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THE ECOLOGY OF BACTERIAL COMMUNITIES IN AMAZONIAN FLOODPLAIN LAKES

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THE ECOLOGY OF BACTERIAL COMMUNITIES IN AMAZONIAN FLOODPLAIN LAKES

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Orientador: Prof. Dr. Hugo Sarmento

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Prof. Dr. Hugo Miguel Preto de Morais Sarmento

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“The most dangerous worldviews are the worldviews

of those who have never viewed the world.”

Alexander von Humbold

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AD - average degree

AFL - Amazonian floodplain lake

APL - average path length

ATTZ - aquatic-terrestrial transition zones

B - betweenness

ba - drainage basin

BCC - bacterial community composition

BP - bacterial production

C - carbono/carbon

C - closeness centrality

CAP - Canonical Analysis of Principal Coordinates

CC - clustering coefficient

CCB - composição da comunidade bacteriana

CCorA - Canonical Correlation Analysis

CDOM - collored dissolved organic matter

chl-*a* - chlorophyll-a

CTAB - cetyl trimethyl ammonium bromide

D - degree

dbRDA - redundancy analysis based on Bray-Curtis dissimilarity measure PERMANOVA - permutational multivariate analysis of variance

DO - dissolved oxygen

DOC - dissolved organic carbon

DOM - dissolved organic matter

EEM - excitation-emission matrix

ERW - early rising water

FDOM - fluorescence dissolved organic matter

FDR - false discovery rate

FI - fluorescence index

FL - free-living

FOAM - Functional Ontology Assignments for Metagenomes database

Fresh - freshness index

FW - falling water

HIX - humification index

HW - high water

LRW - late rising water

LW - low water

M - modularity

ma - macrophyte banks

MAGs - metagenome-assembled genomes

MEBS - Multigenomic Entropy Based score

MOD - matéria orgânica dissolvida

ND - network diameter

NMDS - non-metric multidimensional scaling

op - open lake

OTU - operational taxonomic unit

PA - particle-attached

PARAFAC - parallel factor analysis

PCoA - Principal Coordinate Analysis

PCR - polymerase chain reactions

RL - river-lake transition zone

sol - Solimões River

SparCC - sparse correlations for compositional data

S_R - slope ratio

$SUVA_{254}$ - specific ultraviolet absorbance at 254 nm

TCA - tricarboxylic acid

TR - aquatic-terrestrial transition zone

TSS - total suspended solids

WRT - water residence time

SUMÁRIO

RESUMO

O bacterioplâncton desempenha papel fundamental no funcionamento de ecossistemas aquáticos e nos ciclos biogeoquímicos. Apesar do crescente interesse em se estudar a ecologia desses microrganismos, pouco se sabe sobre a estrutura de comunidades bacterianas e sobre os fatores que regulam sua composição e atividade, especialmente em redes hidrológicas altamente dinâmicas como as várzeas amazônicas. Utilizando análises ópticas, realizamos uma caracterização das fontes, quantidade e qualidade da matéria orgânica dissolvida (MOD), o principal substrato energético para o bacterioplâncton. Aplicando técnicas de sequenciamento de alto rendimento, nós também caracterizamos a composição de comunidades bacterianas (CCB) em diferentes habitats e estações do ciclo hidrológico anual (pulso de inundação) em lagos de várzea amazônicos (Janauacá e Curuaí) e investigamos o efeito de processos locais e regionais na estruturação dessas comunidades. Nossos resultados revelaram um forte padrão sazonal nas condições ambientais, CCB e MOD. A conexão e troca lateral entre o rio principal e suas várzeas foram um importante mecanismo modulador da CCB através dos processos de dispersão. Além disso, as mudanças sazonais nas condições ambientais do lago foram determinantes para o estabelecimento dessas bactérias dispersas. Em relação à MOD, encontramos acoplamento com a CCB e destacamos o importante papel de uma pequena fração de MOD lábil autóctone e sua rápida remineralização pelo bacterioplâncton como um processo importante que mantém a quantidade de MOD relativa baixa e estável ao longo do ano. Adicionalmente, vimos que as redes de interações entre bactérias apresentaram uma estrutura organizada e que haviam espécies-chave presentes em ambos os lagos de várzea. Nós desenvolvemos uma métrica baseada nas redes de interação (*K-value*) para identificar essas espécies de bactérias, as quais desempenham papel crucial na manutenção da estrutura e diversidade de suas comunidades ecológicas e no funcionamento de seus ecossistemas. Este foi o primeiro estudo aprofundado sobre a CCB na maior bacia fluvial do mundo, e demonstrou que o pulso de inundação é uma importante força motriz de diversos fatores locais e regionais, os quais regulam a estrutura e composição de comunidades microbianas, o que gera implicações para os balanços e ciclos de carbono regionais.

Palavras-chave: bacterioplâncton, composição da comunidade bacteriana, matéria

orgânica dissolvida, várzeas amazônicas, pulso de inundação

ABSTRACT

The bacterioplankton plays a key role in the functioning of aquatic ecosystems and biogeochemical cycles. Despite of the growing interest in studying the ecology of such microorganisms, little is known about how bacterial communities are assembly and which factors regulate their composition and activity, especially in highly dynamic hydrological networks such as the Amazonian floodplains. Using optical properties within dissolved organic matter (DOM), we performed a characterization of the source, quantity and composition of DOM, the main energetic substrate for bacterioplankton. Applying high-throughput sequencing techniques, we also characterized the bacterial community composition (BCC) in different habitats and seasons of the annual hydrological cycle (flood pulse) in Amazonian floodplain lakes (Janauacá and Curuaí), and investigated the role of local filters and regional drivers in shaping these communities. Our results demonstrated a strong seasonal pattern in the environmental conditions, BCC, and DOM. Dispersal processes were important factors in shaping BCC, being affected by the connection and lateral exchange between the main river channel and its floodplains. In addition, the seasonal changes in the lake's environmental conditions were determinant for the successful establishment of dispersing bacteria. We also found a strong coupling between DOM and BCC, highlighting the important role of a small fraction of autochthonous labile DOM and its rapid turnover by bacterioplankton as an important process that keeps the DOM quantity relatively low and stable over the year. Additionally, we observed that bacteria interaction networks had an organized structure and that there were key species present in both floodplain lakes. We developed a network-based score (K-value) to identify these bacterial taxa, which play a crucial role in maintaining the structure and diversity of their ecological communities and in the functioning of their ecosystems. To the best of our knowledge, this was the first in-depth study of a BCC in the world's largest river basin, and demonstrated that the flood pulse modulates several regional and local drivers, which regulate microbial community structure and composition, that could ultimately impact regional carbon budgets and biogeochemical cycles.

Keyword: bacterioplankton, bacterial community composition, dissolved organic matter, Amazonian floodplains, flood pulse

INTRODUÇÃO GERAL

COMUNIDADES BACTERIANAS AQUÁTICAS: ESTRUTURA E FUNÇÃO

Apesar de seu tamanho reduzido, os procariotos compõem uma fração significativa da biomassa planctônica em ecossistemas aquáticos, desempenhando papéis fundamentais no funcionamento desses sistemas, como nos ciclos biogeoquímicos, remineralização de nutrientes, cadeia trófica microbiana e produção primária (COTNER & BIDDANDA, 2002).

A análise da diversidade procariota era um ponto limitante até há alguns anos atrás, já que a caracterização morfológica e as abordagens de cultivo de bactérias são muito restritas e não possibilitam capturar a real biodiversidade dos microrganismos (PACE, 1997). Porém, nos últimos anos, a ecologia microbiana sofreu uma revolução impulsionada pelas tecnologias de sequenciamento de DNA de alto-rendimento (*high-throughput sequencing*, SOGIN et al., 2006). Desde então, estas novas ferramentas estão nos auxiliando à compreender a estrutura, evolução e ecologia do mundo microbiano, e se revelaram extremamente inovadoras na descoberta de novos genes, proteínas, enzimas e outras moléculas bioativas. O uso dessas técnicas aliadas às ferramentas bioinformáticas permitem a caracterização de comunidades microbianas diretamente de amostras ambientais, inclusive de táxons pouco abundantes (microrganismos não-cultiváveis em laboratório - e.g. SUNAGAWA et al., 2015; LOGARES et al., 2012).

Esses avanços na caracterização de comunidades microbianas revelaram por exemplo, que elas são extremamente diversas e que podem ser divididas em dois compartimentos básicos: a biosfera microbiana rara e a abundante. Aos componentes da biosfera abundante estão atribuídas um maior número de funções ecossistêmicas. Esses microrganismos geralmente crescem de forma mais ativa, uma vez que estão mais adaptados às condições locais, e apesar de serem mais facilmente identificados por técnicas moleculares, são raramente cultivados em laboratório (PEDROS-ALIÓ, 2006). Já os componentes da biosfera rara, apesar de serem pouco abundantes, abarcam a maior e mais diversa porção de táxons de um dado ambiente. As funções ecológicas da grande maioria desses microrganismos permanecem obscuras (SHADE et al., 2014). Acredita-se que alguns microrganismos raros estão provavelmente a caminho da extinção local ou são taxóns transitórios (PEDROS-ALIÓ, 2012). Eles podem ser ativos, fornecendo

funções importantes ao ecossistema, ou podem estar dormentes, aguardando condições ambientais favoráveis para o seu crescimento (CAMPBELL et al., 2011; EPSTEIN, 2009).

Esforços têm sido feitos não só na caracterização da composição de comunidades bacterianas (CCB) aquáticas, mas também para entender como essas comunidades variam ao longo do tempo e sob diferentes gradientes ambientais (READ et al., 2014; DOHERTY et al., 2017; COTTRELL et al., 2015). De maneira geral, esses estudos vêm revelando que CCBs são altamente dinâmicas em diferentes escalas temporais e espaciais. Por exemplo, foi demonstrado que há diferenças composicionais aos nível de filo e classe nas comunidades bacterianas entre ecossistemas marinhos e de água doce, com uma maior homogeneidade taxonômica nos oceanos (BARBERÁN & CASAMAYOR, 2010). Mesmo entre ecossistemas de água doce, há uma clara diferença na composição das comunidades bacterianas, como demonstrado para lagos em diferentes zonas climáticas, i.e. temperados, boreais e tropicais (COTTRELL et al., 2015; HUMBERT et al., 2009).

Em geral, as CCBs são estruturadas por quatro processos principais: (I) seleção local (*species sorting*), diferenças de aptidão determinística entre as espécies em relação à variáveis ambientais abióticas e interações bióticas; II) por processos de dispersão (*mass effect*), movimentos de imigração e emigração de organismos em uma região; (III) deriva ecológica (*drift*), mudanças estocásticas na abundância de espécies; III) e especiação (*speciation*), formação de nova espécies em escala de tempo evolutiva (VELLEND, 2010; LEIBOLD et al., 2004). Conjuntamente, esses processos vêm sendo aplicados para investigar os fatores que atuam na estruturação na composição de comunidades microbianas em diversos ecossistemas aquáticos (COMTE et al., 2017; LANGENHEDER & RAGNARSSON, 2007; LANGENHEDER & SZEKELY, 2011; LINDSTROM & LANGENHEDER, 2012; SZEKELY & LAGENHEDER, 2014; ZHA et al., 2016), e vêm revelando que elas são moldadas por uma combinação destes processos, que variam de acordo com as características de cada ecossistema.

Por exemplo, foi demonstrado que a CCB em regiões boreais seguiu uma estrutura espacial unidirecional impulsionada pelo recrutamento de espécies do solo em córregos de cabeceira (dispersão). No ambiente, essas espécies eram filtradas

pelas condições ambientais ao longo do gradiente do rio, como tempo de residência da água e pH, resultando em um diminuição na riqueza taxonômica a jusante (RUIZ-GONZÁLEZ, 2015; 2017; NIÑO-GARCÍA, 2016 – Figura 1). O pH, juntamente com a temperatura e tempo residência, foram fortemente relacionados às variações na distribuição de táxons bacterianos em lagos de regiões temperadas (LINDSTRÖM et al., 2005).

A quantidade e qualidade da matéria orgânica dissolvida (MOD) também foi sugerida, através de estudos experimentais e de campo, como um importante fator regulador da composição e atividade de comunidades microbianas aquáticas (LOGUE et al., 2016; CRUMP et al., 2003; KRITZBERG et al., 2006). Diferenças no peso molecular dos substratos orgânicos tiveram o potencial de selecionar táxons bacterianos específicos, uma vez que compostos de baixo peso molecular podiam ser utilizados por um amplo espectro de bactérias, enquanto que a capacidade de degradar compostos com alto peso molecular, uma função menos amplamente distribuída (LOGUE et al., 2016).

Deve-se considerar também que os microrganismos não estão isolados nos ecossistemas, e formam complexas redes de interações ecológicas que incluem interações positivas, negativas e nulas (FAUST & RAES, 2012). A predação é um exemplo de interação negativa, onde uma parte é beneficiada e outra prejudicada. No universo microbiano ela é mais comumente conhecida como *grazing* e causa efeitos diretos e indiretos na CCB, através da predação seletiva, ou influência da predação via eliminação de competidores (PERNTHALER, 2005). Essas interações entre os microrganismos também são abordadas, por exemplo, pelo Conceito de Co-ocorrência e redes ecológicas (PROUXL et al., 2005), e apesar de terem sido inicialmente introduzidas para macro-organismos, vêm sendo atualmente aplicadas para o estudo de comunidades microbianas aquáticas (impulsionadas pelos avanços de ferramentas moleculares e analíticas - PROUXL et al., 2005; BANERJEE et al., 2018). Essas abordagens permitem um estudo aprofundado de como os microrganismos interagem entre si e como isso afeta a diversidade e o equilíbrio de comunidades ecológicas.

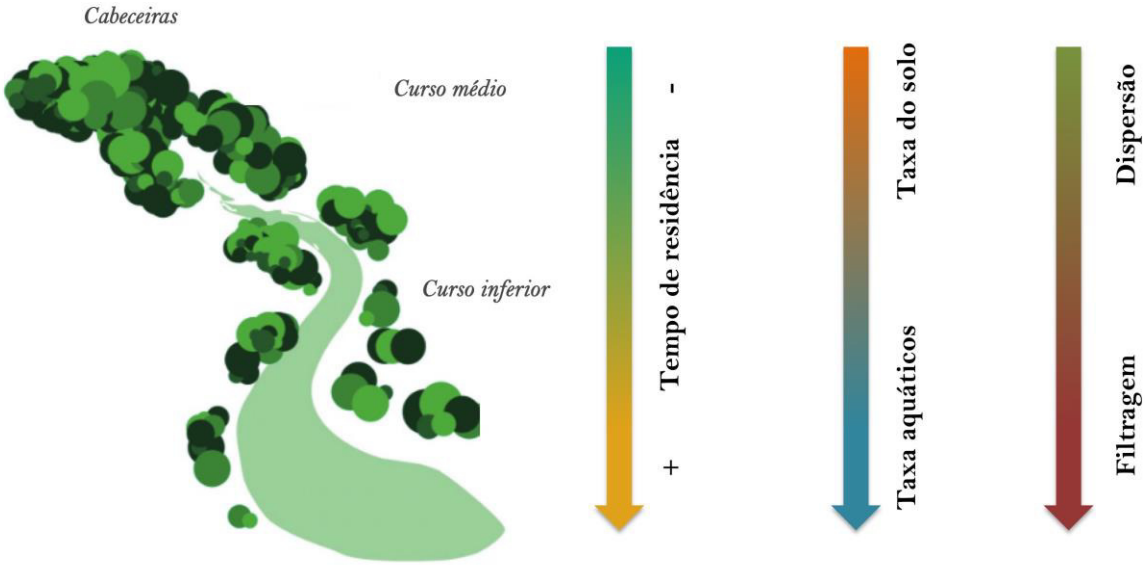

Figura 1: Esquema demonstrando o padrão direcional na estruturação de comunidades microbianas aquáticas em redes hidrológicas boreais

INTERAÇÕES ENTRE COMUNIDADES BACTERIANAS E A MATÉRIA ORGÂNICA DISSOLVIDA

A MOD é a principal forma de C encontrada nos sistemas aquáticos, sendo constituída de uma heterogênea e complexa mistura de substâncias húmicas, ácidos carboxílicos, aminoácidos, entre outros compostos que variam em grau de reatividade (ROIHA et al., 2016; COBLE et al., 2014). Ela desempenha importantes papéis nesses ecossistemas, como na biodisponibilidade de metais, atua no tamponamento do pH, na atenuação da luz que incide a coluna d'água e é o principal substrato energético para as bactérias heterotróficas (CORY & MCKNIGHT, 2005; COBLE et al., 2014; AZAM et al., 1983).

