol CH}_4 \text{ m}^{-2} \text{ hr}^{-1}$.

Data Analysis

Response of net methane flux, potential methane oxidation and methanogenesis rates were examined for outliers at each level of *Corbicula* density using Grubbs test (Grubbs, 1950) and outliers were removed. *Corbicula* biomass was calculated using shell length-dry mass regression (Bullard, 2010). Biomass estimates accounted for size differences between *Corbicula* and were included as a continuous explanatory variable

for each model, rather than density. Generalized least squares regression was used to model the response of net methane flux, methanogenesis and potential methane oxidation to *Corbicula* biomass, allowing variance to differ between *Corbicula* density levels. Correlations between variables explaining potential methane oxidation (*Corbicula* biomass, final DO and sediment organic matter) were examined using Pearson's Correlation. Significantly correlated variables were included in separate models to test their effect on potential methane oxidation. For two-way models, non-significant terms were dropped to improve model fit. Therefore, the simplest models were used to explain the effect of *Corbicula* biomass on potential methane oxidation. Results were reported as change in net methane flux, methanogenesis or potential CH₄ oxidation per gram dry mass of *Corbicula* soft tissue, hereafter referred to as biomass ($\mu\text{mol CH}_4 \text{ m}^{-2} \text{ hr}^{-1} \text{ g}^{-1}$).

Pore water CH₄ concentrations from Latham Park were natural log transformed to improve assumptions of normality and equal variance. A mixed effects model was used to test for effects of *Corbicula* biomass and block on pore water methane concentration, where day and Rhizon® were treated as repeated measures for each basket. Model coefficients were back-transformed after analysis. Results are presented as percent change in pore water methane concentration g⁻¹ of *Corbicula* biomass, accounting for change in pore water methane concentration between blocks in the study reach. Pearson's Correlation was used to test for correlation between sediment %C, DTR and water depth at Latham Park. Sediment %C for one basket containing no *Corbicula* was identified as an outlier by Grubbs test and excluded from analysis.

Changes in sediment DTR, water depth, sediment %C and active channel width between blocks represent change in those parameters over the study reach that could directly or indirectly affect methane cycle processes and pore water methane concentration. Therefore, middle and downstream blocks were compared to the upstream block (y-intercept) but not to each other. Differences in DTR and water depth between blocks were tested using ordinary least squares regression. Differences in sediment %C and active channel width between blocks were tested with generalized least squares regression, which allowed variance to differ between blocks. Results are reported as percent change from upstream block. All statistical analyses were performed in R (R Core Team, 2014), considered significant at $\alpha < 0.05$, and marginally significant at $0.05 < \alpha < 0.09$.

CHAPTER III

RESULTS

Laboratory Experiments

Methane concentrations decreased in net CH₄ flux treatments between 0 and 5 hours (Figure 4), except for one outlier in the high-density treatment which was removed. Negative flux estimates from 0 to 5 hours suggest that CH₄ oxidation rates exceeded methanogenesis rates. GLS regression showed that *Corbicula* has a slightly positive effect on net CH₄ flux from microcosms. Estimated net CH₄ flux in the absence of *Corbicula* was $-1.026 \pm 0.203 \mu\text{mol CH}_4 \text{ m}^{-2} \text{ hr}^{-1}$ ($t = -5.045$, $p < 0.001$, Table 2, Figure 5). Mean net CH₄ flux increased $0.0056 \pm 0.0017 \mu\text{mol CH}_4 \text{ m}^{-2} \text{ hr}^{-1} \text{ g}^{-1}$ *Corbicula* biomass ($t = 3.319$, $p = 0.008$, Table 2, Figure 5). There was a positive interaction between biomass and sediment %C, resulting in a 6.67% increase in estimated CH₄ flux per gram biomass compared with the model where *Corbicula* biomass was considered alone ($t = 2.772$, $p = 0.020$, Table 2). Final DO decreased $0.050 \pm 0.005 \text{ mg/L}$ per gram *Corbicula* biomass ($t = -10.49$, $p < 0.001$, Table 2, Figure 6). However, there was no significant effect of final DO on net CH₄ flux ($t = -1.086$, $p = 0.303$, Table 2). No *Corbicula* were found in microcosms outside of assigned densities.

Methanogenesis treatments showed near zero change in methane concentration over the course of the experiment, suggesting that little or no methanogenesis occurred (Table 2, Figure 7). Two microcosms assigned zero *Corbicula* in the methanogenesis

treatment were found to contain one *Corbicula* each (shell lengths 17 and 11mm). No other microcosms were found to have *Corbicula* outside of assigned densities. Both remaining replicates assigned to the zero-density treatment, one in the low-density treatment and two in the high-density treatment exhibited slightly negative methane flux from 0 to 5 hours. Because negative flux suggests CH₄ oxidation exceeded methanogenesis, CH₄ oxidation was likely not completely inhibited by CH₃F in these microcosms.

Methane concentrations decreased in potential CH₄ oxidation treatments over the course of the experiment (Figure 8). Two microcosms assigned to the zero-density treatment were found to contain one *Corbicula* each (shell lengths 18 and 21.5mm). No other *Corbicula* were found outside of assigned densities. Methane flux was negative for all microcosms, indicating greater rates of oxidation than methanogenesis in CH₄ amended microcosms. Methane concentrations at initial sampling (time 0) were much lower in medium and high-density treatments than zero and low densities (Figure 8).

There was no significant correlation between sediment organic matter and *Corbicula* biomass ($p = 0.334$, $r = 0.279$) or final DO ($p = 0.185$, $r = -0.377$). *Corbicula* biomass had a significant negative effect on final DO. Mean DO was 4.60 ± 0.43 mg/L in microcosms without *Corbicula* ($t = 10.617$, $p < 0.001$, Table 3). There was a 0.02 mg/L decrease in DO g⁻¹ *Corbicula* biomass ($t = -2.527$, $p = 0.025$, Table 3, Figure 9) and there was a significant response of potential methane oxidation to DO. Potential methane oxidation increased by 29.12 ± 8.34 $\mu\text{mol CH}_4 \text{ m}^{-2} \text{ hr}^{-1}$ as DO increased ($t = 3.492$, $p = 0.004$, Table 3, Figure 10). *Corbicula* biomass alone had a marginally significant

negative effect on potential methane oxidation. Potential methane oxidation in microcosms without *Corbicula* was significantly greater than zero ($271.42 \pm 69.49 \mu\text{mol CH}_4 \text{ m}^{-2} \text{ hr}^{-1}$, $t = 3.906$, $p = 0.002$, Table 3). Potential CH_4 oxidation decreased by $1.415 \pm 0.678 \mu\text{mol CH}_4 \text{ m}^{-2} \text{ hr}^{-1} \text{ g}^{-1}$ *Corbicula* biomass ($t = -2.087$, $p = 0.057$, Table 3, Figure 11). However, there was a significant interaction between biomass and sediment %C suggesting that the effect of *Corbicula* biomass on potential methane oxidation is approximately 9% weaker ($1.278 \pm 0.508 \mu\text{mol CH}_4 \text{ m}^{-2} \text{ hr}^{-1} \text{ g}^{-1}$) with increased sediment %C ($t = -2.531$, $p = 0.025$, Table 3).

Field Experiments

Repeated measures analysis revealed that *Corbicula* was responsible for < 1% change overall in pore water methane concentration g^{-1} biomass, and this effect was non-significant ($t = -1.015$, $p = 0.311$, Table 5, Figure 12). The model indicated an increasing trend in pore water methane concentration moving downstream from the upstream block. There was an estimated $134.30 \pm 81.42\%$ increase in methane concentration between upstream and middle blocks, but the increase was not statistically significant ($t = 1.429$, $p = 0.155$, Table 5, Figure 12). However, pore water methane concentration increased by $316.50 \pm 74.07\%$ between upstream and downstream blocks ($t = 2.574$, $p = 0.011$, Table 5, Figure 12). After accounting for change in methane concentration between upstream and middle blocks, the *Corbicula* biomass effect remained insignificant (< 1% change in pore water methane concentration g^{-1} *Corbicula* biomass, $p = 0.80$, Table 5). When accounting for change in pore water methane concentration between upstream and downstream blocks, *Corbicula* biomass had a slightly positive effect on pore water

methane concentration. This $2.29 \pm 1.28\%$ increase in pore water methane concentration g^{-1} *Corbicula* biomass was marginally significant ($t = 1.177$, $p = 0.078$, Table 5, Figure 12), but suggests that *Corbicula*'s effect on pore water methane concentration, may be dependent on site-specific factors within the study reach. However, results of a one-way repeated measures model testing the response of pore water methane concentration in the downstream block only to *Corbicula* biomass also suggested a marginally significant positive relationship between biomass and pore water methane concentration ($1.308 \pm 0.702\% \text{ g}^{-1}$ biomass, $t = 1.759$, $p = 0.085$, Table 5).

Correlation between DTR and water depth was not significant ($p = 0.14$, $r = -0.281$, Figure 13). There was a weak but significant positive correlation between sediment %C and water depth ($p = 0.029$, $r = 0.405$, Figure 14) and a weak but significant negative correlation between sediment %C and DTR ($p = 0.012$, $r = -0.459$, Figure 15).

