Velocity-amplified microbial respiration rates in the lower Amazon River

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Scientific Significance Statement

Most measurements of aquatic respiration in large rivers ignore the influence of river flow on reaction rates, which we hypothesize has resulted in an underestimation of the contribution of microbial respiration to CO2 outgassing in large tropical rivers. Here, we evaluate microbial respiration rates along the lower Amazon River under different simulated flow conditions using rotating incubation chambers. We demonstrated that river velocity and hydrodynamic conditions are key factors controlling microbial metabolism of river-borne organic matter, and that microbial respiration can potentially exceed CO2 outgassing rates in the Amazon River mainstem and be balanced by primary production, outgassing, and other inputs.

Abstract

Most measurements of respiration rates in large tropical rivers do not account for the influence of river flow conditions on microbial activity. We developed a ship-board spinning incubation system for measuring O2 drawdown under different rotation velocities and deployed the system along the lower Amazon River during four hydrologic periods. Average respiration rates in incubation chambers rotated at 0.22 m s\(^{-1}\) and 0.66 m s\(^{-1}\) were 1.4 and 2.4 times higher than stationary chambers, respectively. On average, depth-integrated
respiration rates in chambers spun at 0.22 m s$^{-1}$ and 0.66 m s$^{-1}$ accounted for 64% ± 22% and 104% ± 36% of CO$_2$ outgassing rates, respectively, in mainstem sites. Continuous measurements of in situ pCO$_2$ were also made along with cross-channel profiles of river velocity. A positive correlation between river velocity and pCO$_2$ was observed along the lower river ($r^2 = 0.67$–0.96) and throughout a tidal cycle.

The complex consortium of organic substrates and microorganisms in inland aquatic ecosystems yield high rates of biological respiration relative to marine systems (del Giorgio and Williams 2005). As such, inland waters serve as bioreactors that efficiently transform organic substrates derived from terrestrial and aquatic primary production into carbon dioxide (CO$_2$), fueling CO$_2$ evasion from the world’s rivers and lakes along with lateral inputs (Tranvik et al. 2009; Ward et al. 2017). In large tropical river systems, which, along with their floodplains, contribute disproportionately to global inland water CO$_2$ emissions (Aufdenkampe et al. 2011; Raymond et al. 2013), heterotrophic respiration is often considered to be the primary mechanism for aquatic CO$_2$ super saturation in the river mainstem (Mayorga et al. 2005; Ward et al. 2013). However, in the few circumstances that CO$_2$ outgassing and respiration rates were measured simultaneously in the world’s largest river, the Amazon River, depth-integrated respiration rates could only account for 29% ± 32% of CO$_2$ outgassing in the mainstem and less than 1% in some tributaries (Ellis et al. 2012).

The mismatch between respiration and CO$_2$ outgassing rates is typically attributed to a combination of influence from other CO$_2$ sources to the river such as floodplains (Abril et al. 2014; Melack 2016), the soil–water interface (McClain et al. 2003), and small streams (Johnson et al. 2008). While not discounting these inputs, we hypothesize that incubation-based respiration rates in the Amazon River mainstem have been underestimated.

Resolving the contribution of organic matter (OM) decomposition to regional carbon balances is essential for predicting the response of aquatic systems to future change and requires identification of the biases associated with analytical protocols. Aquatic respiration rates in the Amazon River have been determined by measuring O$_2$ drawdown during dark incubations in small bottles that are shaken periodically (Benner et al. 1995; Ellis et al. 2012; Ward et al. 2013). In less remote systems, the state of the art for modeling ecosystem respiration is based on measurements of in situ conditions such as the concentration and stable isotopic composition of dissolved O$_2$ (Hotchkiss and Hall 2014) or site-specific pCO$_2$ and net ecosystem productivity (Marcarelli et al. 2011; Hotchkiss et al. 2015). However, these techniques are not logistically feasible in the Amazon River, where current velocities can exceed 1.8 m s$^{-1}$, depths can reach nearly 100 m (average cross channel depths ranged from 15.4 m to 51.5 m in this study; Table 1), bank erosion can dislodge trees that one might attach instrumentation to, and an active shipping channel prevents mooring placement.