As bactérias heterotróficas consomem grandes quantidades de C presentes na MOD, as quais são degradadas, armazenadas na biomassa através da produção bacteriana e/ou emitidas através da respiração bacteriana (TRANVIK et al., 2009, GUILLEMETTE et al., 2013 – Fig. 2). Elas também são responsáveis por produzirem e excretarem MOD, naturalmente, durante seu crescimento, divisão e morte celular (KAWASAKI & BENNER, 2006). Há evidências de que a disponibilidade e composição da MOD têm o potencial de favorecer determinados táxons bacterianos enquanto molda a composição e o metabolismo coletivo de comunidades bacterianas, ao mesmo tempo que a composição da MOD é modelada pela

atividade de diferentes grupos bacterianos (AMON & BENNER, 1996; KRITZBERG et al., 2006; ROMERA-CASTILLO et al., 2011). Por isso, nos últimos anos, têm sido feitos grandes esforços para elucidar a contribuição dos diferentes grupos microbianos na transformação da MOD de diversas fontes e vice-versa, sendo essas questões especialmente bem exploradas sob condições controladas em experimentos de incubação (ver KIRCHMAN et al., 2004; KRITZBERG et al., 2006; ROMERA-CASTILLO et al., 2011; JUDD et al., 2006; LOGUE et al., 2016). No entanto, há conhecimento limitado sobre a dinâmica das interações em condições naturais em uma ampla variedade de ecossistemas aquáticos (OSTERHOLZ et al., 2016; AMARAL et al., 2016).

Foi demonstrado experimentalmente (KRITZBERG et al., 2006; JUDD et al., 2006; LOGUE et al., 2016) e empiricamente (OSTERHOLZ et al., 2016; AMARAL et al., 2016) que há relação entre a CCB e o processamento da MOD (KRITZBERG et al., 2006; JUDD et al., 2006; LOGUE et al., 2016). A qualidade do substrato orgânico afetou não só a CCB, mas também sua atividade, e.g. respiração e produção de biomassa (KRITZBERG et al., 2006). A CCB também tem efeito na degradação de MOD, pois apesar de terem degradado a mesma quantidade de MOD de origem terrestre, as bactérias diferiram no padrão temporal e em quais compostos foram preferencialmente degradados (LOGUE et al., 2016).

Questões relacionadas à interação MOD-CCB são particularmente relevantes para sistemas tropicais de várzea, uma vez que a alta complexidade da rede hidrológica e forte sazonalidade culminam em uma elevada diversidade de substratos orgânicos, que quando processados afetam os balanços globais de C (REGNIER et al., 2013; BORGES et al., 2015). Em ecossistemas amazônicos, embora o conhecimento dos processos de transformação microbiana da MOD venham sendo estudados de forma pontual (BENNER et al.,1995; VIDAL et al., 2015), poucos são os estudos que abordam a CCB aquáticas (GHAI et al., 2011; SATINSKY et al., 2015) e ainda, a dinâmica de interações entre a estrutura dessas comunidades e da MOD, as quais moldam o funcionamento dos ecossistemas e ciclos biogeoquímicos.

Estudos prévios na bacia amazônica usando técnicas isotópicas e elementares (principalmente realizadas durante o Experimento Carbono no Rio Amazonas - CAMREX) revelaram que insumos derivados de fontes terrestres

(alóctones) dominam o reservatório de MOD (ERTEL et al., 1986; HEDGES et al., 1986, 1994). Em especial às várzeas, a MOD pode ser dividida em dois grupos distintos: um grande reservatório de MOD mais refratário de fontes alóctones, especialmente plantas C3 (C terrestre de terras altas, insumos ribeirinhos, lixiviados de florestas alagadas e solos) e um pequeno reservatório de MOD lábil derivado de macrófitas C4 e fitoplâncton, que é consumido e rapidamente transformado por bactérias heterotróficas (WAICHMAN, 1996; ELLIS et al 2012; AMARAL et al 2018; MORTILLARO et al., 2016; MELACK & FORSBERG, 2001).

Uma das formas de se estudar a MOD é através das suas propriedades ópticas, as quais provêem informações sobre a quantidade, fonte e qualidade das porções quimicamente distintas dentro da MOD reativa à luz (CORY & MCKNIGHT, 2005; CAMMACK et al., 2004; STEDMON et al. 2003). Apesar de não permitir uma caracterização à nível molecular sobre os compostos presentes na MOD ou ainda revelar padrões detalhados na quimiodiversidade, técnicas ópticas fornecem uma impressão digital da MOD e provêm informações quanto a sua labilidade, aromaticidade (absorbância específica – WEISHAAR et al., 2003), peso molecular (*slope ratio*) e quantidade de material húmico e protéico (picos de matrizes de excitação e emissão – STEDMON & BRO, 2008).

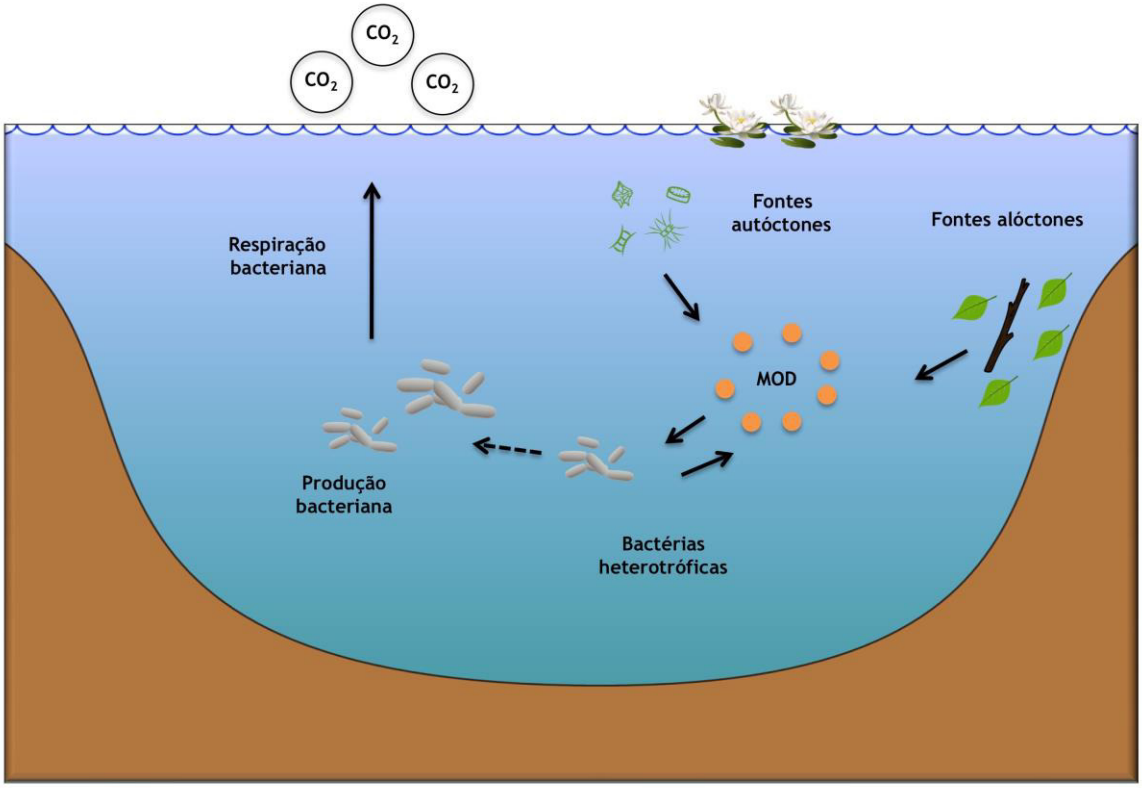

Figura 2: Ilustração esquemática das formas de interação entre a matéria orgânica dissolvida e o bacterioplâncton

ESPÉCIES-CHAVE EM MICROBIOMAS

O conceito de espécie-chave (*keystone species*) foi originalmente proposto por Robert Paine após extensivos estudos experimentais examinando redes alimentares em zonas entremarés de costões rochosos no noroeste do Pacífico (PAINE, 1969). Desde então, esse termo vem sendo utilizado para descrever espécies de animais e plantas que desempenham um papel crucial na manutenção da estrutura e diversidade de suas comunidades ecológicas, afetando sua estabilidade, robustez e resiliência. Porém, ao longo dos anos esse conceito seguiu diferentes linhas de pensamento (COTTEE-JONES & WHITTAKER, 2012) e atualmente não há uma definição operacionalmente aceita em Ecologia, especialmente em Ecologia Microbiana.

Apesar do grande esforço em se estudar esse conceito para diversos grupos animais e vegetais (ESTES & PALMISANO, 1974; NAIMAN et al. 1986; DELIBES- MATEOS et al., 2011), sua aplicação em microbiologia é bem recente (BANERJEE et al., 2018). Uma espécie-chave microbiana foi proposta como sendo aquela altamente conectada, que individualmente ou em uma guilda afeta o equilíbrio de comunidades microbianas, e a sua remoção causa uma grande mudança na estrutura e função de todo o microbioma (BANERJEE et al., 2018).

São diversas as metodologias utilizadas para se identificar espécies-chave, as quais incluem esforços empíricos e ferramentas computacionais (experimentos de adição/remoção, algoritmos, etc). Uma das formas mais utilizadas é através de análises de padrões de co-ocorrência e redes de interação (PROUXL et al., 2005). Apesar de haver discussão sobre os vieses desses métodos (ver RÖTTIERS & FAUST, 2018), métricas de redes têm sido utilizados para identificar possíveis espécies-chaves microbianas em diversos ecossistemas (STEELE et al., 2011; LUPATINI et al., 2014; VICK-MAJORS et al., 2014; ZHAO et al., 2017).

Investigando a CCB e as redes de interação entre bactérias de solos brasileiros (de diferentes biomas e tipos de uso da terra), Lupatini e colaboradores encontraram que membros pertencentes aos filos abundantes Actinobacteria e Proteobacteria eram espécies-chave compartilhadas em uma ampla variedade de solos. Porém, cada solo apresentou espécies-chave particulares, pertencentes aos

filos Chloroflexi, Acidobacteria, Bacteroidetes e Firmicutes (LUPATINI et al., 2014). Para sistemas de água doce, um estudo baseado em padrões de co-ocorrência de microrganismos em lagos polares indicou que as comunidades eram bem estruturadas e apresentavam um padrão modular não-randômico. Além disso, foram identificadas unidades taxonômicas operacionais (OTUs) chave em cada módulo, essas pertenciam à diferentes táxons, e.g. membros do gênero *Pelagibacter*, da família Flavobacteriaceae e da ordem Oceanospirillales (VICK-MAJORS et al., 2014).

Considerando que a atividade de microrganismos afeta o funcionamento dos ecossistemas, estudar as complexas interações entre os microrganismos torna-se indispensável para um melhor entendimento dos processos que regem o funcionamento dos ecossistemas. Uma prática aconselhável e promissora para o campo da Ecologia Microbiana, é a elaboração de novas técnicas para identificação de espécies-chave (e.g. metagenômica e metatranscriptoma) aliadas à validação empírica (BANERJEE et al., 2018). Conjuntamente, essas devem considerar à alta diversidade e complexidade de comunidades microbianas naturais, e o fato de que a maior parte dos microrganismos não são cultiváveis.

O PAPEL DOS SISTEMAS DE VÁRZEA AMAZÔNICOS NO CICLO DO CARBONO

Os sistemas aquáticos continentais apesar de se estenderem por apenas uma pequena fração da superfície terrestre (cerca de 3% de todos os continentes - DOWNING et al., 2006), são compartimentos altamente ativos no ciclo global do carbono (C), atuando na mineralização, transformação e armazenamento de C de origem terrestre, e no seu transporte para os oceanos (COLE et al., 2001; TRANVIK et al., 2009). Do ponto de vista metabólico, esses podem ser classificados em autotróficos (sumidouros), quando a fixação de C através da fotossíntese é dominante, ou heterotróficos (fontes para a atmosfera), quando os processos de oxidação de C (e.g. respiração) são maiores do que a produção primária (DODDS & Cole, 2007).

Os ecossistemas aquáticos continentais mundialmente distribuídos são supersaturados em CO_2 (heterotróficos), independentemente de sua zona climática (SOBEK et al., 2005), apresentando concentrações, em média, três vezes maiores

em relação à atmosfera (COLE et al., 1994). Ambientes aquáticos continentais tropicais são particularmente relevantes nos ciclos biogeoquímicos globais, uma vez que são responsáveis por aproximadamente 60% do total das emissões de C de águas continentais (AUFDENKAMPE et al., 2011). Na bacia Amazônica, a maior bacia fluvial do mundo, as estimativas mostram que o metabolismo aquático é predominantemente heterotrófico, com taxas de emissão de CO_2 comparáveis ao total de emissões globais de córregos e rios (MELACK, 2016; RAYMOND et al., 2013), e cerca de 10 vezes superior à quantidade de C perdida para os oceanos (RICHEY et al., 2002). Isso ocorre devido ao significativo aporte de material orgânico alóctone, ou seja, em razão da decomposição de materiais oriundos de sistemas terrestres adjacentes, os quais entram por escoamento ou lixiviação nos sistemas aquáticos e lá são processados através da decomposição microbiana e foto-oxidação. Além disso, ocorre entrada direta de CO_2 dissolvido na água, produzidos pelas vegetação inundada (ABRIL et al., 2014). Esses materiais complementam as fontes autóctones, representadas por produtores primários - macrófitas e fitoplâncton. A oxidação de ambos os substratos orgânicos (autóctones e alóctones) em CO_2 por bactérias heterotróficas resulta na super-saturação de CO_2 relativo ao equilíbrio atmosférico, comumente observados nos sistemas aquáticos amazônicos (RICHEY et al., 2002; MAYORGA et al., 2005; AMARAL et al., 2018) e representa um importante fluxo de CO_2 no balanço regional de C (WARD et al., 2013).