Mean upstream water depth was 7.40 ± 1.04 cm ($t = 7.12$, $p < 0.001$, Table 6, Figure 16). Water depth increased by $38.89 \pm 20.40\%$ in the middle block, but this change was marginally significant ($t = 1.906$, $p = 0.068$, Table 6, Figure 16). Water was $47.30 \pm 19.86\%$ deeper downstream ($t = 2.381$, $p = 0.025$, Table 6, Figure 16). Mean sediment %C was 0.73 ± 0.05 in the upstream block ($t = 14.01$, $p < 0.001$, Table 6, Figure 17). %C in the middle block was not significantly different ($t = -0.253$, $p = 0.802$, Table 6) but %C in the downstream block was $59.80 \pm 20.24\%$ higher than upstream ($t = 2.955$, $p = 0.007$, Table 6, Figure 17). Sediment DTR decreased moving downstream. Upstream DTR was 70.90 ± 2.60 cm ($t = 26.81$, $p < 0.001$, Table 6, Figure 18). DTR decreased by $14.22 \pm 5.41\%$ in the middle block ($t = -2.623$, $p = 0.014$, Table 6, Figure 18) and by

26.46 ± 5.28% in the downstream block ($t = -5.017$, $p < 0.001$, Table 6, Figure 18). Active channel width was 5.10 ± 0.75m in the upstream block ($t = 6.975$, $p < 0.001$, Table 6, Figure 19). There was a marginally significant increase in active channel width in the middle block (36.6 ± 17.0%, $t = 2.154$, $p = 0.075$, Table 6, Figure 19), due to the presence of a sandbar near the right bank. Downstream channel width was not significantly different than upstream ($t = -1.017$, $p = 0.322$, Table 6, Figure 19). The sandbar, along with differences in sediment %C and DTR within the study reach, may have influenced *Corbicula*'s effect on pore water methane concentration.

CHAPTER IV

DISCUSSION

Laboratory Methane Cycle Process Rates

Laboratory experiments suggest that *Corbicula* had a significant positive effect on net CH₄ flux from sediments. Effect size increased slightly but significantly when considered in conjunction with sediment %C. This 6.67% increase suggests that *Corbicula*'s effect on net methane flux is positively related to sediment %C, and results of potential CH₄ oxidation treatments suggest *Corbicula* increase net CH₄ flux by reducing CH₄ oxidation rates. At least one other study has suggested a positive effect of bivalves on CH₄ flux from lotic sediments (Benelli *et al.*, 2017). However, Benelli *et al.* studied *Sinanodonta woodiana*, which is much larger than *Corbicula*, in a Northern Italian stream with “fluffy” sediment, where organic matter content was over 20%.

The effect of *Corbicula* on CH₄ flux from sediment measured in the present study is much less than measured effects of *S. woodiana*. Given estimated mean biomass of 38 g m⁻² in the low-density treatment of 510 individuals m⁻², which corresponds with densities observed in NBC, *Corbicula* may increase net methane flux from sediment by 5.12 – 5.47 μmol CH₄ m⁻² day⁻¹, depending on sediment organic matter content. *S. woodiana* biomass was 95 ± 10 g m⁻² (mean ± se), with 6 individuals m⁻² (Benelli *et al.* 2017). This larger invasive bivalve increased CH₄ flux from sediments by 101.89 ± 48.24 μmol CH₄ m⁻² hr⁻¹ (Benelli *et al.* 2017), an effect approximately 190 times greater than

estimated for *Corbicula* in the present study. The difference in response between the present study and Benelli *et al.* is likely due, in part, to differences in sediment particle size and organic matter content, as well as animal body size. Sediment organic matter is positively related to CH₄ emissions from streams (Crawford and Stanley, 2016) and presence of methanogen DNA (Smith, 2013). Sediment organic matter above 20% likely contributed to high CH₄ flux from sediment reported by Benelli *et al.*

The size of individual bivalves also drives indirect ecological effects (Majdi *et al.*, 2014), and direct effects, where metabolic rate and respiration generally increase with individual biomass (Brown *et al.*, 2004). *S. woodiana* can reach shell lengths of 30cm (Pou-Rovira *et al.*, 2009), over 16 times the average length of *Corbicula* in this study. The larger mussel is capable of increasing CH₄ flux in the water column by 12.91 ± 5.67 $\mu\text{mol CH}_4 \text{ m}^{-2} \text{ hr}^{-1} \text{ g}^{-1}$ in microcosms containing only *S. woodiana* and water, suggesting that methanogenesis may be directly stimulated by *S. woodiana* metabolism (Benelli *et al.* 2017). Direct and indirect effects of bivalves on methane cycle processes appears to vary greatly with size and environmental conditions, including sediment particle size and organic matter content. Further discussion of *Corbicula*'s relationship with CH₄ cycle processes may help generate predictions to be tested in future experiments and compared with effects of larger bivalves.

Results of net CH₄ oxidation treatments are inconsistent with the hypothesis that *Corbicula*'s effect is driven by bioturbation at low densities, where displacement of sediment is hypothesized to increase potential CH₄ oxidation by enhancing sediment oxygen uptake from surface water. There was no indication of a negative relationship

between *Corbicula* and net CH₄ flux at any density level, suggesting that *Corbicula*'s effect on net CH₄ flux was not driven by bioturbation. The positive relationship observed between *Corbicula* and net CH₄ flux is more likely driven by respiration. As clams consume oxygen, less is available for oxidation of CH₄ already present in the sediment. This explanation is supported by the negative relationship observed between *Corbicula* biomass and dissolved oxygen. However, laboratory experiments did not test the response of net CH₄ flux to the full gradient of *Corbicula* densities found in NBC.

Mean *Corbicula* density in NBC was not directly represented in this experimental design. The low-density treatment of one clam per microcosm approximated densities of 510m⁻², which is closer to maximum densities observed in NBC (550m⁻²) in July 2016. Bioturbation may play a role in decreasing CH₄ flux from sediments by facilitating oxidation at densities between 0 and 500m⁻². Generally, *Corbicula* downstream dispersal is loosely constrained by water chemistry in the stream, but at least one study found assemblages were more common in areas with higher dissolved oxygen concentrations (Pereira *et al.*, 2017), although Pereira *et al.* did not find DO levels below 1-3 mg/L, which is known to negatively affect *Corbicula* growth (Belanger, 1991). *Corbicula* can exude mucus strands which facilitate downstream dispersal when conditions are not favorable (Prezant and Chalmerwat, 1984). This may help explain why Pereira *et al.* found dispersal was skewed towards higher DO levels. *Corbicula* exude mucus strands in NBC (personal observation) and may disperse themselves downstream from a dense assemblage before DO levels become dangerously low. However, deviations from

hypothesized relationships between *Corbicula* and net CH₄ flux cannot be explained fully by *Corbicula* biomass alone.

Both %C and DO were significant covariates in models estimating *Corbicula*'s effect on net CH₄ flux and potential oxidation rates. Specifically, potential oxidation rates were positively influenced by DO and DO was negatively influenced by *Corbicula* biomass. *Corbicula* biomass alone had a marginally significant negative effect on potential CH₄ oxidation. This negative effect was lessened by 9%, from -1.415 ± 0.678 $\mu\text{mol CH}_4 \text{ m}^{-2} \text{ hr}^{-1} \text{ g}^{-1}$ to -1.287 ± 0.508 $\mu\text{mol CH}_4 \text{ m}^{-2} \text{ hr}^{-1} \text{ g}^{-1}$ when accounting for interaction between biomass and sediment %C. While sediment %C alone did not have a significant effect on potential CH₄ oxidation, it appeared to slightly reduce the negative effect of *Corbicula* biomass. If aerobic decomposition rates are slightly higher with more sediment organic matter, then more oxygen may be consumed, lowering potential CH₄ oxidation rates.

In Stream Concentrations

Pore water methane concentration in the study reach ranged from < 2 mg/L to > 10,000 mg/L. Variation in pore water methane concentration was not significantly related to depth of the pore water sampled within each basket or to temporal changes over the course of the 3-day experiment. Rather, location within the study reach was the most significant predictor of pore water methane concentration, where methane concentration increased by 316% between upstream and downstream blocks along the 50m reach (Figure 12). High variability of methane concentration within and between study blocks confounded direct interpretation of *Corbicula*'s impact on pore water methane

concentration. Field experiments did not provide evidence of a mechanistic effect of *Corbicula* biomass alone on stream pore water methane concentration, as hypothesized. However, variation in methane concentration and active channel characteristics between blocks enable potential controls on methane cycle processes to be explored. Effects of *Corbicula* biomass on pore water methane concentration likely depend on context within the stream channel.

Water depth, sediment DTR, %C and active channel width differed between blocks. Water depth increased slightly between upstream and downstream blocks along the study reach (Figure 16). Water depth is inversely related to ebullition from lakes (Bastviken *et al.*, 2004). However, the present study in NBC took place in shallow stream with conditions very different from lakes studied by Bastviken *et al.* Data regarding water depth and its relationship to methane concentration in streams appears to be lacking. DTR decreased along the study reach from upstream to downstream blocks (Figure 18), indicating a decrease in the depth of fine sediments. DTR is known to be positively related to CH₄ emissions from streams (Crawford and Stanley, 2016) where increased depth of fine sediments facilitates anoxic microsite formation (Stanley *et al.*, 2016). Interestingly, pore water methane concentration was highest in the downstream block, where DTR was lowest, suggesting that DTR was not driving the downstream increase in pore water methane concentration. Mean sediment %C was highest in the downstream block (Figure 17), suggesting that decomposition may be fueling methanogenesis at anoxic microsites and increasing pore water methane concentration.

Hyporheic exchanges within the sandbar in the middle block could have increased methane concentration in the downstream reach. Hyporheic zones are dynamic areas of exchange between groundwater and surface water environments (Krause *et al.*, 2011). Hyporheic processes influence nutrient and oxygen availability (Holmes *et al.*, 1994) and redox gradients are shaped by hyporheic residence time (Zarnetski *et al.*, 2011). Long residence times are associated with a transition from aerobic to anaerobic processes and residence times increase toward the downstream end of hyporheic flow paths (Zarnetski *et al.*, 2011). These trends are consistent with observation of changes in CH₄ in the Latham Park study reach. Methane concentrations were lowest upstream of the sandbar, where CH₄ oxidation is more likely favored. As hyporheic residence time increases downstream, especially moving through a sandbar, anaerobic metabolism and methanogenesis would have been facilitated and CH₄ oxidation would have been inhibited, resulting in higher pore water methane concentration observed in the downstream block.