For this reason, we developed a ship-board rotating incubation system (Supporting Information Fig. S1) that allows samples to be disturbed at variable speeds, which we hypothesize reflects the mixing conditions (i.e., ability to maintain particles in suspension) associated with different river flow velocities based on insight from fluid dynamics experiments (e.g., Ivanova et al. 2004). This system provides data in real-time allowing for adaptability in the field and observations of temporally variable O$_2$ drawdown rates throughout the incubation. Respiration experiments were performed using traditional methods (Benner et al. 1995) and this new system along the lower Amazon River (Fig. 1). CO$_2$ outgassing rates were simultaneously measured in floating chambers (Sawakuchi et al. 2017) to evaluate the contribution of depth-integrated respiration to CO$_2$ evasion. While there are uncertainties associated with floating chambers (Kremer et al. 2003; Matthews et al. 2003), we have chosen a similar approach used by Ellis et al. (2012) for consistency, and when properly designed and managed, floating chambers are a cost-effective way of obtaining site-specific gas transfer velocities for specific environments, as opposed to using models developed for other systems (Cole et al. 2010; Gálfalk et al. 2013; Lorke et al. 2015). pCO$_2$ was also measured throughout the sampling campaign and river velocity was measured across each sampling station using an acoustic Doppler current profiler (ADCP). We hypothesize that respiration rates respond to flow regime in relation to the extent of particle suspension, which results in a positive relationship between pCO$_2$ and river velocity.

**Methods**

Four expeditions were performed along the lower Amazon River from April 2014 to March 2016 during low, rising, high, and falling river discharge periods (Fig. 1). The study domain spanned from Óbidos to the last two well-constrained channels near Macapá (Fig. 1). The region from Óbidos to the river mouth encompasses roughly 13% of the basin’s total surface area of $5.83 \times 10^6$ km$^2$. This region is characterized by expansive floodplains, covering about 17% of the lower Amazon basin (Hess et al. 2015), and a main river channel that becomes wider and slower toward the mouth, covering ~1.3% of the lower basin’s surface area (Sawakuchi et al. 2017). The effects of tides dampen river flow even upstream of Almeirim and reverse the river’s flow near the mouth during peak rising tide (Ward et al. 2015). The clear waters of the Tapajós and Xingu Rivers downstream of Óbidos are in contrast to the turbid
Table 1. River depth, velocity, discharge, Reynolds’s number (Re), CO₂ outgassing rates (Sawakuchi et al. 2017), and depth-integrated respiration rates measured in BOD bottles, in stationary incubation chambers (R_{STAT}), and chambers spinning at 0.22 m s⁻¹ (R_{SP}) and 0.66 m s⁻¹ (R_{SP}):

![Table content](https://example.com/table1)

*Respiration rates were determined based on a CO₂ production to O₂ drawdown ratio of 0.32 ± 0.08 for Amazon River mainstem sites and 0.46 ± 0.13 for the Tapajos and Xingu Rivers based on stable isotope probing experiments performed during the same study period (Ward et al. 2016).

--- Data required for calculations not available.

Amazon River waters and allow for high levels of primary production relative to the mainstem (Wissmar et al. 1981; Moreira-Turcq et al. 2003; Ward et al. 2016).

Dark incubation experiments were performed to determine the rate of biological oxygen consumption using three different techniques: (1) triplicate 60 mL biological oxygen
demand (BOD) bottles with O$_2$ measured via Winkler titration (Benner et al. 1995; Ellis et al. 2012), (2) triplicate 60 mL BOD bottles with O$_2$ measured with a YSI ProODO optical probe, and (3) duplicate 2.85 L incubation chambers (48 cm height, 34 cm circumference) interfaced to YSI Exo 2 sonde optical dissolved O$_2$ probes (Supporting Information Figs. S1, S2).