Em geral, estudos vêm demonstrando que os altos fluxos de CO_2 da coluna d'água para a atmosfera na Amazônia são sustentados pela decomposição de MOD terrestre recentemente produzida (menos de 5 anos), sendo que a maior fração de MOD transportada para os oceanos é antiga, variando de dezenas a milhares de anos de idade (MAYORGA et al., 2005). Nesse contexto, as várzeas possuem papel de destaque, uma vez que produzem grandes quantidades de C fresco pela vegetação inundada e macrófitas flutuantes, que podem ser rapidamente processadas ainda nas várzeas ou transportadas em forma de CO_2 por dezenas e centenas de quilômetros rio abaixo antes de serem emitidas (ABRIL et al., 2014).

É importante se destacar que estimativas de balanço de C são difíceis de serem acessadas na bacia Amazônica devido à alta complexidade da rede hidrológica. Esta inclui um mosaico de habitats aquáticos (florestas inundáveis, áreas de lagos abertos, bancos de macrófitas flutuantes, etc.), periodicamente

inundados pelo transbordamento lateral de rios e pelo regime de chuvas, ambos regulados pelas variações no pulso hidrológico anual (*Flood Pulse Concept* - JUNK et al., 1989). Considerando que grande parte dos estudos biogeoquímicos na Amazônia são incompletos, há atualmente um esforço crescente para se refinar o balanço geral desse elemento com a inclusão das áreas inundadas e de diversas escalas espaciais e temporais (AMARAL et al., 2018; BARBOSA et al., 2016; ABRIL et al., 2014).

OBJETIVOS

Esta pesquisa teve como objetivo geral:

- Descrever a composição de comunidades bacterianas de sistemas de várzea amazônicos em diferentes fases do pulso de inundação e investigar quais são os principais fatores locais (i.e. filtragem ambiental e interação com outros microrganismos) e regionais (dispersão) que regulam sua estrutura.

A tese está estruturada em 3 capítulos (Fig. 3), os quais têm como objetivos específicos:

- Capítulo 1: I) estimar a contribuição relativa de diferentes fontes de dispersão bacterianas para uma mesma comunidade em um lago de várzea amazônico; II) investigar como o pulso de inundação afeta as condições ambientais e como essas mudanças, por sua vez, afetam a composição da comunidade bacteriana (CCB); e III) identificar quais são os principais táxons envolvidos em diferenças na CCB ao longo do ciclo hidrológico.
- Capítulo 2: I) estimar a quantidade, fonte e composição da MOD usando propriedades ópticas (absorbância e fluorescência) em um lago de várzea amazônico; II) avaliar a existência de acoplamento entre a composição da MOD e a CCB ao longo do ciclo hidrológico.
- Capítulo 3: I) avaliar se os padrões de interação entre comunidades microbianas são iguais entre ecossistemas com características semelhantes – sistemas de várzea amazônicos; II) identificar espécies-chave nesses sistemas; e III) investigar em quais funções ecológicas

elas estão envolvidas.

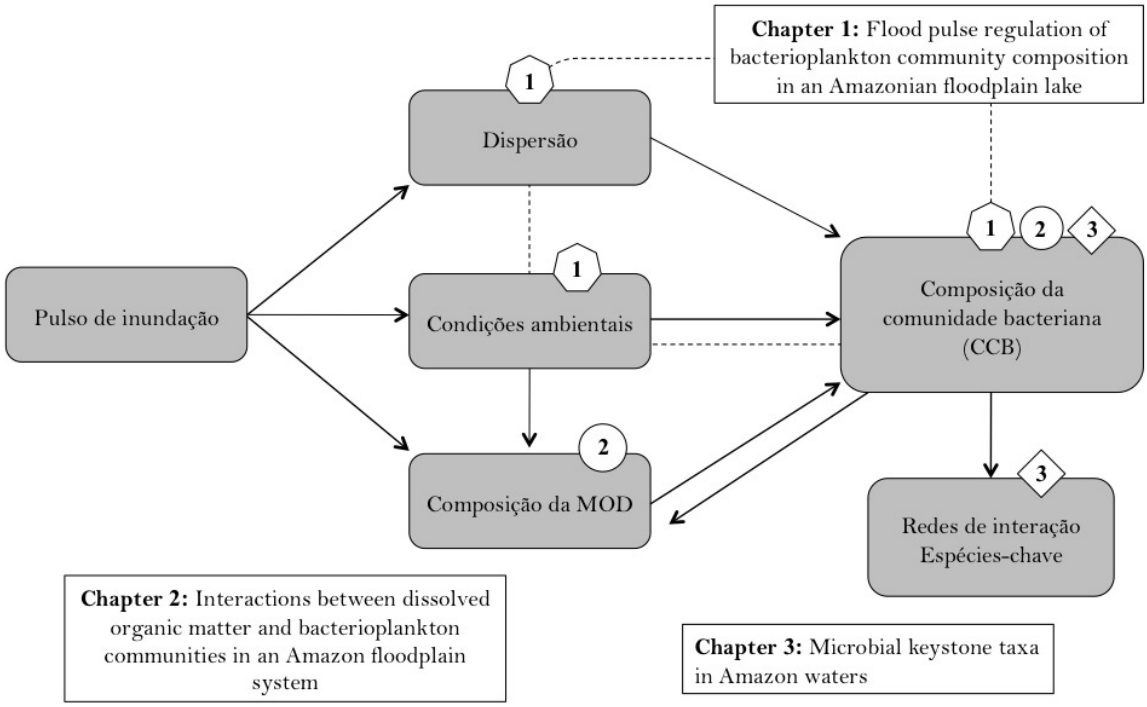

Figura 3: Diagrama da tese destacando os principais temas abordados em cada um dos três capítulos

JUSTIFICATIVA

A bacia Amazônica é a maior bacia fluvial do mundo, estendendo-se por uma área de aproximadamente $6x10^6$ Km^2. Sua grande magnitude de área e volume afetam o processamento de material orgânico terrestre e de seu transporte para o oceano, contribuindo significantemente para os ciclos biogeoquímicos globais. A Amazônia é conhecida pela grande diversidade da fauna e flora, porém, pouco se sabe sobre a biodiversidade de microrganismos.Os estudos existentes se limitaram a investigar apenas a dinâmica espacial ou restringiram-se a amostras do canal principal do rio e/ou afluentes(GHAI et al., 2011; SATINSKY et al., 2015; DOHERTY et al., 2017). Nenhum estudo considerou os fatores locais e regionais responsáveis pela estruturação de comunidades microbianas e ainda, a interação entre a composição de substratos orgânicos (MOD) e a CCB.

As várzeas amazônicas consistem em um mosaico de habitats de terras úmidas, incluindo florestas periodicamente inundadas, bancos de macrófitas flutuantes e ambientes de águas abertas, periodicamente inundados pelo transbordamento lateral de rios e chuvas associado a variações sazonais no pulso hidrológico anual (*Flood Pulse Concept* - JUNK et al., 1989). Considerando toda esta complexidade espacial e sazonal dos sistemas de várzea Amazônicos e o papel chave do bacterioplâncton nos ciclos do carbono e nutrientes, o conhecimento da composição e metabolismo das comunidades bacterianas, assim como dos processos que as regulam (por exemplo, dispersão, filtros ambientais e interações biológicas) são essenciais para um melhor entendimento sobre os processos ecológicos que regem a biogeoquímica do carbono na Amazônia.

Ainda, por serem sistemas altamente produtivos e fortemente regulados pelo pulso de inundação, várzeas são interessantes para se estudar a transição entre sistemas aquáticos distintos e o ambiente terrestre e o efeito da sazonalidade nas condições ambientais como por exemplo, origem e composição da matéria orgânica. E finalmente, para se avaliar como essas mudanças no gradiente hidrológico podem afetar a estrutura das comunidades microbianas aquáticas, nomeadamente os principais decompositores da MOD.

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CAPÍTULO 1

"Flood pulse regulation of bacterioplankton community composition in an Amazonian floodplain lake"

Flood pulse regulation of bacterioplankton community composition in an Amazonian floodplain lake

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Abstract

1- Understanding spatial and temporal dynamics of microbial communities is a central challenge in microbial ecology since microorganisms play a key role in ecosystem functioning and biogeochemical cycles. Amazonian aquatic systems comprise a dynamic mosaic of heterogeneous habits, but are understudied and there is limited information about the mechanisms that shape bacterial community composition (BCC).

2- There is a consensus that environmental selection (species sorting) and dispersal processes (source-sink dynamics) act in concert to shape the composition of these communities, but the relative importance of each mechanism may vary dramatically through time and between systems.

3- Applying 16S rRNA gene amplicon high-throughput sequencing, we studied factors and processes that modulate BCC in an Amazonian floodplain lake (AFL) and used source-tracking models to trace the main dispersal sources of microorganisms in the whole floodplain system during a full hydrological cycle.

4- Our source-tracking models indicated that dispersal processes were predominant, explaining most of the BCC variability throughout the study period. We observed more sources contributing to the sink community during the falling water than rising water period, when contributions from the Solimões River dominated.

5- There was a clear seasonal pattern in BCC, closely related to environmental variables, suggesting that the successful establishment of dispersing bacteria also depends on environmental filtering that is linked to water flow.

6- In summary, source-sink dynamics and species sorting were strongly affected by water exchange and connectivity with the main river that varied throughout the flood pulse cycle. Our results demonstrated the influence of lateral transport and temporal dynamics on BCC in AFLs that could ultimately impact regional carbon budgets and biogeochemical cycles.

1. Introduction

Microbes are the most diverse and ubiquitous organisms within our biosphere. In aquatic systems they play key roles in ecosystem functioning and biogeochemical cycles, nutrient remineralisation and primary production (Cotner & Biddanda, 2002). Understanding how microbial communities are structured is one of the greatest challenges in aquatic microbial ecology. The development of high-throughput sequencing techniques is now allowing us to unravel this hitherto unexplored biodiversity and understand the processes behind temporal and spatial patterns observed across various habitats (Xu, 2006; Read et al., 2015; Humbert, Dorigo, Cecchi, Le Berre, Debroas, & Bouvy, 2009).

Microbial ecologists have been testing whether ecological theories and concepts originally developed for macro-organisms also apply to microorganisms. The metacommunity concept (Leibold et al., 2004) came from metapopulation theory (MacArthur & Wilson, 1967), and makes predictions about a set of local communities with a pool of potentially interactive species that are linked by dispersal (Leibold et al., 2004). The metacommunity concept considers habitat heterogeneity and describes how communities are structured by species responses to environmental filters and local selection (species sorting), and also the role of dispersal processes, immigration and emigration, to rescue species from local competitive exclusion (mass effect, source-sink dynamics).

The metacommunity concept has previously been applied to investigate factors that structure aquatic BCC (Lagenheder & Ragnarsson, 2007; Lindstrom & Langenheder, 2012; Zha, Berga, Comte, & Langenheder, 2016; Comte, Berga, Severin, Logue, & Lindström, 2017; Langenheder & Szekely 2011; Szekely & Langenheder, 2014). In general, BCC is shaped by a combination of different mechanisms, which might vary with the environmental characteristics of each system (Szekely & Langenheder, 2014). However, few studies have addressed these questions in tropical river-floodplain systems (but see Lemke et al., 2009; Tessler et al., 2017) where high temporal variability and spatial heterogeneity add additional layers of complexity.

A good framework for addressing this topic is the source-sink theoretical model that describes how variations in environmental conditions may affect population growth or decline (Mouquet & Loreau, 2003; Mouquet, Miller, Daufresne,

& Kneitel, 2006). Source-sink dynamics have been successfully applied to aquatic microbial communities, but their role seems to be smaller than that of resident bacterial growth (species sorting). A study conducted in a complex boreal aquatic network along an entire terrestrial/aquatic continuum found that BCC followed a directional spatial structure driven by the soil species recruitment (source-sink dynamics) and that these species were filtered by environmental conditions along the gradient (species sorting - Ruiz-González, Niño-Garcia, & del Giorgio, 2015). Another study, which also considered seasonal variations, investigated the importance of external and internal dispersal sources (precipitation, inlet inflow, sediment resuspension and mixing) to the BCC of a dimictic temperate lake and found that previous community structure had a more important role in determining the recent community composition than dispersal sources (Comte et al., 2017).

The Amazon River basin contains a complex hydrological network, with large main river channels and numerous interconnected sub-systems including permanent lakes, floodplains, wetlands, *igarapés* (shallow channels that run through the adjacent forest) and *igapós* (flooded area with typical hydrophilic vegetation), forming the world's largest river system with a drainage basin area of 6.1×10^6 km^2 (Richey et al., 1990). The connectivity and material exchange between floodplain systems and the river are strongly affected by water flux direction and relative contribution of different water inputs, which depend on the floodplain catchment area and the flood pulse (the annual variation in the water level), as demonstrated for the same study area (Lake Janauacá) (Bonnet et al., 2017).

A range of studies has demonstrated that floodplain systems play a crucial role in the structure and dynamics of the Amazon River system (Melack & Forsberg, 2001; Abril et al., 2014). Usually, these complex systems have high primary and secondary production (Melack & Forsberg, 2001; Forsberg, Melack, Richey & Pimentel, 2017). The flooded vegetation produces and exports large amounts of inorganic and organic carbon to the river that can be transported over great distances before being metabolized and released to the atmosphere (Abril et al., 2014).

In the Amazon basin, most studies have investigated only the spatial dynamics of BCC (Tessler et al., 2017) or were restricted to sampling either the river's main channel and tributaries or the plume/estuary (Ghai et al., 2011; Satinsky et al., 2015). In contrast, few studies have considered both spatial and seasonal

scales (Doherty et al., 2017). Most importantly, no studies have evaluated the mechanisms that shape bacterial community assemblages in floodplain systems and how local communities are connected into a regional metacommunity.

Given the importance of large tropical floodplain river systems, and the key role of bacteria in the degradation of the organic matter and other globally relevant biogeochemical cycles, this study aimed to: i) track the relative contribution of bacterial dispersal sources into a sink community in an Amazonian floodplain lake (AFL); ii) investigate how the flood pulse changes environmental conditions and how these changes in turn affect BCC; and iii) identify key taxa involved in the major shifts in BCC throughout the hydrological cycle of an AFL. To accomplish this we sampled monthly between June 2015 and May 2016 in the channel that formed the only permanent surface connection between the lake and the main river. We sampled this site because it showed the microbiota entering or leaving the lake. We hypothesized that the flood pulse strongly affects environmental conditions, as well as the relative contribution of the different sources of bacteria to the channel. During high water periods, when water from the Solimões River enters the lake through the channel, we expected an increase in bacterial sources from the river and a decrease in contribution from other sources, while in drier conditions when river discharge is lower, waters from the drainage basin and from the channel's previous community were expected to be the main contributors to BCC.