Corbicula biomass may not be the major driver of pore water methane concentration in this stream, but its effect on methane concentration did change along the study reach. Specifically, *Corbicula* biomass appeared to be positively correlated with methane concentration in the downstream block, where pore water methane concentration was highest. Though only marginally significant statistically, the effect of *Corbicula* on pore water methane concentration in the downstream block is noteworthy when compared to estimates of *Corbicula*'s effect in middle and upstream blocks. Both suggested a slightly negative effect of *Corbicula* on pore water methane concentration,

but neither was statistically significant. Further, pore water methane concentration increased 2.3% per gram biomass in the downstream block. Considering the range of *Corbicula* densities found in NBC (0-550m⁻²) and the heterogeneity of this urban stream channel (i.e. presence and location of sandbars), *Corbicula*'s effect on pore water CH₄ could be negligible where densities are low and sediments are well oxygenated (i.e., non-significant effects in upstream and middle blocks). Conversely, pore water methane concentration could increase up to 92%, where *Corbicula* reaches high densities in hyporheic tailwaters.

While the scale of this experiment is quite small, results suggest *Corbicula* may further increase pore water methane concentration where methanogenesis has already been stimulated via hyporheic flow paths. This result is especially relevant for urban streams, where erosion and sedimentation lead to formation of sandbars that increase channel heterogeneity. Hyporheic flow paths also affect nitrogen cycling (Holmes *et al.*, 1994; Zarnetski *et al.*, 2011) and relationships between methane and nitrogen cycles are not fully understood (Bodelier and Steenbergh, 2014; Stanley *et al.*, 2016). These relationships are further complicated by intensive nitrogen loading in urban streams (Bernhardt *et al.*, 2008) and invasive benthic consumers which are known to drive nutrient cycles (Atkinson *et al.*, 2017) and CH₄ flux (Benelli *et al.*, 2017).

Conclusions

Laboratory results of this study highlight the potential role of *Corbicula* in stimulating CH₄ flux from sediments by reducing CH₄ oxidation rates. Deviation from hypothesized results suggests potential for seasonal differences in *Corbicula*'s effect on

CH₄ cycle processes. These differences may be driven by changes in the hyporheic environment. Results from field experiments suggest that *Corbicula*'s effect on *in situ* methane concentration, and underlying CH₄ cycle processes, may vary with active stream channel characteristics and associated hyporheic flow paths. Specifically, increasing sediment %C and decreasing DO appear to enhance effects of *Corbicula* biomass on methane concentration within and CH₄ flux from stream sediments. As anthropogenic activities continue to impact stream channel morphology, carbon and oxygen dynamics, future studies should consider the role of benthic consumers in CH₄ cycle processes within these heterogeneous ecosystems.

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APPENDIX A
TABLES AND FIGURES

Table 1. Microcosms

Corbicula density for each microcosm and *Corbicula* density and biomass scaled per square meter for each microcosm and density level. There were originally 4 replicates for each microcosm treatment and density level. Final replicates refer to the total number of replicates used in analysis, after accounting for deviations in assigned *Corbicula* density, leaky microcosms, dead clams and outliers.

Microcosm treatment	Corbicula density	Corbicula density m ⁻²	Corbicula biomass (g m ⁻²)	Final replicates
Net CH ₄ flux	0	0	0	3
	1	510	37.59 ± 2.65	4
	2	1020	73.77 ± 4.22	3
	3	1530	122.12 ± 2.17	2
Methanogenesis	0	0	0	2
	1	510	28.37 ± 5.21	4
	2	1020	78.97 ± 2.07	3
	3	1530	117.36 ± 2.59	4
Potential CH ₄ oxidation	0	0	0	2
	1	510	39.08 ± 4.01	5
	2	1020	75.10 ± 3.45	4
	3	1530	118.06 ± 2.06	3

Table 2. Net Methane Flux Results

Generalized least squares regression response of net methane flux ($\mu\text{mol CH}_4 \text{ m}^{-2} \text{ hr}^{-1}$) to *Corbicula* biomass (g m^{-2}), final DO (mg/L) and interaction between biomass and sediment %C. Generalized least squares response of final DO (mg/L) to *Corbicula* biomass (g m^{-2}) in net CH_4 flux treatments. Effect size is reported by the model coefficient as change in response variable ($\mu\text{mol CH}_4 \text{ m}^{-2} \text{ hr}^{-1}$ or mg/L DO) (mean \pm se).

Response variable	Explanatory variable	Model coefficient	D.F.	t-value	p-value
Net methane flux	Intercept (biomass)	-1.026 ± 0.203	2,10	-5.045	<0.001
	biomass	0.0056 ± 0.0017	2,10	3.319	0.008
	Intercept (biomass:%C)	-0.933 ± 0.185	2,10	-5.038	<0.001
	biomass:%C	0.006 ± 0.002	2,10	2.772	0.02
	Intercept (final DO)	-0.203 ± 0.137	2,10	-1.484	0.169
Final DO	Final DO	-0.055 ± 0.051	2,10	-1.086	0.303
	Intercept	8.629 ± 0.511	2,10	16.87	<0.001
	biomass	-0.050 ± 0.005	2,10	-10.49	<0.001

Table 3. Potential Methane Oxidation Results

Generalized least squares regression response of potential CH₄ oxidation ($\mu\text{mol CH}_4 \text{ m}^{-2} \text{ hr}^{-1}$) to *Corbicula* biomass (g m^{-2}), final DO (mg/L) and interaction between biomass and sediment %C. Generalized least squares response of final DO (mg/L) to *Corbicula* biomass (g m^{-2}) in potential CH₄ oxidation treatments. Effect size is reported by the model coefficient as change in response variable ($\mu\text{mol CH}_4 \text{ m}^{-2} \text{ hr}^{-1}$ or mg/L DO) (mean \pm se).

Response variable	Explanatory variable	Model coefficient	D.F.	t-value	p-value
Potential CH ₄ oxidation	Intercept (biomass)	271.40 \pm 69.49	2,13	3.906	0.002
	Biomass	-1.415 \pm 0.678	2,13	-2.087	0.057
	Intercept (biomass:%C)	242.90 \pm 48.74	2,13	-2.531	0.025
	Biomass:%C	-1.28 \pm 0.51	2,13	-2.531	0.025
	Intercept (final DO)	21.26 \pm 28.71	2,13	0.741	0.472
	Final DO	29.10 \pm 8.34	2,13	3.492	0.004
Final DO	Intercept	4.60 \pm 0.43	2,13	10.617	<0.001
	Biomass	-0.020 \pm 0.008	2,13	-2.527	0.025

Table 4. Block Characteristics in Latham Park

Methane concentrations (mg/L), temperature (°C) and DO (mg/L) between blocks and sampling days in Latham Park. Methane concentrations are mean \pm se. DO was only measured on day three.

Block	Day	Pore water CH ₄	Surface water		
			CH ₄	Temperature	DO
Upstream	one	58.39 \pm 29.81	34.27 \pm 0.41	32.2	
	two	73.79 \pm 39.12	38.77 \pm 0.28	30.0	
	three	218.59 \pm 111.08	29.49 \pm 0.97	31.0	11.75
Middle	one	339.79 \pm 175.76	42.82 \pm 0.84	32.6	
	two	502.47 \pm 214.10	43.40 \pm 0.47	30.0	
	three	585.13 \pm 369.18	34.11 \pm 0.56	31.0	11.32
Downstream	one	262.29 \pm 62.42	43.46 \pm 0.42	33.0	
	two	269.33 \pm 55.34	45.15 \pm 0.60	30.4	
	three	450.18 \pm 226.56	34.97 \pm 0.82	30.7	9.30

Table 5. Effects of *Corbicula* Biomass and Block

Two-way repeated measures model results show response of natural log-transformed pore water methane concentration (mg/L) to *Corbicula* biomass (g) and position within the 50m study reach. One-way repeated measures model tests *Corbicula*'s effect in the downstream block only. Values were back-transformed to estimate percent change in pore water methane concentration. Effects sizes were calculated as % change in pore water methane concentration g^{-1} *Corbicula* biomass (mean \pm se).

Model	Explanatory variable	Model coefficient	Effect size	t-value	p-value
Two-way model	Intercept	3.390 \pm 0.396	---	8.569	<0.001
	Biomass	-0.009 \pm 0.009	-0.941 \pm 0.935	-1.015	0.311
	Middle	0.851 \pm 0.596	134.3 \pm 81.42	1.429	0.155
	Downstream	1.427 \pm 0.554	316.5 \pm 74.07	2.574	0.011
	Biomass:middle	0.003 \pm 0.013	0.339 \pm 0.133	0.256	0.800
	Biomass:down	0.023 \pm 0.013	2.288 \pm 1.284	1.774	0.078
One-way model	Intercept	4.817 \pm 0.333	---	14.45	<0.001
	Biomass	0.013 \pm 0.007	1.308 \pm 0.702	1.759	0.085

Table 6. Block Effect

Ordinary least squares results for changes in water depth (cm), sediment DTR (cm) along the study reach. Sediment % C and active channel width (m) were modeled with generalized least squares due to unequal variance between blocks. Effect size is reported as % change in response variable from upstream block (intercept) (mean \pm se).