For the 60 mL BOD incubations, dissolved O$_2$ was measured before and after a $\sim$ 24 h period. O$_2$ drawdown was assumed to be linear. Reported respiration rates represent the average measurement of the titration and optical probe methodologies and variability between the two methods were generally less than between replicates. In the 2.85 L chambers, dissolved O$_2$ measurements were logged on a 10–30 s interval throughout the incubation (Fig. 2). Chambers were affixed to Marconi spin plates and rotated at the minimum and maximum available speeds (20 rpm and 60 rpm, respectively), and an additional chamber was held stationary. The spin plate consists of a horizontally positioned central rotating axis, with attachment points for up to two of our custom chambers and one sonde that rotate around the axis (Supporting Information Fig. S1). O$_2$ sensors inserted inside the chambers prior to filling were attached to the sonde via custom cables. Blank tests were performed with the chambers using Milli-Q water, showing minimal variability (i.e., $\leq$ 1%) in dissolved O$_2$ concentrations (Supporting Information Fig. S3).

Chambers were filled with water from the center of each channel at 50% river depth using a Shurflo submersible pump typically during late morning hours. Fifty percentage depth was chosen as it reflects the average sediment distribution across the river’s depth profile (Filizola and Guyot 2004). Chambers were overfilled for at least three times their volume without bubbling. Chambers were wrapped in reflective tape to exclude light and kept in the shade to maintain temperatures equivalent to river temperature (river and incubation chamber temperatures were within $\sim$ 1°C). Sixty milliliter BOD bottles were filled with water from 50% depth and the surface in triplicate at three stations located across the channel (left and right margins and center) during late morning to early afternoon time periods. BOD bottles were kept in the dark in foil lined Styrofoam coolers in the

Fig. 1. Average CO$_2$ outgassing fluxes along the lower Amazon River (Sawakuchi et al. 2017) compared to depth-integrated respiration rates measured in stationary BOD bottles, incubation chambers spinning at 0.22 m s$^{-1}$ and 0.66 m s$^{-1}$, and calculated velocity-normalized respiration rates based on measured river velocity and the correlation between chamber spin rate and observed respiration rates (g C m$^{-2}$ d$^{-1}$).
Shade. BOD bottles were combusted before use and the chambers were acid-washed, rinsed with deionized water (DI) water, and sample rinsed.

Bacterial growth efficiency, or the amount of biomass produced relative to OM assimilated, ranges from below 0.05 to 0.6 in natural aquatic ecosystems (del Giorgio and Cole 1998), but rates reported for the Amazon River to date assume that all O2 consumed is converted to CO2. Previous experiments showed that 32.0% ± 7.6% and 46.4% ± 13.4% of 13C-labeled vanillin and 13C-labeled plant leachates were converted to CO2 in the lower Amazon River mainstem and lowland tributaries, respectively (Ward et al. 2016). We used these values for calculation of the molar conversion of O2 drawdown to CO2 production under the assumption that the amount of labeled substrate not converted to CO2 was assimilated as biomass or recycled.

pCO2 was measured during one of our discharge surveys across the mouth throughout a tidal cycle using a headspace equilibration chamber interfaced to a Licor 820 Infrared gas analyzer (Frankignoulle et al. 2001). River velocity, average depth, and discharge were measured across the channel at all sampling locations using a Sontek River Surveyor M9 Portable nine-beam 3.0 MHz/1.0 MHz/0.5 MHz ADCP. Cross-channel ADCP transects were performed at 1–2 h intervals for ~3 h in channels affected by tidal variation. CO2 outgassing rates were measured using floating chambers as reported by Sawakuchi et al. (2017). Statistical significance was tested using unpaired t-tests with a 95% confidence interval.