2. Methods

2.1 Study Area and sampling

Lake Janauacá (3°23' S; 60°18' W; altitude 32 m) is an AFL located 40 km southeast of Manaus, in the middle of the Amazon basin (Figure I - 1). With a local watershed area of 770 km^2 and a floodable area ranging between 23 km^2 at low water period to 390 km^2 at high water periods (Pinel et al., 2015). The lake is located along the south margin of the Solimões River and is permanently connected to it by a 12 km-long channel. The channel is the main connection (ground water is another less important permanent connection) and water-exchange path between lake and river during most of the year. The exceptions are high water periods (May-August), when other connections are established along the riverbank in the northern part of the lake (open lake - northern area). The southernmost portion of the lake is

characterized by a dendritic shape (Figure I - 1), which drains predominantly upland forests and agricultural areas. In the northern part, there is a larger portion forming a large open water lake (Figure I - 1), with strong influence from the river during high water periods, as the river floods into the lake. This portion is also dominated by herbaceous macrophytes and the dominant species vary throughout the year.

Subsurface (50 cm) water samples were collected monthly at the channel site (Figure I - 1) between June 2015 and May 2016, covering a full hydrological cycle (totaling 9 field campaigns – with gaps in the monthly sampling only in November and December 2015 and April 2016 – Figure I - 2). We chose this channel sampling site to address questions about the seasonal dynamics in BCC and environmental drivers (species sorting). To track the sources of microorganisms dispersing to the sink community (channel) we sampled the BCC at four additional sites (in campaigns 4, 6, 7 and 9 – Table I - S1): (I) open lake, a site located in the northern region of the floodplain lake that is influenced by the Solimões River; (II) drainage basin, a site located in the southern portion of the lake that is influenced by a forest stream receiving black and clear waters (Sioli, 1984); (III) macrophyte banks located near the margin of the basin in a wind protected area and; (IV) Solimões River, the river mainstream (Figure I - 1).

The study period included an extreme high water period (starting in June 2015 – Figure I - 2) that was followed by an atypically dry period in November and December 2015 (the latter not covered in the present study). During this period the lake was reduced to a draining channel, and it was not possible to sample some of the sites because they had dried out (see satellite images of high and low waters in Figure I - S1).

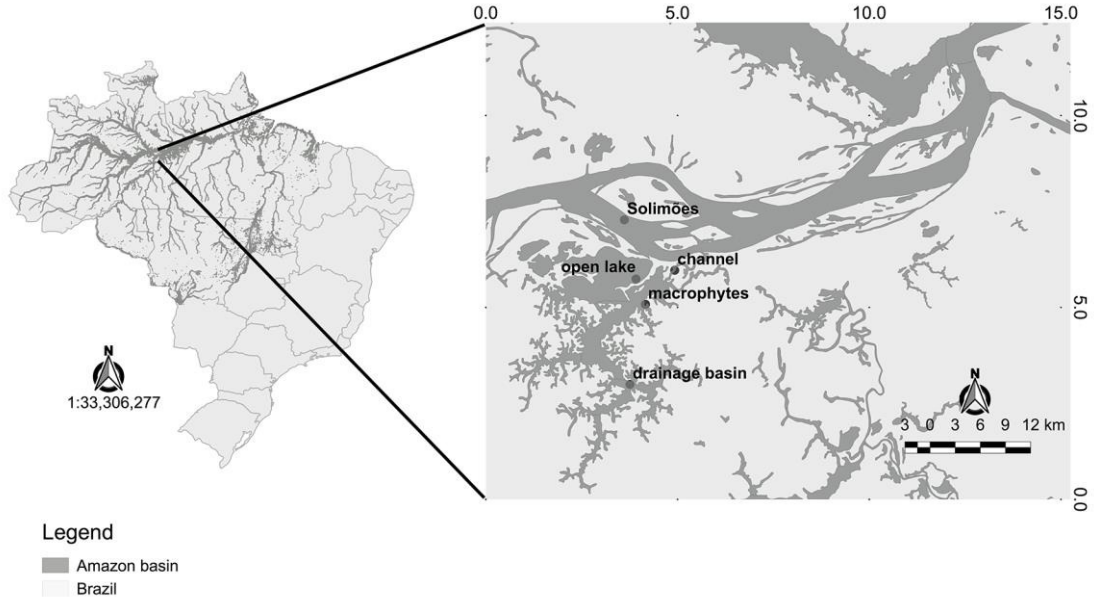


Figure I - 1: Map of the study area Lake Janauacá showing sampling sites: open lake, drainage basin, macrophytes, channel, Solimões. This map represents the lake in the high water period.

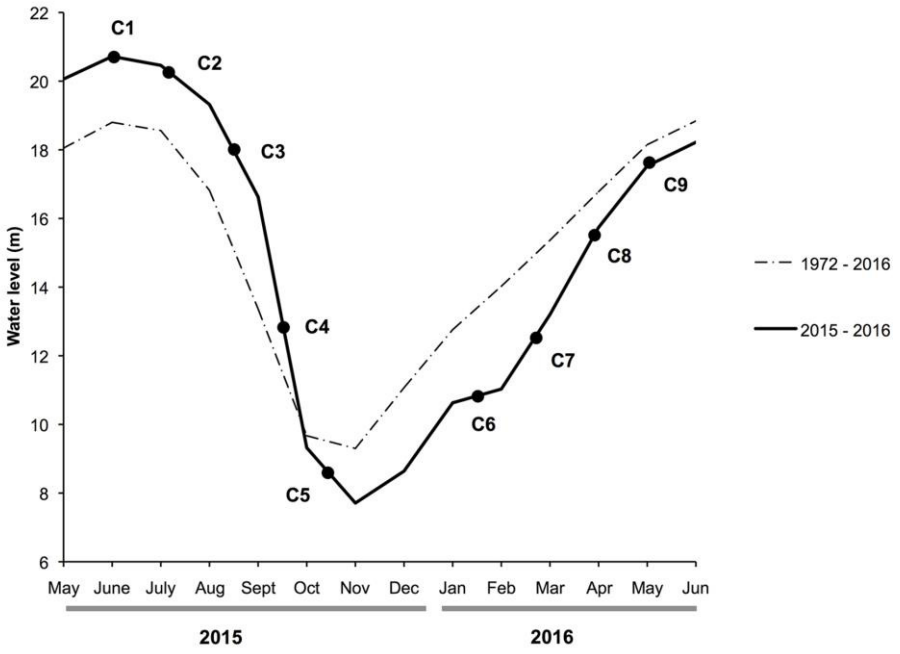
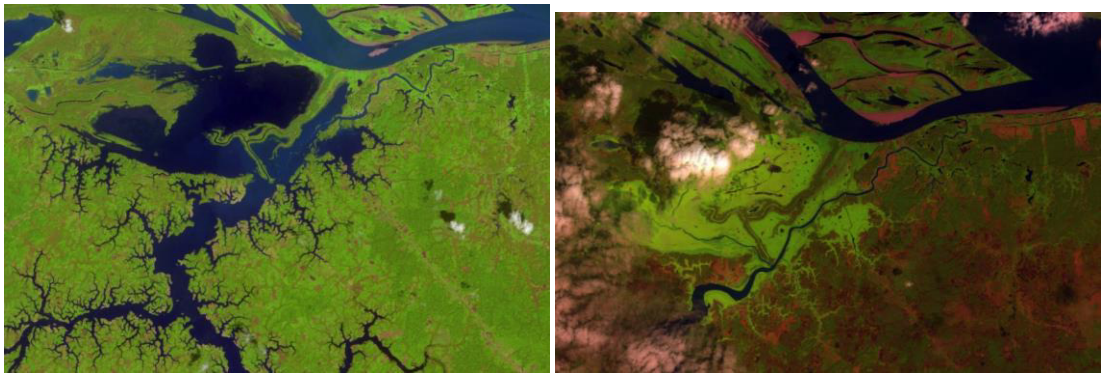


Figure I - 2: Dashed line shows the historical water level (m) based on 44 years of daily records for the Solimões River at the Manacapuru gauging station. Solid line represents mean monthly water levels from May 2015 to June 2016. Numbers indicate campaigns that took place in different periods of the flood pulse, grouped as: high (Campaigns C1, C2), falling (C3, C4, C5) and rising (C6, C7, C8, C9) water periods. Source: Brazilian National Agency of Waters (http://www.snirh.gov.br/hidroweb/).

Supplementary Table I - S1: Sampling design. Samples were collected monthly at the channel site in all 9 campaigns to investigate seasonal dynamics in BCC and environmental drivers (*). Also, to track dispersal sources to the channel community we sampled additional sites (+).

Campaigns	channel	open lake	drainage basin	macrophytes	Solimões
1	*				
2	*				
3	*+				
4	*+	+	+	+	+
5	*+				
6	*+	+	+	+	+
7	*+	+	+	+	+
8	*+				
9	*+	+	+	+	+

*monthly samples;seasonal patterns and drivers +dispersal sources



Supplementary Figure I - S1: Satellite images of lake Janauacá in a high water period (April 2016 – on the left) and in a low water period (December 2015 – on the right). Image (RESOURCESAT-2, sensor LISS3) composition RGB-453: 12/19/15 (Source DGI/INPE; ISRO, 2016) and 06/09/16 (Source ASF/ESA/USGS, 2016). Image cortesy of Rodrigo Nunes.

2.2 Environmental variables, DNA extraction and purification

Water temperature and electrical conductivity were measured using a CTD profiler (CastAway, SonTek, San Diego, CA, USA) sampling at 4 Hz with data reported at 0.3 m intervals. Other physical variables such as pH and dissolved

oxygen (DO) were determined using specific probes (YSI ProODO, Yellow Springs, OH, USA). Water transparency was determined with a Secchi disc. Water sampling and in situ measurements were taken in the morning (between 9 and 12 am). Water level was noted twice a day by local residents using a metric ruler fixed in a series of wood frames distributed along the channel banks.

Samples for chemical analyses were stored in clean insulated flasks kept in thermal boxes until processing (for a maximum of 4 hours). Water for chlorophyll-*a* (Chl-*a*) was filtered through Whatman® GF/F filters using a vacuum pump and the filters were frozen and stored in the dark until analysis. Chl-*a* was determined spectrophotometrically, following filter maceration and extraction in 90% acetone (Wetzel & Likens, 2000). For TSS we weighed the filters (Millipore® 0.45 µm pore size) before and after filtration, once dried at 60^{o}C, and used the subtracted value (in mg) per litre of water volume filtered.

For BCC, lake water was filtered sequentially though 3 µm (Whatman® Nucleopore, UK) (particle-attached fraction - PA) and 0.2 µm pore-size, 47 mm diameter (Millipore® Isopore, USA) (free-living fraction- FL) and DNA samples were stored at -20 ºC in the field station and subsequently at -80 ºC in the laboratory. Total DNA was extracted directly from the filters using phenol-chloroform extraction followed by purification in Amicon columns (Millipore® 100KDa/100.000MWCO). We were not able to extract high-quality DNA from our samples with the widely used MoBio PowerSoil DNA isolation kit (MO BIO Laboratories, Inc, Carlsbad, CA, USA), probably because of the humic-rich nature of Amazonian waters. An additional purification step with 10% cetyl trimethyl ammonium bromide (CTAB) was carried out for a few samples where necessary (samples 1FL, 4PA) to remove humic substances (Schneegurt, Dore, & Kulpa, 2003), when the extract was not susceptible to PCR amplification.

2.3 DNA amplification and sequencing

The V3/V4 region of the 16rRNA gene was amplified with the bacterial primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACT ACHVGGGTATCTAATCC-3') (Herlemann et al., 2011). Polymerase chain reactions (PCRs) were performed twice in a 25 µl reaction volume containing 12.5 µl of KAPA High - fidelity Hotstart ready mix (KAPA Biosystems, Boston, MA, USA), 0.3 µM of

each primer (forward and reverse), 10 µl of PCR-grade water, and 10 ng of DNA. Reactions were started with an initial step of 95ºC for 3 min, followed by 25 cycles of 98ºC for 20s, 62ºC for 15s, 72ºC for 15s and finally 72^{o} for 60s.

Subsequently, PCR products were purified with magnetic beads AMPURE XP kit (Beckman Coulter) and indexed with Nextera XT kit V2 (Illumina, Inc, San Diego, CA, USA). An additional step of purification with magnetic beads was performed, and then a combined pool was prepared by mixing 5 µl from each library. High-throughput sequencing was performed on an Illumina Miseq2000 instrument (Laboratório Multiusuário Centralizado para Sequenciamento de DNA em Larga Escala e Análise de Expressão Gênica, Univerdade Estadual Paulista, Jaboticabal, São Paulo, Brazil). All sequences were submitted to the BioSample database (https://www.ncbi.nlm.nih.gov/sra/SRP127556).

2.4 Sequence processing and exploratory analyses

We performed the quality filtering, denoising and removal of potential chimeras and non-bacterial sequences using UPARSE (Edgar, 2013) in a previously implemented pipeline (Logares et al., 2014; Logares, 2017). Paired-end reads were merged with PEAR (Zhang, Kobert, Flouri, & Stamatakis, 2014). All sequences shorter than 100 bp (base pairs) were discarded and the full-length dereplication was carried out with USEARCH. Merged sequences were clustered into operational taxonomic units (OTUs) using UPARSE, applying a threshold of 97% identity (Quast et al., 2012). Chimeric sequences were filtered out with USEARCH (Edgar, 2010). Taxonomic classification was done with BLASTn against SILVA 119.1 (Zhang & Fang, 2000) with at least 75% of similarity. For further analyses, all chloroplasts and Archaea sequences were removed and to enable comparisons between samples, the OTU table was randomly subsampled (rarefied) based on the sample with the least number of reads (10,341 sequences). Then, we calculated the relative abundance by dividing the number of reads of each OTU by the total number of reads in each rarefied sample (10,341).

To assess the effect of environmental variables (log transformed) on channel BCC we used the distance-based redundancy analysis (dbRDA) based on Bray-Curtis dissimilarity measure. Hypotheses about differences in the structure of bacterial communities between FL and PA and among seasons were evaluated using

permutational multivariate analysis of variance (PERMANOVA) and the results visualized using ordination (non-metric multidimensional scaling (NMDS).

All data analyses were carried out in R version 3.3.3 (R Core Team, 2016) using the R package vegan (Oksanen et al., 2016). Figures were drawn using the package ggplot2 (Wickham, 2009).

2.5 Identifying the sources of dispersing bacterial

To identify and determine the contribution of a set of sources to the bacterial community in the channel (sink) we used a Bayesian approach. Analyses were conducted in SourceTracker 0.9.5 software with MacQIIME (version 1.9.1, Knights et al., 2011). For each campaign, we ran a different model with a specific set of sinks and sources, considering also the size fraction (FL and PA), resulting in four models: FL fraction in falling (campaign 4 – model 1) and rising waters (campaigns 6, 7 and 9, models 2, 3 and 4, respectively). The same procedure was used for the PA size fraction: PA fraction in falling (campaign 4 – model 1) and rising waters (campaigns 6, 7 and 9, models 2, 3 and 4, respectively). For all models (FL and PA), the sink was always the bacterial community in the channel and the potential sources were: the resident communities sampled at the same site during the preceding campaign (e.g. for campaign 4, the preceding community was that collected during campaign 3), or bacterial communities sampled in macrophyte banks (ma), open lake (la), drainage basin (ba), Solimões River (sol), or unknown sources.