Response variable	Block	Model coefficient	Effect size	D.F.	t-value	p-value
Water depth	Intercept	7.40 \pm 1.04	---	2,26	7.12	<0.001
	Middle	2.87 \pm 1.40	38.89 \pm 20.41	2,26	1.91	0.068
	Downstream	3.50 \pm 1.38	47.30 \pm 19.86	2,26	2.38	0.025
Sediment DTR	Intercept	70.90 \pm 2.60	---	2,26	26.8	<0.001
	Middle	-10.07 \pm 3.37	-14.22 \pm 5.42	2,26	-2.62	0.014
	Downstream	-18.75 \pm 4.08	-26.46 \pm 5.28	2,26	-5.02	< 0.001
Sediment %C	Intercept	0.73 \pm 0.05	---	3,26	14.0	<0.001
	Middle	-0.014 \pm 0.060	-1.98 \pm 7.82	3,26	-0.253	0.802
	Downstream	0.437 \pm 0.15	59.80 \pm 20.24	3,26	2.96	0.007
Active channel width	Intercept	5.10 \pm 0.75	---	3,6	6.80	<0.001
	Middle	1.87 \pm 0.87	36.6 \pm 17.0	3,6	2.15	0.075
	Downstream	-0.77 \pm 0.75	-15.0 \pm 14.8	3,6	-1.02	0.322

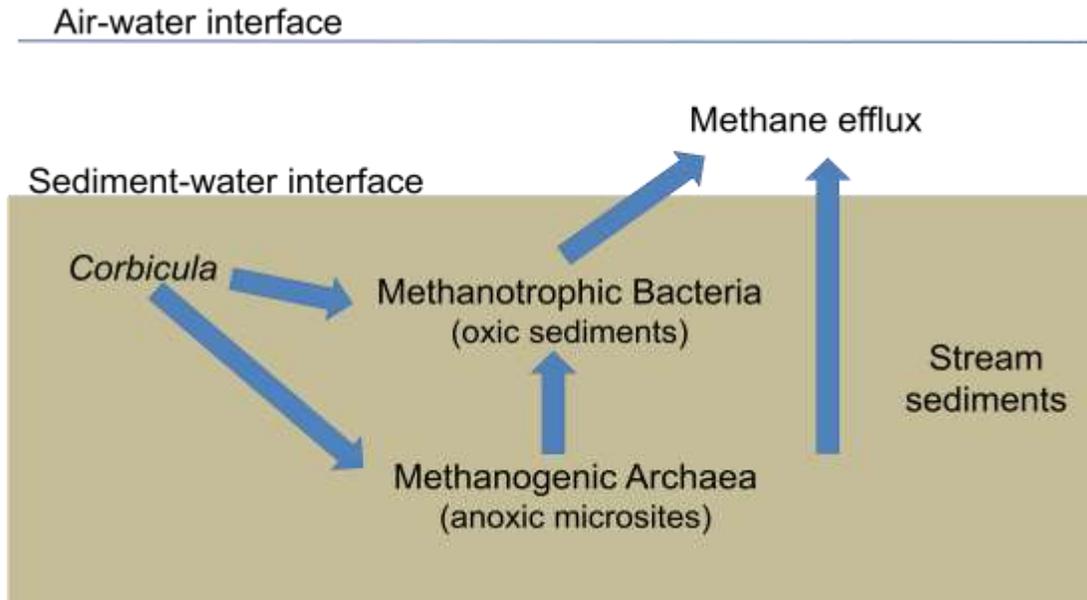


Figure 1. Conceptual Model of *Corbicula* in Stream Sediment.

Corbicula may affect methanogenesis, CH₄ oxidation and flux from stream sediments through respiration or bioturbation.

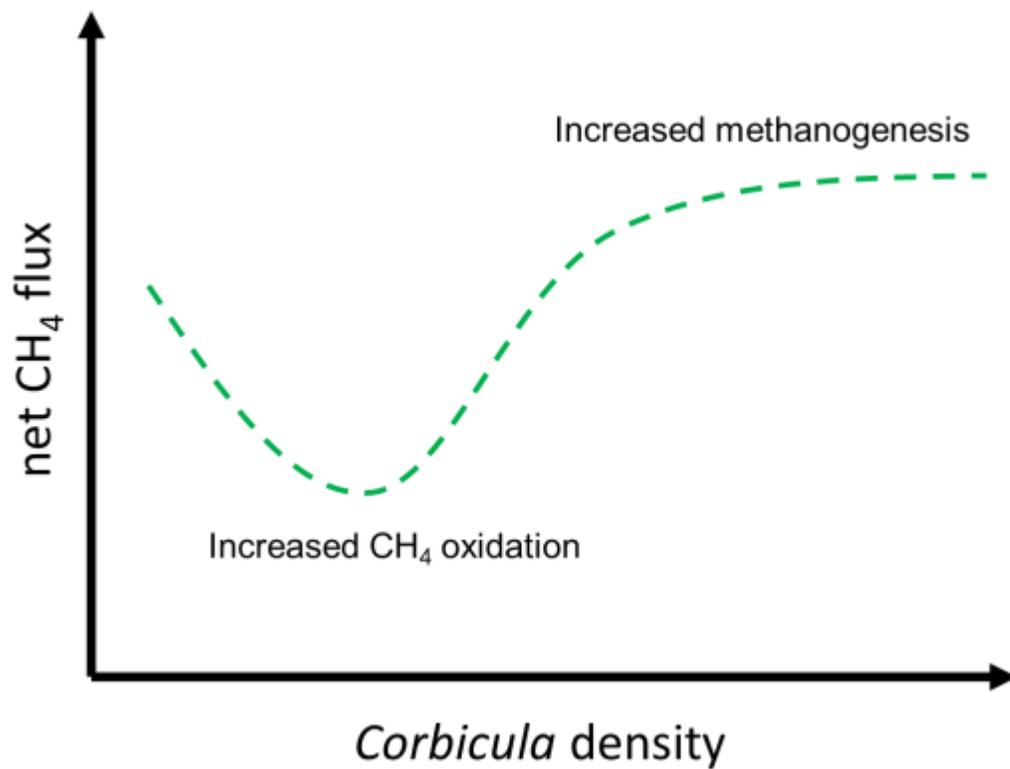


Figure 2. Conceptual Model for Laboratory Experiments.

Hypothesized effects of *Corbicula* on net CH₄ flux. CH₄ oxidation may be enhanced by bioturbation at low densities, lowering net CH₄ flux. At high densities, respiration consumes oxygen, facilitating formation of anoxic microsites and increasing net CH₄ flux.

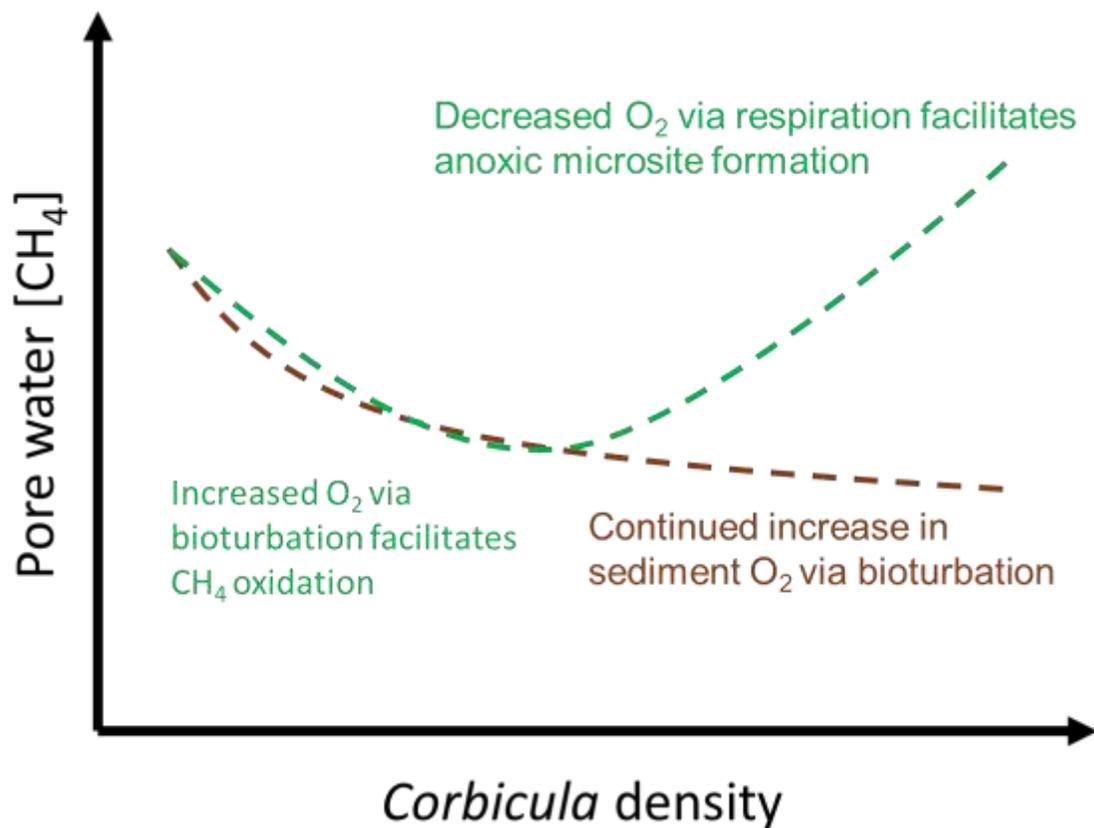


Figure 3. Conceptual Model for Field Experiments.

Hypothesized effects of *Corbicula* density on pore water methane concentration in North Buffalo Creek at Latham Park. Bioturbation may facilitate CH₄ oxidation at low densities, reducing pore water methane concentration. Respiration consumes oxygen at high densities, facilitating formation of anoxic microsites. Alternatively, bioturbation continues to deliver oxygen to sediments, further increasing CH₄ oxidation.