**Results and discussion**

**Linkage between river velocity and respiration**

Evidence from rotating incubation experiments suggests that river respiration rates are dependent on the extent of particle suspension, which was controlled by rotation speed in the case of our experiments. In situ particle suspension is achieved when current velocity is equal to or greater than a particle’s settling velocity in still water (Rubey 1933). In all experiments performed there was a linear drawdown of O2 in the chambers, which was more temporally variable in the stationary chamber, and the rate of drawdown was positively correlated to the spin rate ($r^2 = 0.76–1.00$; Fig. 2; Supporting Information Table S1). Average respiration rates across all sites measured in the chambers rotated at 0.66 m s$^{-1}$ ($R_{3x}$) and chambers rotated at 0.22 m s$^{-1}$ ($R_{1x}$) were 2.4 and 1.4 times faster than in the stationary chambers ($R_{STAT}$) (Table 1).

We hypothesize that this relationship between rotational velocity and respiration rates exists because of the importance of interactions between suspended particles, dissolved constituents, and free-living and particle-bound microbes in driving aquatic metabolism. Particles are important receptors for microbial exoenzymes (Catalán et al. 2015) that enable processes such as priming effects to work effectively in aquatic environments (Ward et al. 2016). Further, the microbes associated with particles are significantly more metabolically active than free-living microbes along the lower Amazon River (Satinsky et al. 2015). The physiology and biological oxygen demand of *Escherichia coli* cells are directly dependent on small scale fluid motion, whereas stagnant fluids result in low activity (Coleman et al. 2003; Al-Homoud et al. 2007).

Bottle effects are another issue leading to underestimations of respiration rates determined by 60 mL BOD incubations ($R_{BOD}$). $R_{BOD}$ was 2.7, 3.7, and 6.5 times slower than $R_{STAT}$, $R_{1x}$, and $R_{3x}$, respectively (Table 1). Although there is no direct evidence, this difference could perhaps be due to the development of anoxic micro-environments surrounding settled particles that limit microbial activity as opposed to a larger matrix volume with more heterogeneous conditions even when held stationary. Similar observations have been made with respect to primary production measurements in the ocean. For example, primary production rates were an
order of magnitude higher in 4 L compared to 30 mL bottles, which was attributed to enhanced algal mortality and a shift in the balance between production and carbon consumption (Gieskes et al. 1979).

The critical methodological issues resulting in respiration rate underestimations have been described above. The next challenge, which our experiments cannot fully address, is how to estimate actual in situ respiration rates based on the river velocity, especially considering the wide range of velocities observed along the lower river throughout seasons, tidal cycles, and within tributaries. For example, river velocity integrated across the channel and with depth ranged from 0.03 m s$^{-1}$ at low water in the Xingu River to 1.76 m s$^{-1}$ at Óbidos (Table 1). Velocity was highest at Óbidos, decreasing from an average of $1.36 \pm 0.41$ m s$^{-1}$ to $0.64 \pm 0.09$ m s$^{-1}$ across the mouth. An approach would be to normalize respiration rates in our chambers to in situ velocities using the observed linear correlation between chamber rotation velocity and respiration rates. However, this is not a straightforward calculation considering we do not fully understand how the fluid dynamics (and associated particle motion) within our chambers relate to turbulence and flow structure in the river itself. While ADCP measurements can begin to reconcile flow velocity structure, there is currently no robust theoretical basis for describing turbulent mixing in nature, and we rely on semi-empirical lab investigations (Gualtieri 2010). Given these logistical realities, our key point is that the main factor likely driving our observations of rotation-dependent respiration rates is that the particles in the fluid are in motion and suspended, regardless of how the motions are generated.

For a rough evaluation of whether flow is laminar or turbulent in our chambers and the river, which results in particle motion and mixing, we did a calculation of Reynolds’s numbers (Re) based on equations in the literature for rotational Re for the chambers and straight-flowing, uniformly round channel Re for the river, each factoring for velocity (rotational velocity in the case of chambers), radius of the system, kinematic viscosity, and water density (Reynolds 1883; Holman 2002; Childs 2010). Rotational Re within our chambers ranged from $1.4 \times 10^4$ to $4.2 \times 10^4$. Considering turbulent flow in rotating cylinders begins at Re values from 40 to 60 (Childs 2010), our chambers experienced turbulent flow at all rotation rates, and increasing the rotational velocity resulted in an equivalent increase in the dominance of inertial vs. viscous forces, inducing greater mixing at higher Re values as observed in other types of rotating cylinders (Sanders et al. 1981; Reich and Beer 1989; Bluemink et al. 2005).