2.6 Differences in bacterial community composition between seasons

A group significance test was used to compare the number of reads of various taxonomic levels in sample groups and to check whether they were significantly different (Kropf, Heuer, Grüning, & Smalla, 2004). The tests were performed in MacQiime (script group_significance.py - http://qiime.org/scripts/group_significance.html) using rarefied tables to compare all taxonomic levels (Phylum, Class, Order, Family and Genus) between fractions (PA and FL) and among seasons (high, falling and rising waters) in channel samples. Firstly, we removed taxa that were not present in at least 10% of our samples. Subsequently, the group significance test showed which taxa were differentially represented in each group based on a Kruskal–Wallis test, and calculated a P value

corrected (using the Benjamini-Hochberg false discovery rate (FDR) procedure) for multiple comparisons. We considered P-values ≤ 0.1 as significantly different. We did not consider unclassified taxa in this analysis.

3. Results

3.1 Tracking sources of bacteria during the flood pulse

The sampling design had a good coverage of the possible sources of dispersal to the bacterial communities observed in the channel, as the proportion of unknown sources was low, averaging less than 7.5% (excepting model 1 - PA fraction with 16.7% from unknown sources). Bacterial dispersal sources, as well as their contributions to the sink communities varied according to the size fraction and season (Table I - 1, Figure I - 3).

The sources for the BCC observed at the Lake channel were more diverse during the falling water period (campaign 4) than in other periods. The lowest source-diversity was registered during the rising water period.

During the falling water period, the drainage basin had the largest contribution to BCC in both PA and FL, followed by the open lake. The previous community and Solimões River were important sources for PA but not FL communities (Table I - 1). Whereas, during the rising water period (campaigns 6, 7 and 9) we observed only few dominant sources. Firstly, the contribution from Solimões River increased for both PA and FL. In contrast, open lake, macrophytes and drainage basin communities had no or very low contributions to the sink for both PA and FL. The contribution of the previous community was especially high in campaign 7.

Table I – 1: Results from Source- Tracker analysis showing the contribution of different sources to the sink (channel community) in both size fractions (free- living [FL] and particle- attached [PA]) across four campaigns (falling 4, rising 6, 7 and 9).

Model	Season	Sources	Sink: channel FL Contribution (%)	Sink: channel PA Contribution (%)
1	falling 4	previous PA	0.3	10.3
		previous FL	0.2	8.8
		drainage basin PA	8.9	23.6
		drainage basin FL	49.8	0.7
		open lake PA	2.7	9.8
		open lake FL	25.5	9.0
		macrophytes PA	4.0	0.0
		macrophytes FL	0.0	3.8
		Solimões PA	0.2	12.7
		Solimões FL	3.9	4.9
		Unknown	4.6	16.7
2	rising 6	previous PA	10.7	11.1
		previous FL	0.0	0.3
		macrophytes PA	0.0	0.0
		macrophytes FL	0.2	0.1
		Solimões PA	52.8	60.5
		Solimões FL	29.9	21.7
		Unknown	6.5	6.4
3	rising 7	previous PA	23.7	42.6
		previous FL	14.3	28.2
		drainage basin PA	0.0	0.0
		drainage basin FL	6.1	0.6
		open lake PA	0.0	0.0
		open lake FL	0.0	0.0
		macrophytes PA	0.0	0.0
		macrophytes FL	0.0	0.0
		Solimões PA	48.7	24.3
		Solimões FL	0.0	0.0
		Unknown	7.2	4.3
4	rising 9	previous PA	5.8	8.6
		previous FL	14.5	24.4
		drainage basin PA	0.0	0.1
		drainage basin FL	0.0	0.0
		open lake PA	0.0	0.0
		open lake FL	0.0	0.0
		macrophytes PA	0.1	0.4
		Solimões PA	20.3	14.6
		Solimões FL	55.3	44.7
		Unknown	3.9	7.4

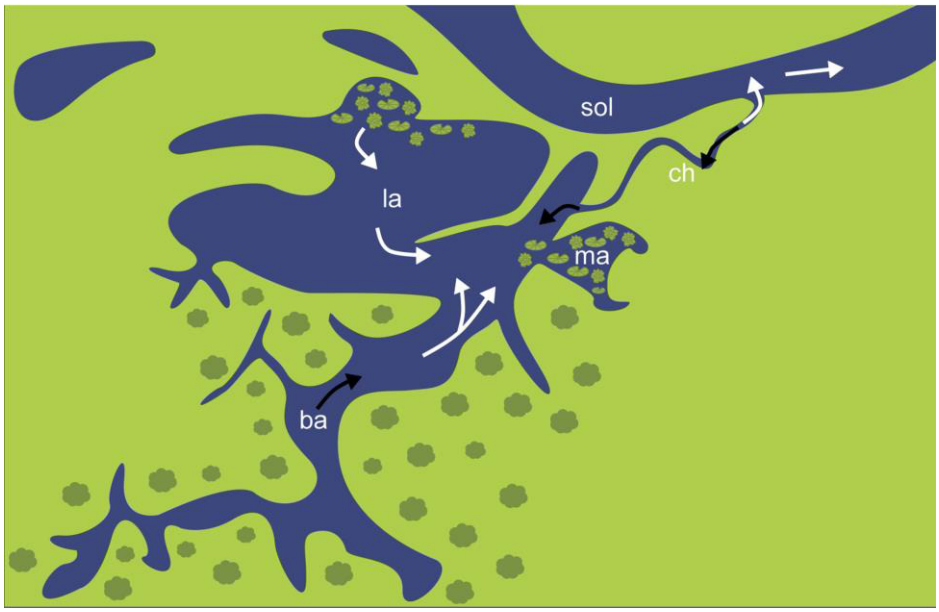


Figure I - 3: Schematic illustration of Lake Janauacá showing the sink (ch) and dispersal sources. The white arrows indicate the typical water flux dynamics in falling water and the black arrows in rising water periods. Abbreviations: drainage basin (ba); channel (ch); macrophytes (ma); Solimões (sol); open lake (la).

3.2 Environmental variables and their relationship with bacterial communities

The flood pulse clearly affected the physical and chemical properties of the channel water (Table I - S2). The high water period was characterized by increased water level and decreased DO (average of 1.76 mg L^{-1}). During this period, we registered low concentrations of TSS, intermediate Chl-*a* concentrations (3.73 and 16.2 µg L^{-1}, compared with the maximum and minimum 65.26 and 0.90 µg L^{-1}, respectively – table I - S2) and higher water transparency (Secchi depth > 1m) compared to the other periods investigated. During the falling water period, Chl-*a* concentration reached the annual maximum value (65.26 µg L^{-1}) in our study. Consequently, DO concentrations increased slowly from campaign 4 to reach a maximum value at the end of the season (campaign 5). During the rising water, conductivity and TSS increased to values ranging between 48.7 and 72.1 µS cm^{-1} and 102 to 162 mg L^{-1}, respectively. In contrast, water transparency and Chl-*a* concentrations reached their lowest annual values during this period (average Secchi disk depth of 14 cm and Chl-*a* ~ 2.5 µg L^{-1}). DO concentrations decreased again

towards the end of the rising water period (campaigns 8 and 9).

The dbRDA model with temperature, pH, DO, Chl-*a*, conductivity, Secchi and water level (Figure I - 4, note that TSS was excluded because it was correlated with conductivity) explained 72% of the variation in channel BCC for all campaigns and size fractions (all 7 axes, constrained proportion=0.72) in the channel site. Most variation was explained by the first and second axes (CAP1=35.5%; CAP2=15.6%). Campaigns 1, 2 and 3 (both PA and FL) were grouped together and were associated with lower temperatures and high water level (Figure I - 4). At the beginning of the falling water period (campaign 3), an inverse relationship with low DO concentrations was observed. Campaigns 4 and 5 were grouped together and were positively related with transparency and Chl-*a* concentrations. During early rising water, campaigns 6 and 7 were positively associated with DO concentrations and high temperatures. Samples from the end of the rising waters (campaigns 8 and 9), were positively related with pH, conductivity, DO and low transparency.

Supplementary Table I - S2: Environmental variables measured in the channel in 9 campaigns. Abbreviations: dissolved oxygen (DO), electrical conductivity (cond), total suspended solids (TSS), chlorophyll-*a* (chla).

Sample	Date	Season	temperature (°C)	secchi (m)	pH	DO ($mg.L^{-1}$)	Cond ($uS.cm^{-1}$)	TSS ($mg.L^{-1}$)	chla ($ug.L^{-1}$)
1	Jun.16	high	29.2	1.2	6.62	1.58	39.7	10.4	3.73
2	Jul.16	high	29.5	1.3	6.67	1.95	42.8	7.33	16.2
3	Aug.16	falling	30.1	1.1	6.49	2.03	69.7	5	9.34
4	Sep.16	falling	30.9	0.9	6.4	2.64	33.8	10	45.39
5	Oct.16	falling	32.6	0.25	7.12	7.12	31.3	84	65.26
6	Jan.17	rising	29.9	0.1	6.36	5.11	64.2	162	1.78
7	Feb.17	rising	29.3	0.1	7.28	5.59	72.1	132	5.21
8	Mar.17	rising	30.0	0.17	6.14	4.32	54.1	103	0.90
9	May.17	rising	28.6	0.20	6.41	2.96	48.7	102	2.1

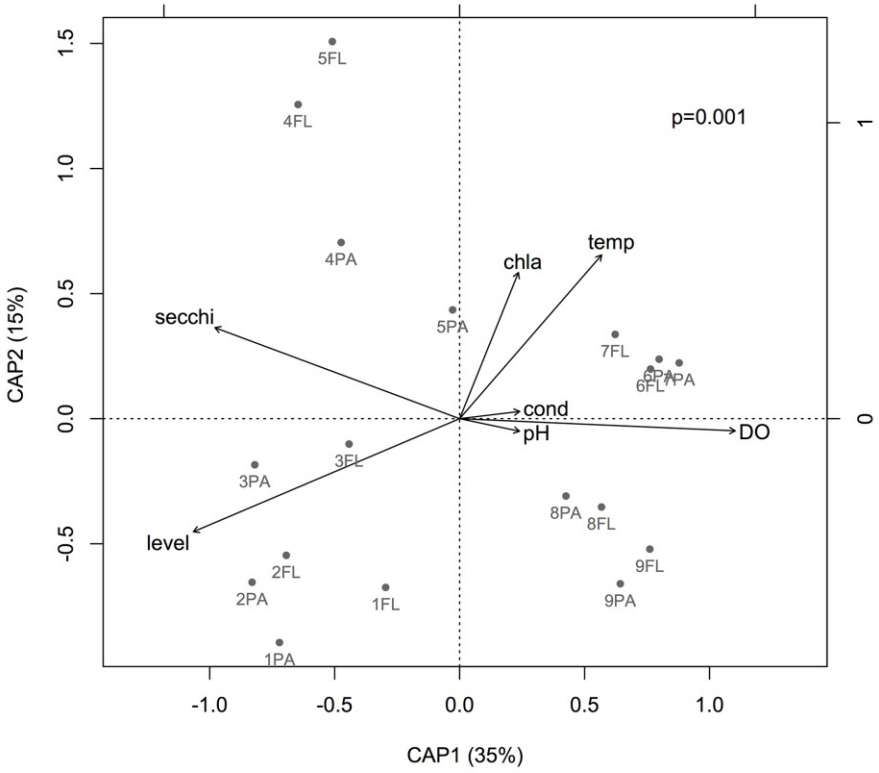


Figure I - 4: Distance based redundancy analysis (dbRDA) of OTUs in bacterial communities and environmental variables at the channel site. Samples are identified with the number of the campaign and the size fraction, free-living (FL) and particle-attached (PA). Abbreviations: dissolved oxygen (DO), electrical conductivity (cond), water temperature (temp), chlorophyll-*a* (chla), water level (level).

3.3 Features of Amazonian bacterioplankton

BCC in the channel varied throughout the year, with a clear seasonal pattern partitioning the communities into three different groups (Figure 5). These groups were high water (campaigns 1 and 2), falling water (campaigns 3 to 5) and rising water periods (campaigns 6 to 9) (Figures I - 2, I - 5). In contrast, BCC in FL and PA fractions were not separated in the NMDS bidimensional plot (Figure I - 5). This was confirmed by the PERMANOVA analysis, where seasons were strongly associated with patterns in BCC, explaining 45% of the variation (R^2=0.45, p ≤0.001), but size fractions (FL vs. PA) did not differ.

Few differences were observed between FL and PA fractions, but there were clear seasonal patterns for several phyla (Figure I - 6). Phyla *Actinobacteria*, *Proteobacteria* (classes *Alpha, Beta, Gammaproteobacteria*), *Planctomycetes* and

Cyanobacteria had the highest relative abundances on all campaigns, accounting for at least 75% of the total community (Figure I - 6). Other phyla such as *Chloroflexi, Verrucomicrobia, Acidobacteria, Bacteriodetes* and *Parcubacteria* fluctuated seasonally. *Saccharibacteria, Firmicutes, Armatimonadetes, Gemmatimonadetes* and *Chlorobi* had relative abundances typically ranging from 0.1-1%.

PA and FL fractions did not differ in taxonomic composition (as also pointed out in the community analyses). However, there were seasonal differences for both fractions for all taxonomic levels (phylum, class, order, family and genus) (Table I - S3). We found two main patterns, (i) taxa with significant differences for more than one taxonomic level due to their dominance. For example, *Synechococcus* was an overrepresented genus in our samples, especially during the falling water period. This pattern was also reflected at other levels – *family I*, order *Subsection I*, class and phylum *Cyanobacteria*. (ii) taxa with significant differences for only one taxonomic level. This was the case for the genus *Planctomyces*, which was overrepresented in high water but with no significant differences manifested at higher taxonomic ranks. We found significant differences in the seasonal pattern at different taxonomic levels in the following phyla: *Cyanobacteria*, *Actinobacteria*, *Proteobacteria* (*Alpha, Beta* and *Gamma*), *Planctomycetes*, *Verrucomicrobia*, *Armatimonadetes* and *Chlorobi*.

The phylum *Armatimonadetes*, the genus *Planctomyces,* classes *Alphaproteobacteria* (most represented by the family *Methylocystaceae*) and *Gammaproteobacteria* (most represented by the family *Methylococcales*) were significantly overrepresented at high water (Table I - S3). In contrast, *Cyanobacteria* (genus *Synechococcus* and *Merismopedia*) and *Chlorobi* were significantly more highly represented during falling water. Members of *Verrucomicrobia* (genus *Opitutus*) and *Betaproteobacteria* (family *Comamonadaceae* were significantly overrepresented during rising water compared to other seasons (Table I - S3).

Overall, *Actinobacteria* tended to be the phylum with highest relative abundances across all samples, but also increased during rising water, mostly related to an increase in the *AcI clade (hgcI)*. In contrast, the *Actinobacteria* class *Thermoleophilia* and *Acidimicrobiia* were overrepresented during falling water.