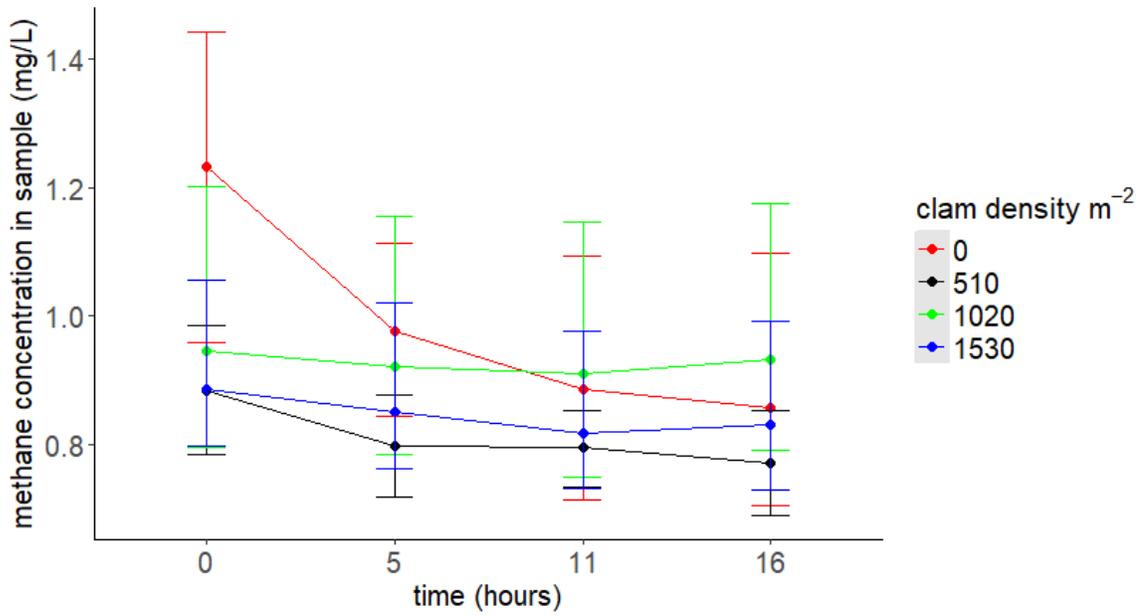


Figure 4. Net Methane Flux Time Course.

Methane concentrations in microcosms with 100% stream water (net methane flux) over the course of the experiment. Flux for each microcosm were calculated from 0 to 5 hours.

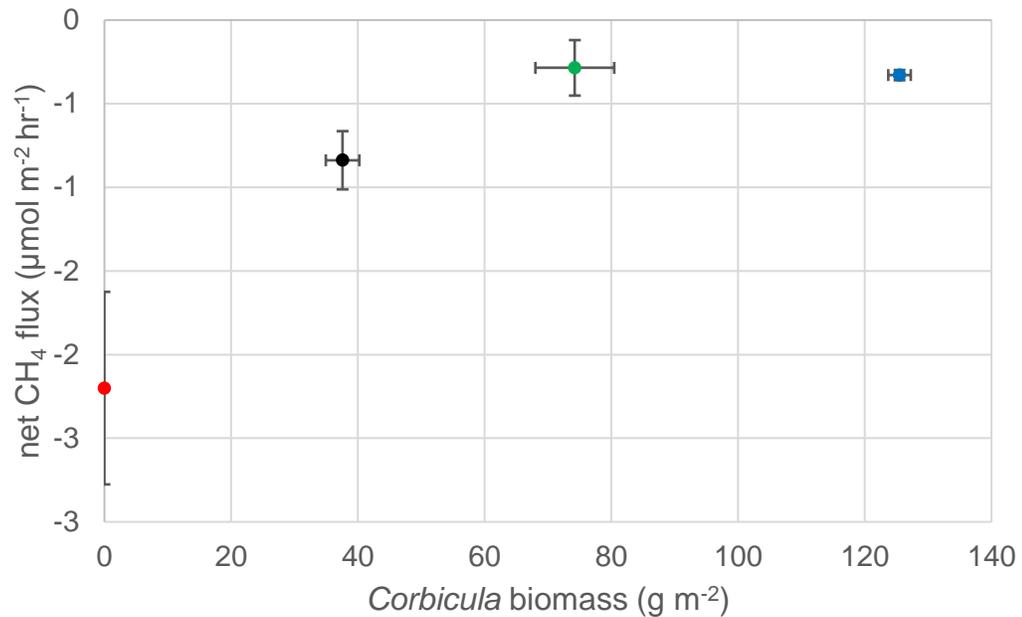


Figure 5. Net Methane Flux Response to Biomass.

Methane flux from microcosms with 100% stream water (net methane flux). GLS estimated net methane flux in the absence of *Corbicula* was $-1.027 \pm 0.203 \mu\text{mol CH}_4 \text{m}^{-2} \text{hr}^{-1}$ ($t = -5.045$, $p < 0.001$, Table 2). Mean net methane flux increased $0.0056 \pm 0.0017 \mu\text{mol CH}_4 \text{m}^{-2} \text{hr}^{-1} \text{g}^{-1} \text{Corbicula biomass}$ ($t = 3.319$, $p = 0.008$). Points represent mean CH₄ flux at each *Corbicula* density level (individuals m⁻²), 0 (red), 510 (black), 1020 (green) and 1530 (blue). Error bars show standard error for biomass (x-axis) and net CH₄ flux (y-axis) at each density level.

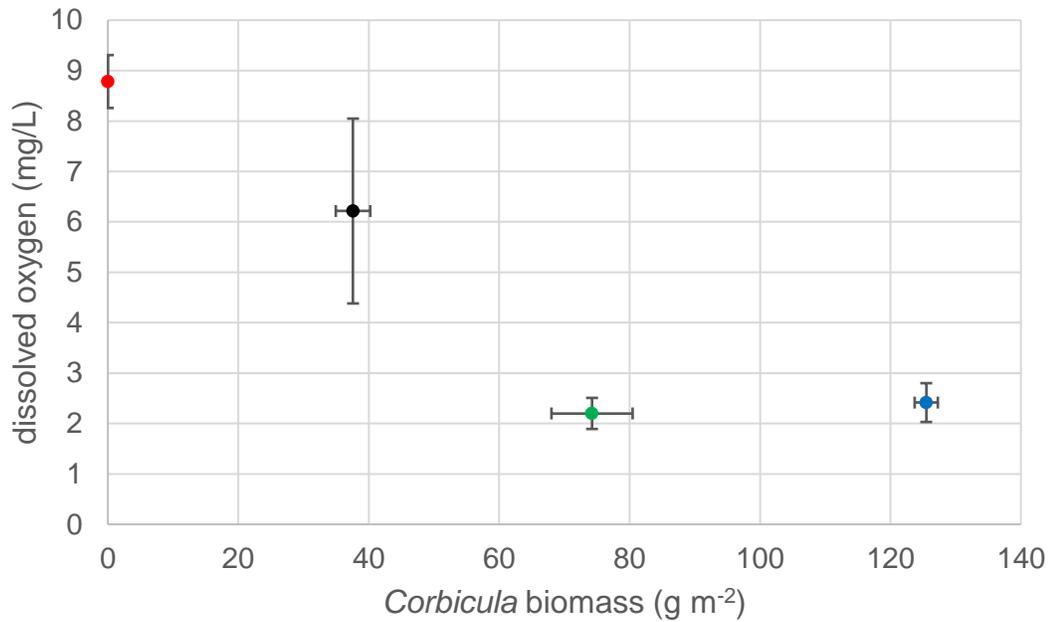


Figure 6. Relationship between DO and Biomass in Net Flux Treatments.

Response of dissolved oxygen (mg/L) to *Corbicula* biomass in microcosms estimating net CH₄ flux. GLS estimated DO was 8.629 ± 0.511 mg/L ($t = 16.87$, $p < 0.001$, Table 2) in microcosms without *Corbicula*. Mean DO decreased by 0.050 ± 0.005 mg/L per gram *Corbicula* biomass ($t = -10.49$, $p < 0.001$, Table 2). Points represent mean DO (mg/L) at each *Corbicula* density level (individuals m⁻²), 0 (red), 510 (black), 1020 (green) and 1530 (blue). Error bars show standard error for biomass (x-axis) and DO (y-axis) at each density level.

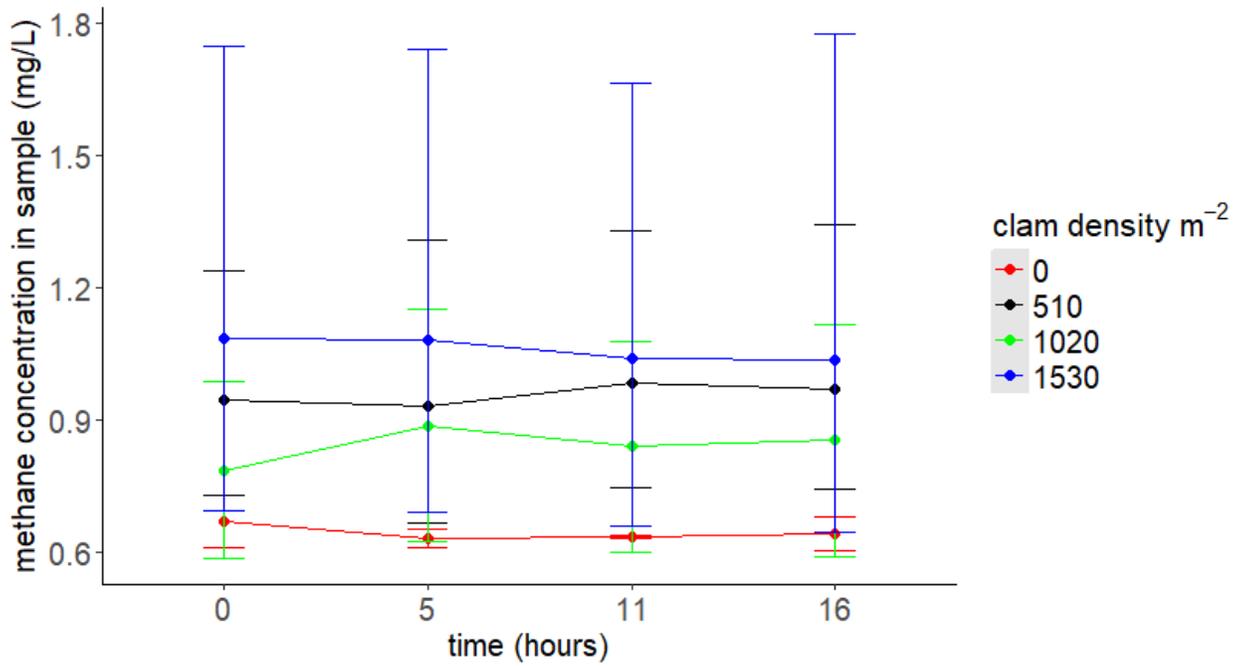


Figure 7. Methanogenesis Time Course.