Re ranged from $1.1 \times 10^6$ for the Tapajós River during low discharge to $2.7 \times 10^8$ at Óbidos during high discharge (Table 1). These Re values are well above the critical Re value for transition from laminar to turbulent flow in a straight flowing pipe (Re = 1000-2000) (Reynolds 1883; Holman 2002). While we did not evaluate in situ mixing rates, the extent to which river water and its dissolved and particulate load mixes is known to be directly linked to Re and the associated velocity and channel geometry (Bouchez et al. 2009; Gualtieri 2010; Potter et al. 2016). Further work is needed to quantify the relationship between river turbulence, velocity, and respiration to constrain spatiotemporal dynamics across the hydrodynamically complex lower river.

Carbon dioxide and river velocity linkages

Considering that dissolved CO$_2$ isotopic signatures in the lowland regions of the Amazon basin show little carbonate weathering-derived CO$_2$ entering from the surrounding landscape into the lower river, the concentration of dissolved CO$_2$ in the lower Amazon River mainstem is a dynamic balance between inputs from respiration of allochthonous and autochthonous OM (Mayorga et al. 2005), floodplains (Mellack et al. 2009; Abril et al. 2014), and sediments (Devol et al. 1987) and outputs from gas evasion (Richey et al. 2002; Sawakuchi et al. 2017) and in situ primary production (Engle et al. 2008; Silva et al. 2009; Gagne-Maynard et al. 2017). CO$_2$ concentrations were lowest in the tributaries, with an average of $1470 \pm 967$ ppm compared to $3111 \pm 1487$ ppm in the upstream lower Amazon River mainstem sites (i.e., from Óbidos to Almeirim) and $2247 \pm 1006$ ppm across the mouth (Fig. 3).

pCO$_2$ in the mainstem consistently decreased downstream of Óbidos during each hydrologic period indicating that CO$_2$ losses occur more rapidly than the inputs along the lower river. River velocities also decreased downstream of Óbidos as the river channel(s) widen and disperse the river’s discharge over a larger area (Table 1). An additional effect of the width and orientation of the lower Amazon River is increased gas transfer velocities due to a longer fetch and higher winds compared to further upstream, which maintains high outgassing rates even as pCO$_2$ decreases (Sawakuchi et al. 2017). Although it could be coincidental, a positive linear relationship was observed between pCO$_2$ and river velocity during each sampling period (Fig. 3A). Although there is no direct evidence, it is possible that the downstream decrease in pCO$_2$ is a result of decreasing respiration rates as the river slows down and outgassing rates remains high.

Similar observations were made during a series of transects performed throughout a tidal cycle across the river mouth (Fig. 3B). pCO$_2$ had a distinct cross channel pattern, with higher pCO$_2$ observed in a deep trough with faster water movement near the right margin (Fig. 3C). The magnitude of the maximum and minimum values observed across the river profile during each transect was linked to river velocity. The highest CO$_2$ concentrations were observed during ebb tide, reaching a maximum of 1642 ppm (Fig. 3). The right margin pCO$_2$ peak was 29% lower during the slowest river velocity transect (1174 ppm). Likewise, the minimum
pCO₂ observed during these two transects was 24% lower for the same low velocity transect (995 ppm) relative to the high velocity transect (1314 ppm). The average river velocity was 0.80 m s⁻¹ and 0.24 m s⁻¹ (e.g., negative value indicates river was flowing in reverse) during the maximum and minimum velocity transects, respectively, with the velocity being ~70% lower for the low velocity transect. For comparison, R₁ₓ was 52% lower than R₃ₓ during the same rising period in the North Channel of Macapá as a result of a 67% decrease in rotation velocity (Table 1).