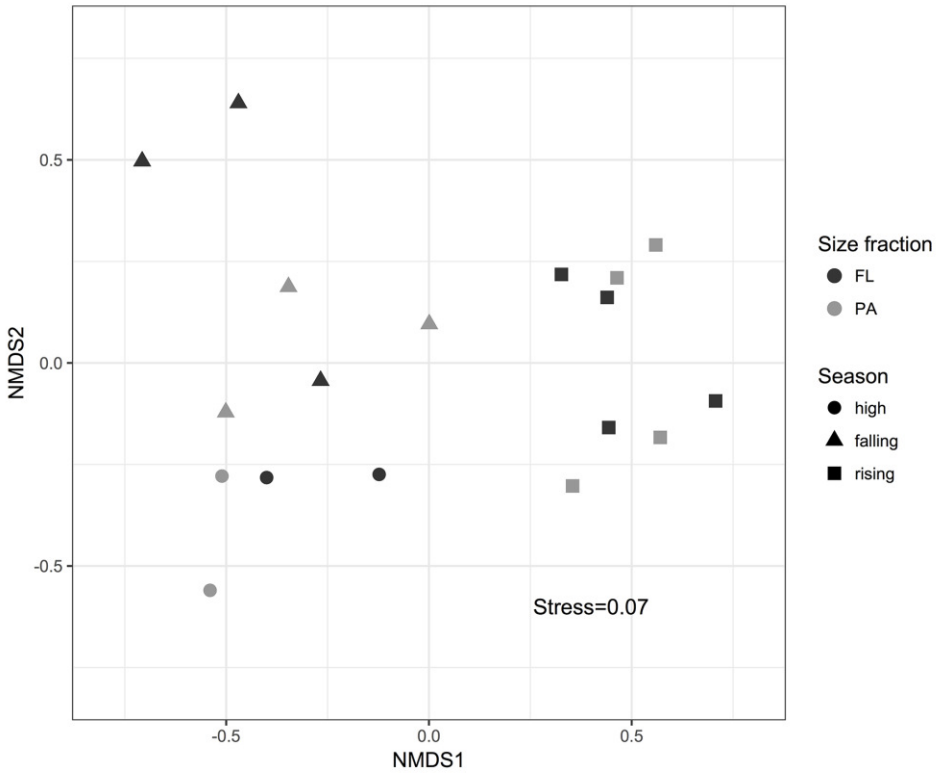


Figure I - 5: Two-dimensional plot of non-metric multidimensional scaling (NMDS) ordination of bacterial OTUs in the channel. Symbols shape are indicating seasons, high, falling and rising water periods, and colors (gray and black) are indicating the size fraction, free-living (FL) and particle-attached (PA).

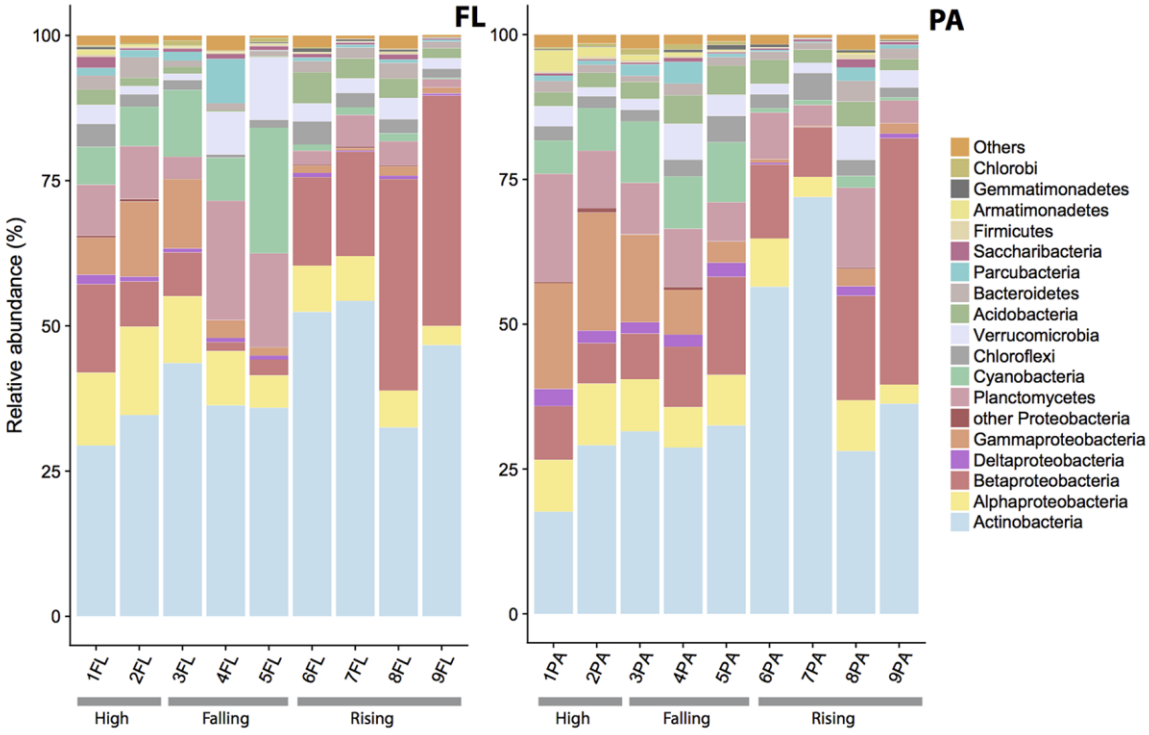


Figure I - 6: Average relative abundance (%) of the main phyla in the channel's bacterial community in each campaign, 1 to 9 in free-living (FL) and particle-attached (PA) communities. Note that *Proteobacteria* are divided among *Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria*, *Gammaproteobacteria* and others *Proteobacteria.*

Supplementary Table I - S3: Results from the group significance test. Level is bacterial taxonomic level; Taxa is bacterial taxa; Test-Statistic is the value of the Kruskal-Wallis test statistic; P is significance; FDR_P is the p-value corrected by the Benjamini-Hochberg false discovery rate procedure; high, rising and falling water periods represented by the group mean (the mean number of reads of each taxonomic level for each season).

Level	Taxa	Test-Statistic	P	FDR_P	high	rising	falling
Phylum	Cyanobacteria	14.74	0.001	0.02	682.25	105.25	1215.17
Phylum	Spirochaetae	13.52	0.001	0.02	4.75	0.38	7.83
Phylum	Chlorobi	12.88	0.002	0.02	34.5	10.63	74.33
Phylum	Armatimonadetes	11.19	0.004	0.04	170	11.63	37.17
Phylum	Candidatus_Berkelbacteria	9.78	0.008	0.06	26.25	0.75	41.50
Class	Cyanobacteria	14.74	0.001	0.05	628	99.13	1204.00
Class	Spirochaetes	13.52	0.001	0.05	4.75	0.38	7.83
Class	Chlorobia	12.88	0.002	0.05	34.5	10.63	74.33
Class	Opitutae	12.63	0.002	0.05	42.25	149.63	35.17
Class	Thermoleophilia	12.39	0.002	0.05	38	9.00	50.33
Class	Gammaproteobacteria	12.05	0.002	0.05	1497.75	127.63	737.83
Class	Armatimonadia	11.06	0.004	0.06	135.5	5.00	18.50
Class	Berkelbacteria_bacterium_GW2011_GWE1_39_12	10.78	0.005	0.06	3.5	0.00	1.00
Class	Actinobacteria	10.69	0.005	0.06	1951	4341.50	2431.33
Class	Bacteroidetes_vadinHA17	10.14	0.006	0.08	9	0.25	5.83
Class	Acidimicrobiia	9.83	0.007	0.08	841	509.13	1072.17
Class	Alphaproteobacteria	9.21	0.010	0.10	1224	633.88	881.83
Order	SubsectionI	14.74	0.001	0.06	589	86.75	1092.33
Order	Solirubrobacterales	14.65	0.001	0.06	3.25	0.00	9.67
Order	Spirochaetales	13.52	0.001	0.06	4.75	0.38	7.83
Order	Chlorobiales	12.88	0.002	0.06	34.5	10.63	74.33
Order	Opitutales	12.70	0.002	0.06	25	144.25	26.00
Order	GIF9	11.90	0.003	0.06	0.75	0.00	0.00
Order	Mycoplasmatales	11.90	0.003	0.06	0.75	0.00	0.00
Order	Gaiellales	11.83	0.003	0.06	34.75	9.00	40.67
Order	Frankiales	11.37	0.003	0.07	1630	4136.25	2078.17
Order	Methylococcales	11.08	0.004	0.07	1178	51.00	587.83
Order	Armatimonadales	11.06	0.004	0.07	135.5	5.00	18.50
Order	Acidimicrobiales	9.83	0.007	0.10	841	509.13	1072.17
Order	Burkholderiales	9.80	0.007	0.10	687.25	2115.38	566.83
Order	Corynebacteriales	9.38	0.009	0.11	16.75	4.00	21.83
Order	Syntrophobacterales	9.34	0.009	0.11	6.25	0.50	5.33
Family	Elev-16S-1332	16.15	0.000	0.10	0	0.00	5.83
Family	FamilyI	14.74	0.001	0.10	627.75	99.13	1204.00
Family	Armatimonadaceae	12.91	0.002	0.10	135.5	2.50	18.50
Family	Opitutaceae	12.70	0.002	0.10	25	144.25	26.00
Family	Phaselicystidaceae	12.23	0.002	0.10	7.5	0.00	1.50
Family	CABC2E06	12.16	0.002	0.10	76	2.25	9.67
Family	Mycoplasmataceae	11.90	0.003	0.10	0.75	0.00	0.00
Family	Sporichthyaceae	11.57	0.003	0.10	1613.25	4133.88	2073.33
Family	Comamonadaceae	11.30	0.004	0.10	303	1205.00	234.00
Family	Alcaligenaceae	11.15	0.004	0.10	5.5	0.63	6.83
Family	Methylococcaceae	11.08	0.004	0.10	1060	46.75	571.83
Family	Methylocystaceae	11.08	0.004	0.10	618.5	83.13	347.17
Genus	Synechococcus	14.74	0.001	0.09	557.25	71.25	991.50
Genus	Luteolibacter	13.98	0.001	0.09	1	0.00	7.33
Genus	Armatimonas	12.91	0.002	0.09	135.5	2.50	18.50
Genus	Candidatus_Planktophila	12.84	0.002	0.09	0.25	12.25	0.50
Genus	Opitutus	12.70	0.002	0.09	25	144.25	26.00
Genus	Methylovulum	12.69	0.002	0.09	2	0.00	0.33
Genus	Oligoflexus	12.69	0.002	0.09	0	0.00	1.50
Genus	Phaselicystis	12.23	0.002	0.09	7.5	0.00	1.50
Genus	Planctomyces	12.14	0.002	0.09	541.75	46.38	158.00
Genus	Candidatus_Bacilloplasma	11.90	0.003	0.09	0.75	0.00	0.00
Genus	Actinobacteria_bacterium_IMCC26256	11.40	0.003	0.10	139.5	13.38	42.67
Genus	AcI clade	11.37	0.003	0.10	1607	4108.63	2054.00
Genus	Merismopedia	11.08	0.004	0.10	2.75	0.63	18.67

4. Discussion

We evaluated the mechanisms that shaped BCC in this AFL and tracked the main dispersal sources of microorganisms, considering the whole river-floodplain system in different phases of the annual hydrological cycle. We found that different bacterial sources contributed to the BCC over the year and that the importance of each source was related to the specific stage of the hydrological cycle. The changes triggered by the flood pulse shifted environmental conditions which in turn altered dispersing bacterial communities.

4.1 Tracking sources of bacteria along the flood pulse

A water balance for the fluxes between the mainstream (Solimões River) and Lake Janauacá revealed that the contribution of different water inputs varied seasonally with contributions from more diverse inputs during the rising water period (Bonnet et al., 2017). Considering a complete annual cycle, the Solimões River was the main source of water to the lake (~87%), followed by upland water from the drainage watershed (1-9%). Ground water (<1%) and direct precipitation (<5%) had lower contributions. During the low water and early rising water periods, local waters and water remaining from the previous year were the main components of the lake. Their relative contribution to the water budget decreased as Solimões River water entered the lake through the channel (Bonnet et al., 2017). Therefore, dispersal sources were expected to contribute differently throughout the year because of changes in water flow. For bacterial communities, our models revealed a greater contribution from a more diverse range of dispersal sources during the falling water period. The main explanation for this pattern is the direction of water flow during this period, flowing from the southern portion (draining basin) to the river through the channel. BCC from different habitats located in the watershed could be transported by flow into the channel and subsequently to the main river. At this stage, the lake was a probable source of nutrients, organic matter and bacterial taxa for the river. When the water was flowing in the opposite direction with the Solimões feeding the lake, the main source of water to the lake was the main river and the inputs from the local watershed became less important. At this stage we observed an increase in the contribution of Solimões bacterial dispersal sources.

Previous studies reported the directional structuring of BCC along the gradient of the river continuum, with a loss of abundant taxa from headwaters and a decreased taxonomic richness downstream (Ruiz-González et al., 2015; Ruiz-González, Niño-Garcia, Berggren, & del Giorgio, 2017). Here, we found that the flood pulse controlled not only the transport downstream, but also lateral exchange between the main river (Solimões) and the associated floodplain lake (Janauacá). These results suggest that all systems in this complex landscape (and their microbiomes) are linked by water flows and driven by river flood-pulses, promoting exchange and dispersal at least during some periods of the hydrological cycle.

An earlier study in two boreal lakes showed that the most important source of bacteria was the previous (resident) community, while all other dispersal sources (sediments, inlets, the other strata of the lake and precipitation) appeared to have limited immediate effects on the community (Comte et al., 2017). These results suggested that species sorting was more important than dispersal in those lakes. In contrast, we observed that the previous community contributed significantly as a source only during rising water (campaigns 7, 9). Rising water led to longer water residence time (WRT, Bonnet et al, 2017, this study), and this provided more time for bacterial communities to adapt to local conditions and persist in the lake, leading to a higher contribution by previous communities as a source. With increased river discharge and water level, resident bacteria are flushed from the system and dispersing bacteria are exposed to environmental filtering by the new local conditions.

Although the proportion of unknown sources was very low, these sources may represent soil bacteria that enter the system during flooding and that are not filtered by species sorting at that time. Soil bacteria are known to be important sources for downstream temperate aquatic habitats (Crump et al., 2012; Ruiz-González et al., 2015). While this topic has not been explored in tropical river systems and floodplains, we know that the flood pulse is a strong force in controlling the linkage between aquatic and terrestrial systems through the formation of aquatic/terrestrial transition zones (ATTZ – Junk et al., 1989) and further research is required to fully describe this relationship.

Even in periods with high densities (for example, June, July and January), macrophytes were not an important source of dispersal to the bacterial community in

the channel in any of our models. Special chemical and physical conditions in these microhabitats creates an environmental filter that hinders the colonization of non-adapted bacterial taxa. Additionally, there are highly diverse and host-specific BCC forming biofilms attached to plants (Crump & Koch, 2008; Pang et al., 2016; Zhao et al., 2017) that may be less susceptible to transport to surrounding waters.

Here, we found that dispersal processes had a predominant role in shaping BCC in the highly connected hydrological network of the Lake Janauacá. Considering the dynamic hydrology and hydraulic flows in AFLs (*eg*. Lesack & Melack, 1995; Bonnet et al., 2017) and that the flux is not directional, a more complex conceptual framework is needed, where the main river is also an important lateral bacterial source to floodplain systems and to the exchange of microorganisms throughout the annual hydrological cycle.