Methane concentrations in microcosms receiving 3.5% CH₃F amendments (methanogenesis treatments) over the course of the experiment. Flux for each microcosm were calculated from 0 to 5 hours.

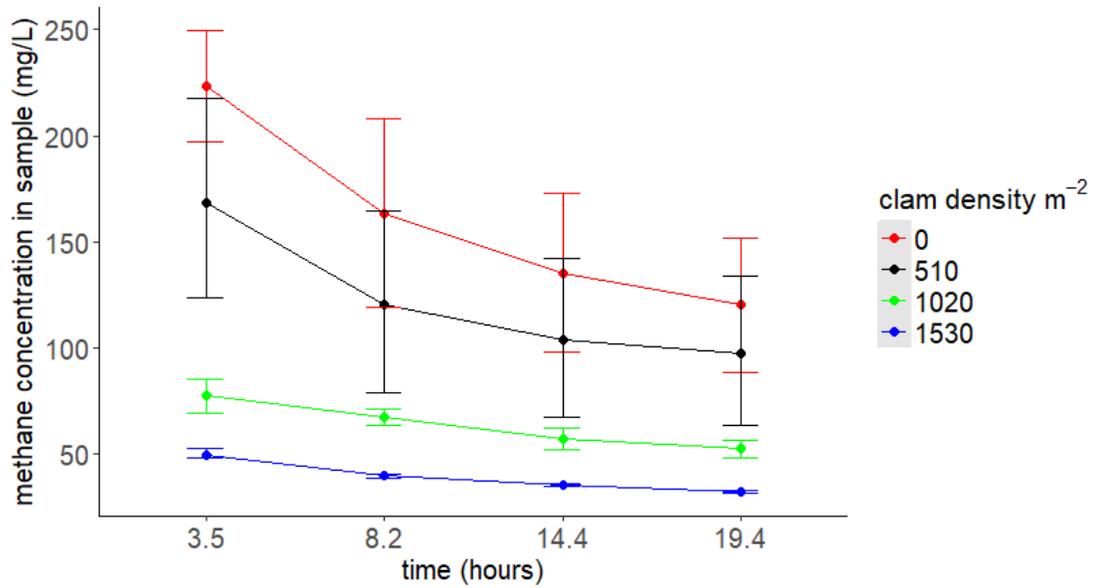


Figure 8. Potential Methane Oxidation Time Course.

Methane concentrations in microcosms receiving CH₄ amendments (potential CH₄ oxidation) over the course of the experiment. Flux for each microcosm was calculated from 0 to 5 hours.

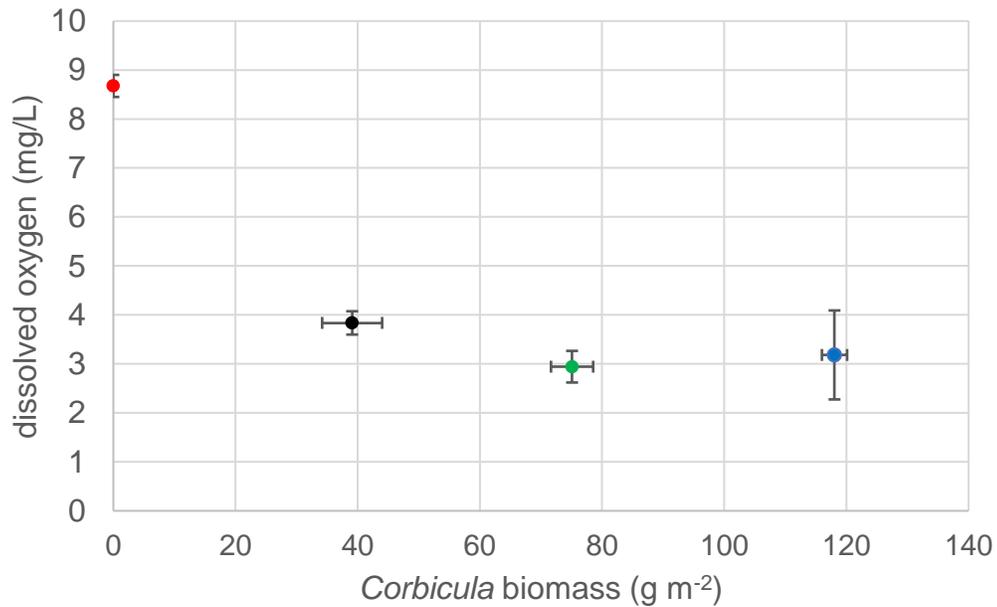


Figure 9. DO and Biomass in Potential Oxidation Treatments.

Response of mean DO to *Corbicula* biomass. GLS showed mean DO was 4.60 ± 0.43 mg/L in microcosms without *Corbicula* ($t = 10.617$, $p < 0.001$, Table 3). Mean DO decreased by 0.020 ± 0.008 mg/L g^{-1} *Corbicula* biomass ($t = -2.527$, $p = 0.025$, Table 3). Points represent mean DO (mg/L) at each *Corbicula* density level (individuals m^{-2}), 0 (red), 510 (black), 1020 (green) and 1530 (blue). Error bars show standard error for biomass (x-axis) and DO (y-axis) at each density level.

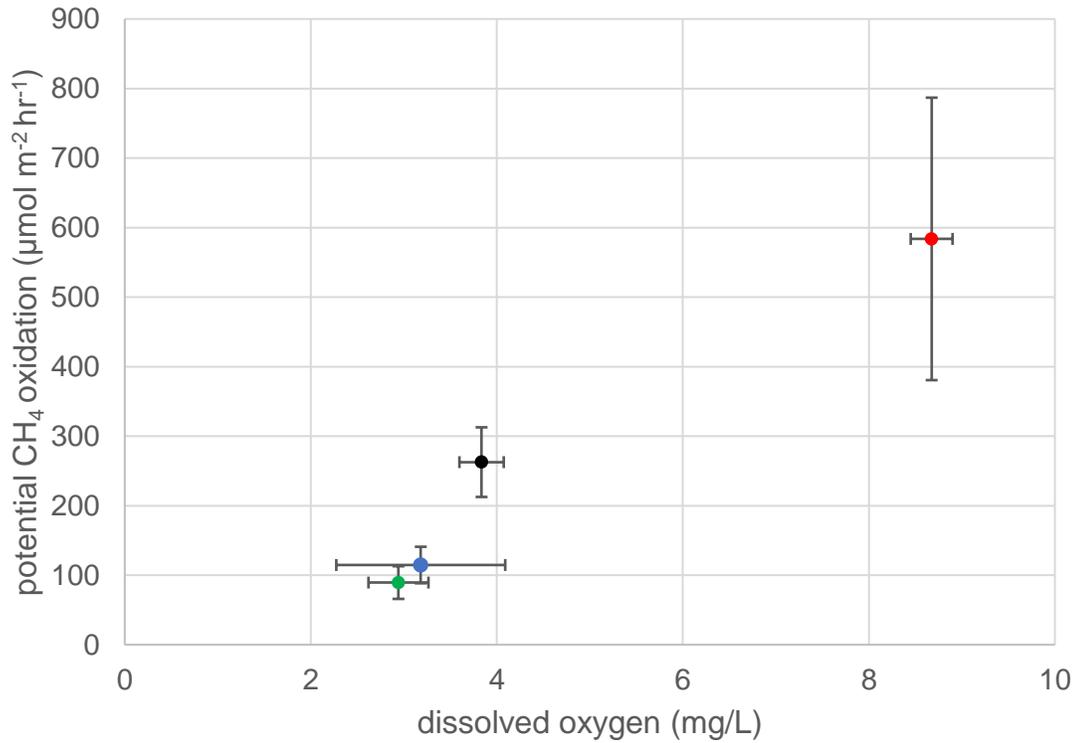


Figure 10. Potential Methane Oxidation and DO.

Response of potential CH₄ oxidation (µmol CH₄ m⁻² hr⁻¹) to dissolved oxygen (mg/L). GLS regression showed potential CH₄ oxidation increased by 29.12 ± 8.34 µmol CH₄ m⁻² hr⁻¹ as DO (mg/L) increased ($t = 3.492$, $p = 0.004$, Table 2). Points represent mean potential CH₄ oxidation and DO at each *Corbicula* density level (individuals m⁻²), 0 (red), 510 (black), 1020 (green) and 1530 (blue). Error bars show standard error for DO (x-axis) and potential CH₄ oxidation (y-axis) at each density level.

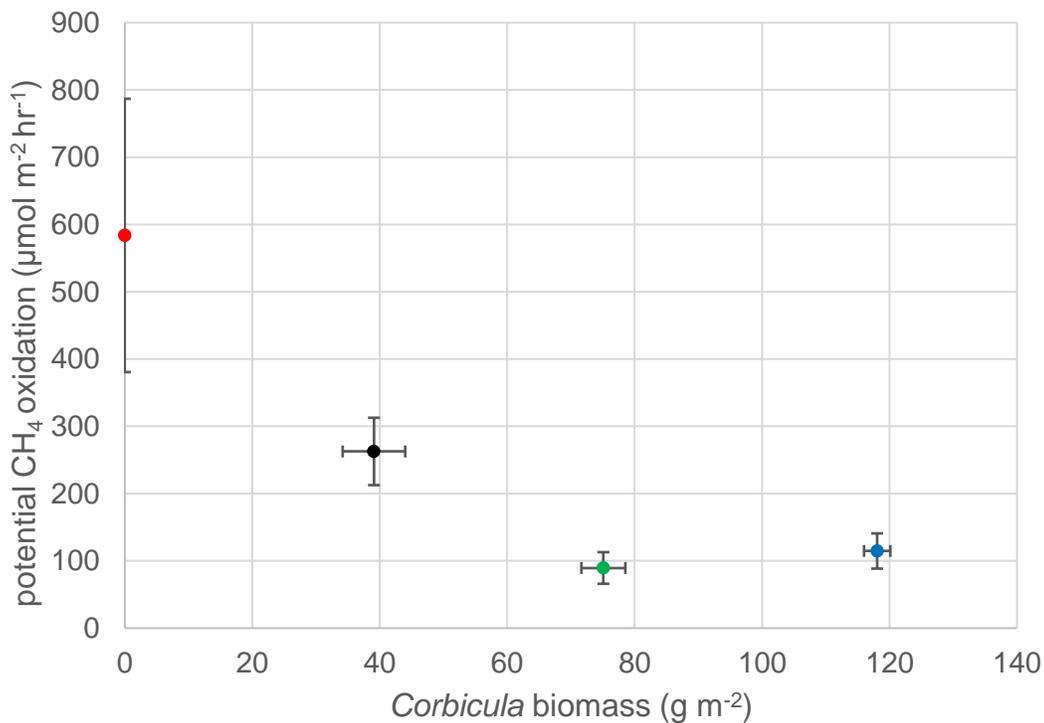


Figure 11. Potential Methane Oxidation and Biomass.