The consistent variability in pCO₂ throughout the tidal cycle is compelling evidence for the reliance of CO₂ production rates on river velocity over short time scales that should be further examined. Outgassing rates should also theoretically be greatest during periods of high river velocity when surface conditions are more turbulent and gas transfer velocities are greater (Raymond and Cole 2001; Alin et al. 2011). Likewise, enzyme kinetics likely play an important role in shaping this hydrodynamic-biological linkage, but it is unclear how these kinetics respond within the chambers vs. in situ.

**Contributions of respiration to river channel CO₂ outgassing**

The amount of CO₂ produced by depth-integrated respiration was calculated across each channel using average depths measured during ADCP transects (Fig. 1; Table 1). R_BOD accounted for 12% ± 7% of average CO₂ outgassing rates in the lower Amazon River mainstem, from Óbidos to Macapá, and 58% ± 54% of CO₂ outgassing rates in the clearwater tributaries similar to past studies (Ellis et al. 2012). R₁ₓ and R₃ₓ accounted for 64% ± 22% and 104% ± 36%, of CO₂ outgassing rates, respectively, in the lower Amazon River mainstem and mouth sites compared to 295% ± 127% and 528% ± 428%, respectively, for the clearwater tributaries (Fig. 1). R₃ₓ respiration rates essentially balanced CO₂ outgassing rates measured in the mainstem and mouth sites, which is, perhaps, not surprising considering the average mainstem river velocity (0.98 ± 0.42 m s⁻¹) was comparable to the 0.67 m s⁻¹ rotation rate (bearing in mind the fluid dynamics caveats previously described). Although it is often assumed that primary production is minimal in the turbid mainstem, evidence from dissolved oxygen isotopes suggests that primary production occurs at roughly 25–50% the rate of respiration both upstream (Quay et al. 1995) and within our study domain (Gagne-Maynard et al. 2017). If these inferred photosynthetic rates are accurate, this could suggest that actual in situ respiration rates are higher than our R₃ₓ rates due to higher river velocities than experienced in our chambers. Likewise, our floating chamber estimates of CO₂ outgassing do not capture all effects of the complex hydrodynamics on gas transfer.

Seasonally, respiration rates in the Amazon River mainstem were highest relative to CO₂ outgassing during rising water (Table 1). For example, during rising water, R₁ₓ accounted for 149% ± 57% of CO₂ outgassing rates on average at the upstream Amazon River sites compared to 46% ± 15% at high water, 96% ± 91% during falling water, and 64% ± 15% at low water. The abundance of reactive OM during rising water in the
Amazon River (Ward et al. 2013, 2015, 2016) likely drives rates of respiration that exceed CO2 outgassing as observed here, resulting in an accumulation of CO2 in the water column. CO2 concentrations peak at high water (Fig. 3) and begin to decline as CO2 is outgassed more rapidly than respiration produces CO2.

There has been a fundamental gap in our ability to quantify the processes that drive aquatic CO2 production in large tropical rivers due to methodological bias. Here, we demonstrate that river velocity and hydrodynamic conditions are key physical factors controlling microbial metabolism of river-borne OM that has not been appropriately considered either conceptually or quantitatively. We have demonstrated that microbial respiration can potentially exceed CO2 outgassing rates in the Amazon River mainstem and be balanced by primary production, outgassing, and other inputs. The apparent linkage between river velocity and respiration may have considerable implications when considering the impact of human activities that alter river flow such as damming and leveeing (Maavara et al. 2017). For example, alterations to inland water residence times by future impoundments and changes to the hydrologic cycle are expected to reduce the overall efficiency of OM breakdown in inland waters (Catalán et al. 2016). It remains unclear how this hydrodynamic-biogeochemical linkage will alter spatial distributions of carbon and energy balances along the land to sea continuum and higher trophic levels.

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