4.2 Seasonal variations in environmental conditions and BCC

The flood pulse produces changes in riverine systems four-dimensionally (in space and time), affecting biogeochemical cycles, productivity, animal and plant distributions and interactions (Thomaz et al., 2007; Oliveira & Calheiros, 2000; Ferreira, 1997). Despite this understanding, little is currently known about how bacterioplankton responds to the flood pulse. We observed a clear seasonal pattern in environmental variables followed by changes in BCC. Seasonal patterns in BCC are well documented in some temperate aquatic systems, where temperature appears to be the main force shaping BCC (Staley et al., 2015; Poretsky, Rodrigues-R, Luo, Tsementzi, & Konstantinidis, 2014; Rösel, Allgaier, & Grossart, 2012). However, few comparable studies have been carried out in tropical systems (Doherty et al., 2017; de Oliveira & Margis, 2015) so it remains unclear whether temperature is also the driving force there.

The low DO concentration found during the high water period is related to the flooding of large lateral areas and the increased surface area subjected to both sediment and planktonic respiration, which consumes DO (Devol, Forsberg, Richey & Pimentel, 1995). Also, daily stratification is commonly observed in the Amazon region due to slight and temporary changes in water temperature and density (Tundisi et al., 1984; Lewis, 1987; Sarmento, 2012) that could result in these low DO concentrations. These conditions may have facilitated the establishment of bacteria

belonging the families *Methylocystaceae* (*Alphaproteobacteria*) and *Methylococcaceae* (*Gammaproteobacteria*). Both are obligate methanotrophs that play an important role as biological filters for methane emissions, supporting high rates of biological methane (CH_4) oxidation, as previously reported for Lake Janauacá (Barbosa et al., 2018).

During the falling water period, depth decreased and the water column mixed, allowing nutrient remobilization from bottom waters and sediments, which culminated in phytoplankton proliferation and an increase in DO concentrations (rapid increase in Chl-*a* concentrations). The relative abundance of photoautotrophs such as *Cyanobacteria* (e.g. genus *Synechococcus* and *Merismopedia*) and *Chlorobi* increased although cyanobacteria are important community members in Lake Janauacá all year round.

Synechococcus was amongst the most abundant genera in our study, being significantly overrepresented in communities during falling water. This genus also had high relative abundances in other AFLs (Toyama et al., 2017), but not in rivers (Doherty et al., 2017; Toyama et al., 2017). High temperatures and the food-web structure of tropical aquatic environments enhance the contribution of these phototrophic picoplankton to primary production (Sarmento, 2012; Domingues et al., 2016). This is due to their higher surface:volume ratio compared to larger phytoplankton cells, which confers an advantage under the oligotrophic conditions common in Amazon waters (Lewis, 1976).

The genus *Planctomyces* (phylum *Planctomycetes*) also featured higher relative abundances during the falling water period. They are found in many aquatic and terrestrial environments, but are usually not very abundant in oxic freshwater ecosystems (Newton et al., 2011). Members of *Planctomycetes* together with other quite abundant phyla found here (*Betaproteobacteria*, *Gammaproteobacteria* and *Verrucomicrobia)* have been reported in association with cyanobacteria blooms in tropical lakes (Woodhouse et al., 2016).

During the rising water period the expansion of flooded areas increases inputs of allochthonous materials from the river and surrounding areas into AFLs (Melack & Forsberg, 2001; Moreira-Turcq et al., 2013). As a consequence, the terrestrial genus *Opitutus* (*Verrucomicrobia)* probably entered the lake with a temporary increase in relative abundance. At that moment, the high TSS concentration in the turbid

Solimões River flowing into the lake had a direct impact on water transparency, which resulted in a drop in cyanobacteria and Chl-*a* concentrations, also reported in other AFLs (Barbosa, de Moraes Novo, Melack, Gastil-Buhl, & Filho, 2010; Forsberg et al., 2017).

Although differences between PA and FL fractions are commonly observed (Jackson, Millar, Payne, & Ochs, 2014; Savio et al., 2015), other Amazon studies have not shown such differences Doherty et al, 2017; Satinsky et al., 2015), and we did not find differences in BCC between FL and PA size fractions here (supported by any of the statistical tests performed). These results could be result from the pore-size of the filters used, which may have been inadequate to separate the full range of particle size in Amazon waters. The concentrations and sizes of particulate and colloidal organic matter in Amazon basin is variable and depends on the system and season analyzed (Benedetti, Ranville, Ponthieu, & Pinheiro, 2002; Hedges et al., 2000). For example, the average concentration of fine particulate organic matter (0.1-63 μm) was six times higher than the coarse particulate concentrations (>63μm) in the Amazon River during a low water period (Hedges et al., 2000). Here, we used 3μm membrane filters and we acknowledge that this may have been too large a pore-size to separate smaller particles with bacteria attached from their FL counterparts. We did not test other filter pore sizes here, but we recommend their use to investigate size-specific patterns in future studies.

Overall, despite growing interest and efforts to study the composition of aquatic bacterial communities, the ecology and function of many components of the microbiota are still largely unknown, especially in structurally complex and inaccessible systems like the Amazon. This makes it difficult to characterize seasonal and spatial patterns in the BCC. From our results, it is clear that the flood pulse controls fluxes of water and materials and environmental conditions that ultimately influence bacterial community assembly. In our metacommunity approach, dispersal processes were predominant and hydrology was the main driving force controlling the contribution of different bacterial sources to the sink community through the year. Lateral exchange with the main river was an important mechanism shaping BCC in the studied AFL, shown by its increased contribution during the rising water period. In addition, changes in environmental conditions during the flood pulse appeared to determine the successful establishment of dispersing bacteria, which showed

seasonal differences at all taxonomic levels (from phylum to genus). In conclusion, our study presents strong evidence that bacterial dispersal between local communities plays a large role in highly connected hydrological networks like Amazonian floodplain-river systems and reinforces the need for further studies.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

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CAPÍTULO 2

“Linking dissolved organic matter composition and bacterioplankton communities in an Amazon floodplain system”

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Linking dissolved organic matter composition and bacterioplankton communities in an Amazon floodplain system

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Abstract

Dissolved organic matter (DOM) is the main substrate for aquatic prokaryotes, fuelling their metabolism and controlling community composition. Amazonian rivers transport and process large fluxes of terrestrial DOM and yet, the link between DOM composition and its main consumers, heterotrophic bacteria, has not been well explored. The aims of this study were to characterize DOM composition and investigate the coupling between DOM and bacterial community composition (BCC) during a complete hydrological cycle in an Amazon floodplain system (lake Janauacá). Our study revealed a clear seasonal pattern in DOM composition through the flood pulse, which affected the ammounts of autochthonous:allochthonous sources and consequently the extent of humification, molecular weight, and aromaticity of the DOM. BCC was tightly coupled to DOM fluorescence, which was also driven by differences in hydrology with distinct fluorescence components and bacterial taxa being more abundant and correlated with a specific hydrological season. This coupling was particularly well reflected for three of the four identified fluorescence components, two terrestrial-humic like components (C1 and C3) and an autochthonous component (C4). Despite clear changes in DOM composition, dissolved organic carbon concentrations tended to be relatively stable throughout the year. Overall, our results suggest that BCC shifts were associated with DOM quality but not with its quantity, and that bacteria preferably use and turnover labile and freshly produced DOM in lake Janauacá.

1. Introduction

Dissolved organic matter (DOM) is a heterogeneous mixture of humic substances, carbohydrates, carboxylic acids, and amino acids, with varying degrees of reactivity (Coble et al., 2014; Roiha et al., 2016). In freshwater lakes and other surface waters, DOM compounds have a strong influence on light attenuation, metal speciation and bioavailability, while also acting as a pH buffer. Importantly, this complex mixture of organic compounds also represents the main substrate and energy source for heterotrophic bacteria (Azam et al., 1983; Coble et al., 2014). The diversity and coupled functional attributes of the inherently complex natural bacterial communities observed in lakes define their potential to process different types of organic matter, and in general, such heterotrophic activity is a major sink for the aquatic DOM pool (Tranvik et al., 2009; Guillemette et al., 2013).

Interactions between heterotrophic bacteria and DOM are complex and may shape the apparent composition of both of these key ecosystem components. There is accumulating evidence, especially regarding incubation experiments under controlled conditions, that the availability and composition of organic substrates favour specific bacterial groups and in this way shape bacterial community composition (BCC) and community metabolism, and vice versa (Kritzberg et al., 2006; Romera-Castillo et al., 2011; Guillemette et al., 2016). Moreover, bacteria do not only consume and degrade DOM, but also produce and release an array of autochthonous organic compounds during cell growth, division, and death (Kawasaki & Benner, 2006), thereby influencing the availability, composition and biogeochemical cycling of C in the biosphere (Battin et al., 2008; Osterholz et al., 2016).

Amazon floodplains consist of a mosaic of wetland habitats including periodically flooded forests, floating macrophytes and open water environments, periodically inundated by the lateral overflow of rivers and rain coupled to seasonal variations in the annual hydrological pulse (Flood Pulse Concept) (Junk et al., 1989). Additionally, waters in the Amazon basin are classified on the basis of their appearance in white, black and clear waters (Sioli, 1984). Whitewaters are very turbid because of high concentrations of suspended solids. In contrast, black waters are characterized by low loads of suspended sediment and high concentrations of humic DOM (Sioli 1984). Finally, clear waters have an intermediary concentration of

both, suspended solids and humic DOM.

In Amazon, large amounts of terrestrial DOM from inundated forests and soil leachates enter floodplains following inundation (allochthonous sources), together with loads of DOM derived from other aquatic primary producers, such as herbaceous plants and phytoplankton (autochthonous sources). Seasonal variations in hydrodynamics and carbon sources result in an enormous diversity of both terrestrial and aquatic DOM potentially available for bacterial metabolic use, ultimately causing CO_2 super-saturation relative to atmospheric equilibrium, commonly observed in Amazon aquatic systems (Richey et al., 2002; Mayorga et al., 2005; Amaral et al., 2018). Considering the quantitative significance of the Amazon basin in terms of area and water volume as well as the important role of floodplains in global C processing, it is of central importance to study the composition and reactivity of carbon compounds as well as linkages to heterotrophic bacteria in these environments under natural conditions.

Previous studies, using isotopic and elemental analyses, revealed that the DOM present in the Amazon rivers system is highly refractory and derived predominantly from terrestrial inputs (allochthonous) (Ertel et al., 1986; Hedges et al., 1986; Hedges et al., 1994; Mayorga et al 2005). However, labile carbon derived from autochthonous sources was also shown to play an important role in the carbon dynamics of these systems (Mayorga et al. 2005). Regarding Amazon floodplain lakes, there are apparently two distinct DOM pools: a large pool of more refractory DOM from C_3 allochthonous plants sources (terrestrial C from uplands, riverine inputs, leached from flooded forests and soils), and a small pool of labile DOM compounds derived from C_4 macrophytes and phytoplankton, which are consumed and rapidly turned over by heterotrophic bacteria (Waichman, 1996; Melack & Forsberg, 2001; Ellis et al 2012; Mortillaro et al., 2016; Amaral et al 2018;).

Here, we present results from a systematic characterization of DOM using optical properties (absorbance and fluorescence) and an investigation of the coupling between the apparent DOM properties and BCC over a complete hydrological cycle in an Amazon floodplain system. We also investigated the uptake of distinct natural DOM sources by the indigenous lake bacterial community. We hypothesized that: (i) DOM quantity would be affected by the strong seasonality in hydrological conditions (variations in water level and connectivity with terrestrial surroundings) typical from

these tropical floodplain systems; ii) as well its origin; iii) and quality; (iv) DOM pool would be composed by a small pool of freshly and labile DOM, with a larger pool of terrestrial DOM, as previously suggested for Amazon waters (Mayorga et al., 2005); (v) bacteria would preferentially consume and mineralize labile DOM from autochthonous sources due its greater accessibility and nutritional quality (Bianchi, 2011; Guillemette et al., 2013); (vi) seasonal changes in DOM quantity and composition would be tracked by parallel changes in BCC because bacteria are the main DOM consumers.

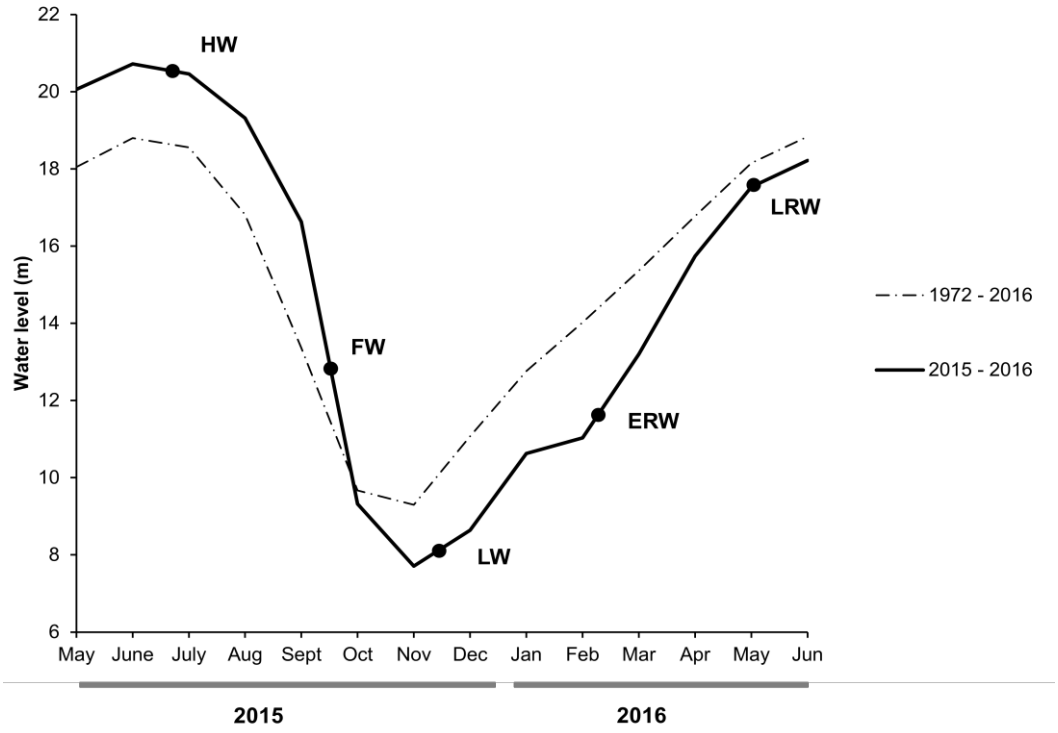
2. Methods

2.1 Study area and sampling

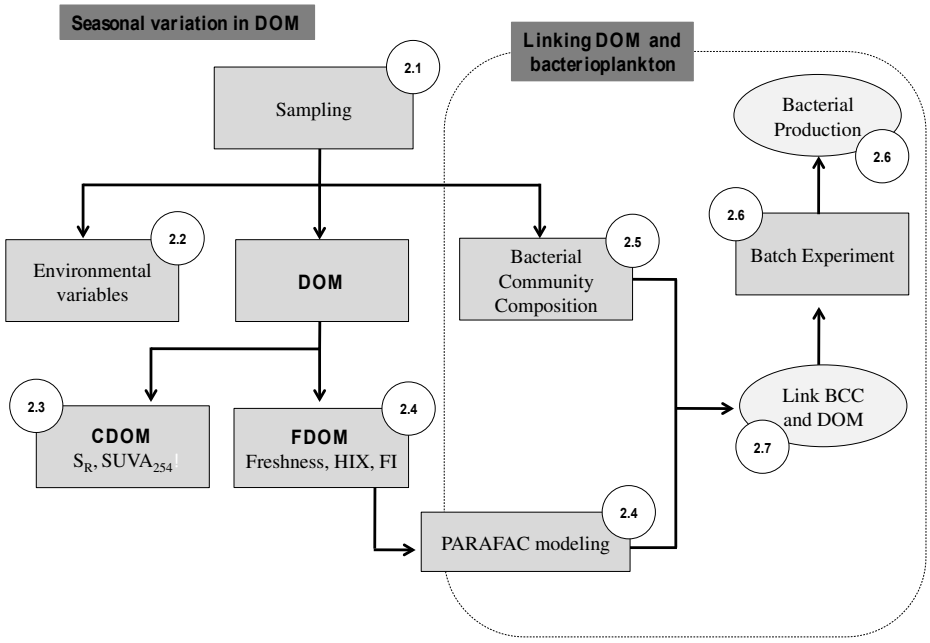
Floodplain lake Janauacá (3°23′ S, 60°18′ W; altitude 32 m) is located in the middle of the Amazon basin, on the south margin of Solimões River, being permanently connect to it year-round by a 12 km long channel (more details can be found in de Melo et al., 2019). We performed seasonal sampling between June 2015 and May 2016, for a total of five campaigns in different phases of the annual hydrological cycle (Supplementary Figure II - S1): i) high water (June 2015 - HW), ii) falling water (September 2015 - FW), iii) low water (November 2015 - LW), iv) early rising water (February 2016 - ERW) and v) late rising water (April 2016 - LRW). Water levels in the lake varied 10 m between low and high water seasons; in synchrony with the Solimões River flood pulse (Fig II - S1).