Response of potential CH₄ oxidation to *Corbicula* biomass. GLS showed mean potential CH₄ oxidation in microcosms without *Corbicula* was $271.42 \pm 69.49 \mu\text{mol CH}_4 \text{ m}^{-2} \text{ hr}^{-1}$ ($t = 3.906$, $p = 0.002$, Table 3). Potential CH₄ oxidation decreased by $1.415 \pm 0.678 \mu\text{mol CH}_4 \text{ m}^{-2} \text{ hr}^{-1} \text{ g}^{-1}$ *Corbicula* biomass ($t = -2.087$, $p = 0.057$, Table 3). Points represent mean potential CH₄ oxidation at each *Corbicula* density level (individuals m⁻²), 0 (red), 510 (black), 1020 (green) and 1530 (blue). Error bars show standard error for biomass (x-axis) and potential CH₄ oxidation (y-axis) at each density level.

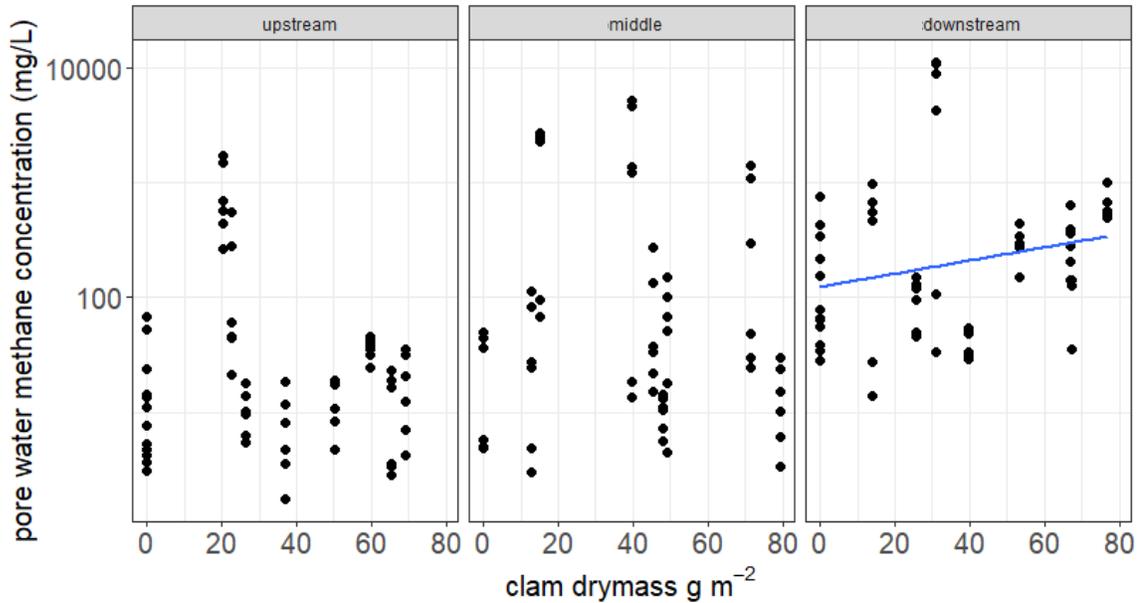


Figure 12. Response of Pore Water Methane to Biomass and Block.

Response of pore water CH₄ concentration to *Corbicula* biomass, as CH₄ concentration changes between blocks in the study reach, accounting for repeated measures of day and Rhizon® depth. Repeated measures mixed effects model showed pore water CH₄ concentration increases approximately $316.50 \pm 74.07\%$ between upstream and downstream blocks ($t = 2.574$, $p = 0.011$). Pore water CH₄ concentration increased $2.29 \pm 1.28\%$ g⁻¹ *Corbicula* biomass when accounting for increased pore water CH₄ concentration in the downstream block ($t = 1.774$, $p = 0.078$).

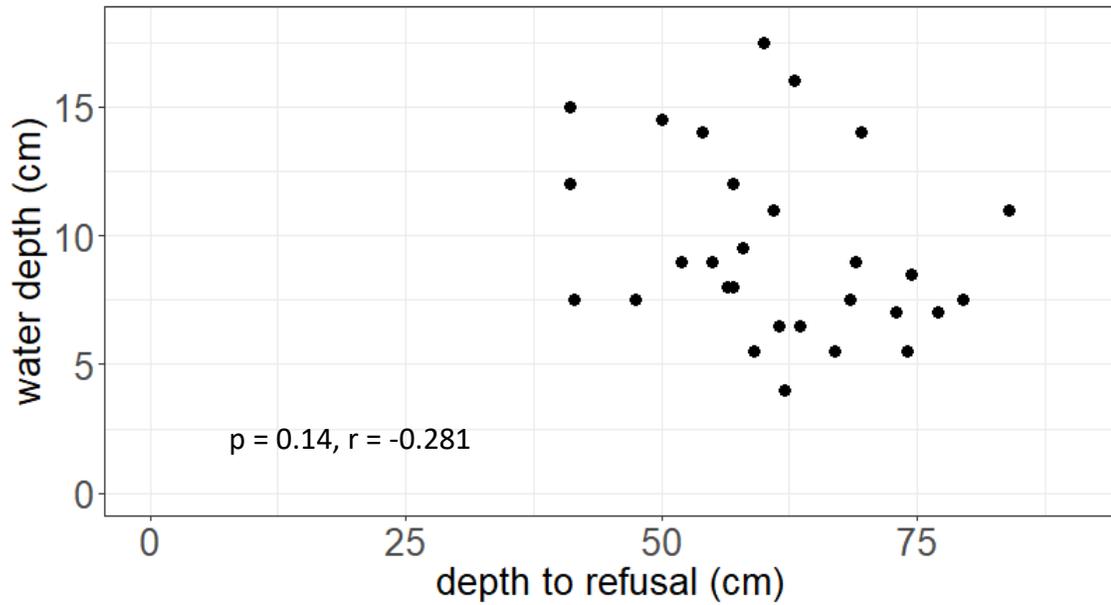


Figure 13. Correlation between Water Depth and Sediment DTR.

There was a slightly negative correlation between water depth and DTR, but this relationship was not significant. Pearson's Correlation $p = 0.14, r = -0.281$.

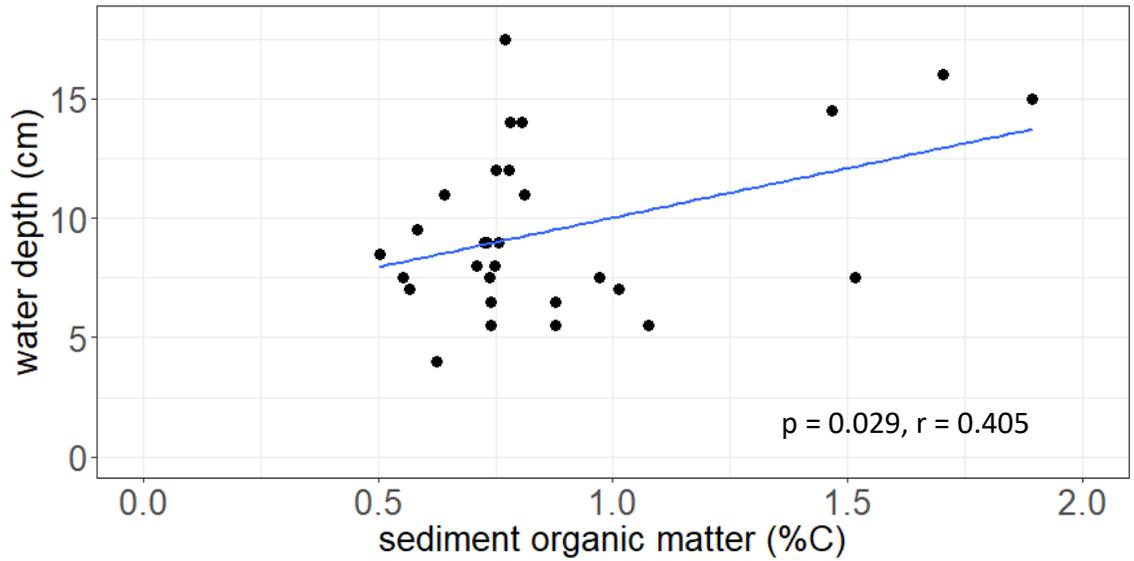


Figure 14. Correlation between Water Depth and Sediment %C.

There was a positive correlation between water depth and sediment %C. Pearson Correlation $p = 0.029$, $r = 0.405$. This correlation appeared to be driven by measurements from the downstream block.

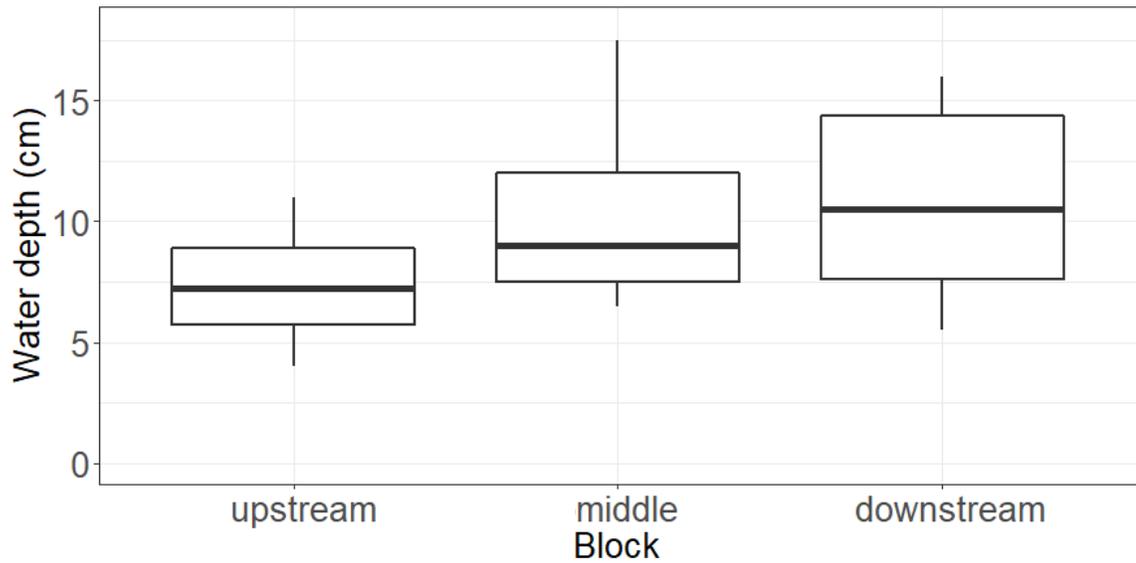


Figure 16. Change in Water Depth between Blocks.

Mean upstream water depth was 7.4 ± 1.04 cm ($t = 7.12$, $p < 0.001$, Table 6). Water depth increased by $38.89 \pm 20.40\%$ in the middle block, but this change was marginally significant ($t = 1.906$, $p = 0.068$, Table 6). Water was $47.30 \pm 19.86\%$ deeper downstream than upstream ($t = 2.381$, $p = 0.025$, Table 6).

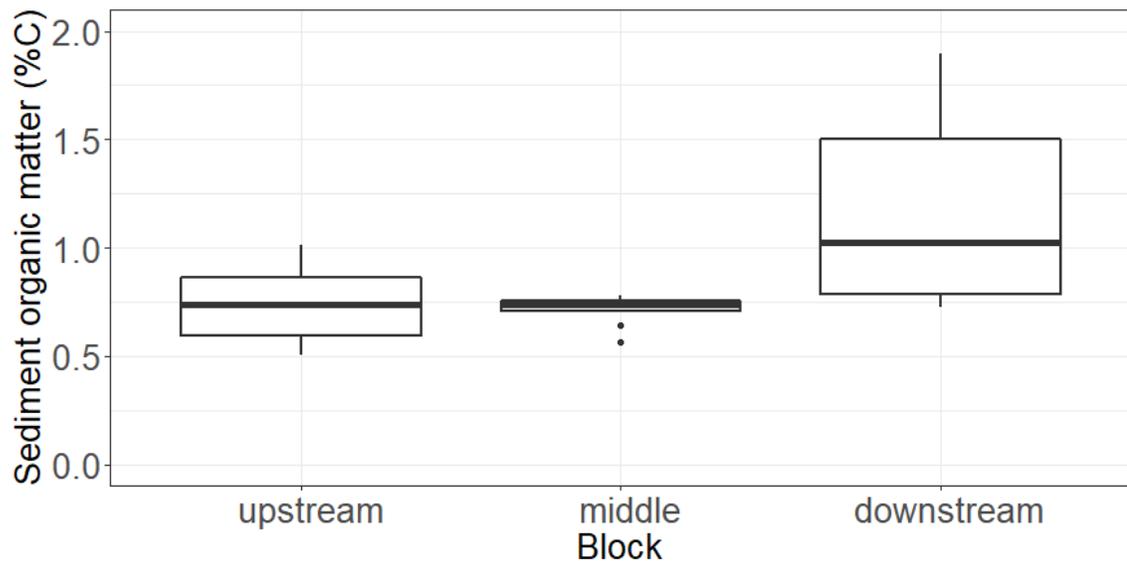


Figure 17. Change in Sediment %C between Blocks.

Sediment organic matter varies between blocks. Mean sediment %C was $0.73 \pm 0.05\%$ in the upstream block ($t = 14.01$, $p < 0.001$, Table 6). %C in the middle block was not significantly different ($t = -0.253$, $p = 0.802$, Table 6) but % C in the downstream block was $59.80 \pm 20.24\%$ higher than upstream ($t = 2.955$, $p = 0.007$, Table 6).

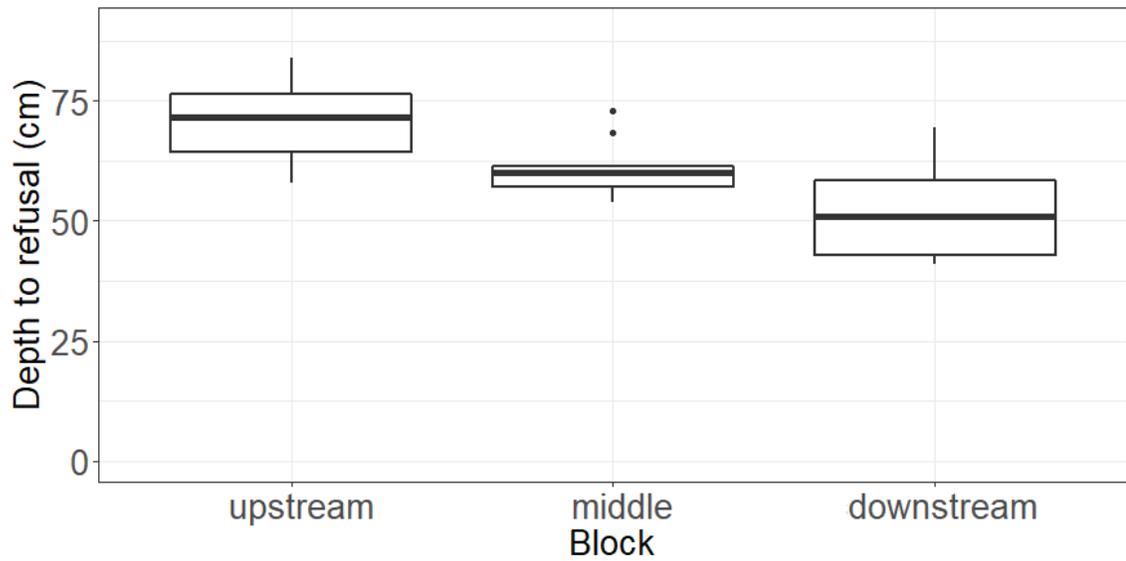


Figure 18. Change in DTR between Blocks.

Upstream DTR was 70.90 ± 2.60 cm ($t = 26.81$, $p < 0.001$, Table 6). DTR decreased by $14.22 \pm 5.41\%$ in the middle block ($t = -2.623$, $p = 0.014$, Table 6) and by $26.46 \pm 5.28\%$ in the downstream block ($t = -5.017$, $p < 0.001$, Table 6).

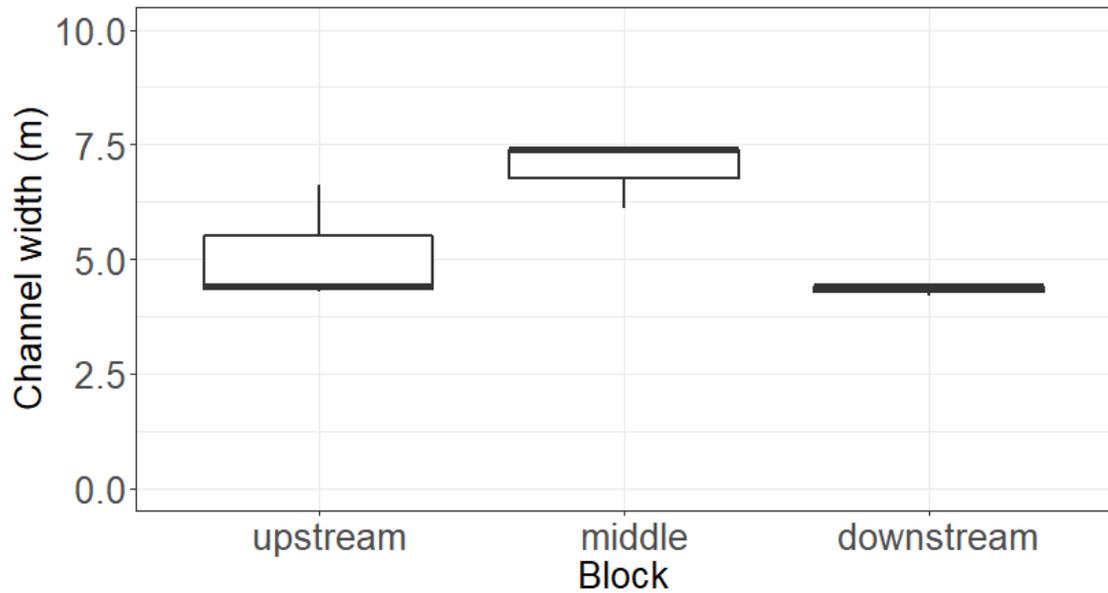


Figure 19. Change in Active Channel Width between Blocks.

Active channel width was 5.10 ± 0.75 m in the downstream block ($t = 6.795$, $p < 0.001$, Table 6). There was a marginally significant increase in active channel width increased $36.6 \pm 17.0\%$ in the middle block compared to upstream ($t = 2.154$, $p = 0.075$, Table 6). There was no significant difference in active channel width between upstream and downstream blocks ($t = -1.017$, $p = 0.322$).