To track changes to DOM composition we used samples from all of these campaigns, but to specifically assess the BCC-DOM coupling we used samples for only four campaigns (excluding the LW campaign). Samples were collected just below the water surface (50 cm depth) at five sites (see de Melo et al., 2019): (1) drainage basin - a region of the lake draining upland areas, characterized by seasonally variable clear and humic waters; (2) open lake Janauacá - an open water area in the northern region of the floodplain lake, strongly influenced by Solimões waters during high water periods; (3) macrophyte bank - site located near to the lake margin, dominanted by different aquatic grass species throughout the year (*Paspalum repens* P.J.Bergius, *Oryza rufipogon* Griff, *Luziola spruceana* Benth. ex Döll, *Eugenia inundata* D.C.; Amaral et al., 2018); (4) channel - the permanent connecting channel between the floodplain lake and the Solimões River (12 km long);

(5) Solimões River - the main source of river water to the lake. Water samples were stored in acid-washed insulated boxes and transported in the dark to a field laboratory for further analysis and preservation within no more than 3 h. Figure II - S2 provides a schematic overview of the methodology employed here, which is detailed below.



Supplementary Figure II - S1: Water level in different phases of the hydrological annual cycle covered by the present study. Dashed line shows the historical water level (m) based on 44 years of daily records for the Solimões River at the Manacapuru gauging station. Solid line indicates observed variation in mean monthly water levels from May 2015 to June 2016. Abbreviations: high water (HW), falling water (FW), low water (LW), early rising water (ERW), late rising water (LRW). Source: Brazilian National Agency of Waters (http://www.snirh.gov.br/hidroweb/).



Supplementary Figure II - S2: Schematic diagram of the research methodology. Note that numbers are indicating the correspondent section in the text.

2.2 Environmental variables

Water temperature and electrical conductivity were measured using a CTD profiler (Castway, Sontek Inst. Co, San Diego, CA, USA). Other physical parameters such as pH and dissolved oxygen (DO) were determined using specific probes (YSI Inst., Yellow Springs, OH, USA, model Pro-ODO) sampling at 4 Hz with data reported at 0.3 m intervals. Water transparency was determined with a Secchi disc. All of these measurements were obtained directly in the field.

Samples for chlorophyll-*a* (chl-*a*) were filtered through GF/F filters (Whatman®, Maidstone, UK) using a vacuum pump and stored frozen in the dark until analysis. We determined the chl-*a* concentration for each filter using a spectrophotometer, following filter maceration and extraction in 90% acetone (Wetzel & Likens, 2000). For calculation we used the trichromatic equations of Strickland & Parsons (1992). Dissolved organic carbon (DOC) samples were filtered through pre-combusted (450–500 °C for 1 h) glass fiber GF/F filters (Whatman®, Maidstone, UK) and stored in pre-cleaned insulated amber glass vials and kept at 4 °C until the analysis (maximum of

two weeks). DOC was determined using a total organic carbon analyzer (TOC-V Shimadzu, Quioto, Japan).

2.3 Coloured dissolved organic matter (CDOM)

Water samples for optical analyses were filtered through 0.2 µm membrane filter (Millipore® Isopore, Burlington, MA, USA) and stored in acid-washed amber glass vials at 4°C for a few days prior to analysis. Absorbance measurements were conducted at room temperature in the dark. Absorbance spectra of CDOM were performed from 200 to 800 nm in a 0.01 m quartz cuvette (1 nm intervals and dwell time of 0.2 s), and compared against ultrapure water blanks using a FS5 spectrofluorometer (Edinburgh Instruments, Livingston, UK). We further estimated the specific ultraviolet absorbance at 254 nm ($SUVA_{254}$) and the slope ratio (S_R) for each sample. The $SUVA_{254}$ (L mgC^{-1} m^{-1}) was calculated dividing the sample-specific absorbance coefficient at 254 nm by the cuvette path length (1 cm) and then by the DOC concentration (mg L^{-1}). $SUVA_{254}$ is commonly used as a proxy of aromatic content, with higher values associated with greater aromaticity (Weishaar et al., 2003). S_R was defined as the ratio of the spectral slope between 275-295nm by the spectral slope between 350-400 nm. S_R was determined from the linear regression of the log-transformed absorbance spectra. This parameter has been negatively associated to DOM molecular weight (Helms et al., 2008).

2.4 *Fluorescence dissolved organic matter (FDOM)*

Following the same basic filtering and storage procedure as for absorbance measurements, we collected excitation-emission matrices (EEM) of the FDOM in 0.01 m quartz cuvettes using a FS5 spectrofluorometer according to the following method; excitation wavelengths (λ_{ex}) ranged from 240 to 450 nm in 10 nm increments, and emission wavelengths (λ_{em}) from 300 to 560 nm in 2 nm increments with a dwell time of 0.25 s, bandwidth of 5 nm. All EEMs were background corrected against a Milli-Q water. Subsequently the sample signal (S) was corrected for the reference (R) lamp signal, to get S/R. All fluorescence spectra were corrected for inner filter effects using the absorbance-based approach (Lakowicz, 2006; Kothawala et al., 2013). Fluorescence intensities of the EEM were calibrated to Raman units by dividing the intensity with the Raman area of Milli-Q water integrated at λ_{ex} of

350 nm, and over an λ_{em} range of 380 to 420 nm (Lawaetz & Stedmon, 2009). We used the software Matlab and the DOMcorr toolbox to perform all corrections (Murphy et al., 2010).

Three additional indices were derived from the matrices: Fluorescence Index (FI), Humification Index (HIX) and Freshness Index (Fresh). The FI was determined as the ratio between emission wavelengths (λ_{em}) at 470 and 520 nm with excitation at 370 nm (Cory et al., 2010). Higher FI values (~1.8) are associated with DOM derived from bacterial and algal sources, while lower values (~1.2) indicate DOM derived from terrestrial material (for example, soil, leaves, wood) (McKnight et al., 2001). Intermediate values (~1.4) indicate a mixture of both sources. The HIX was calculated as the area under the emission spectra between λ_{em} 435–480 nm divided by the peak areas between λ_{em} 300–345 nm + 435–480 nm at an λ_{ex} 254 nm. HIX is a proxy of DOM humic content, where higher values indicate higher concentrations of humic substances (Ohno, 2002). The Freshness Index is an indicator of recently produced DOM, with higher values indicating newly produced DOM. This index was estimated as the ratio of the emission intensity at λ_{em} 380 nm by the maximum emission intensity in the interval λ_{em} 420-435, both at an λ_{ex} of 310 nm (Parlanti et al, 2000).

We performed a parallel factor analysis (PARAFAC) to identify components within the EEM that represent independently varying regions of the EEM and represent different types of fluorescencent DOM using Matlab software and the DOMFluor toolbox (Stedmon et al., 2003). The analysis was developed using EEM for altogether 388 samples from Lake Janauacá. After including non-negativity constraints, we removed an outlier and validated the model using split-half analysis and random initialization (Stedmon et al., 2003). We further identified the nature of each PARAFAC component by comparison to previously reported components using the OpenFluor database (Murphy et al., 2014).

C1 exhibits both UVC and UVA excitation maxima (260 and 350 nm, respectively) and an emission peak at 480 nm, similar to peak C, which is described as humic-like material exported from terrestrial sources (Coble, 1996, Figure II - 1). This component is characterized by DOM rich in aromatic compounds and of high molecular weight and is widespread in aquatic environments (Stedmon et al., 2003; Williams et al., 2010). C2 has been described as a proxy for terrestrial DOM, rich in

fulvic acids and is often referred to as A + M peaks (Coble, 1996). C3 represent terrestrially derived DOM of intermediate molecular weight (Lambert et al., 2016). Finally, C4 has properties similar to tryptophan-like fluorescence, an amino acid signal indicative of autochthonous sources (Stedmon et al., 2003).

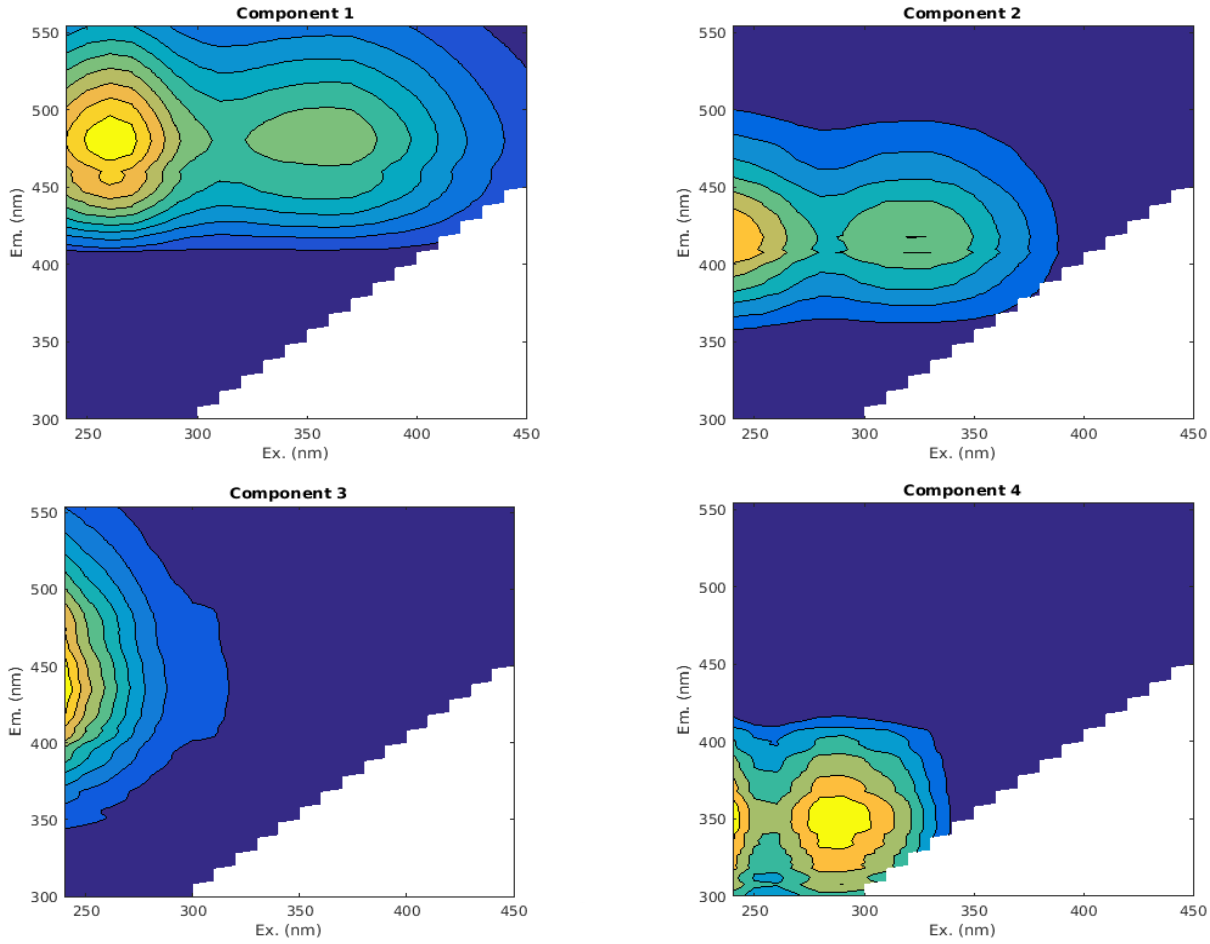


Figure II - 1: Excitation-emission spectra of four fluorescing components (C1, C2, C3 and C4) describing the major fluorescing regions as identified in our study using parallel factor analysis (PARAFAC).

2.5 Bacterial Community Composition (BCC)

Samples for determination of BCC were first prefiltered through 3 µm pore size polycarbonate membranes (Whatman® Nucleopore, Maidstone, UK) to remove larger particles. Filtrates were subsequently passed through 0.2 µm polycarbonate membranes (Millipore® Isopore, Burligton, MA, USA) to collect the free-living

bacterial size-fraction. Membranes were stored at -80 ºC until bacterial DNA was extracted from filters using the phenol-chloroform method followed by purification with Amicon Ultra-4 centrifugal filters (Millipore® 100kDa) (Ganesh et al., 2014). Using primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACT ACHVGGGTATCTAATCC-3') we amplified the V3/V4 region of the 16rRNA gene (Herlemann et al., 2011). We performed all PCR reactions with KAPA High - fidelity Hotstart ready mix (KAPA Biosystems, Boston, MA, USA) with an initial step of 95ºC for 3 min, 25 cycles of 98ºC for 20s, 62ºC for 15s, 72ºC for 15s and finally 72° for 60s. Subsequently, PCR products were purified with magnetic beads in the AMPURE XP kit (Beckman Coulter, Pasadena, CA, USA) and Indexed with Nextera XT kit V2 (Illumina, Inc, San Diego, CA, USA). An additional step of purification with magnetic beads was performed, and then a combined pool was prepared by mixing 5 µl from each library. High-throughput sequencing was performed on an Illumina Miseq2000 instrument using a paired-end approach and 2 x 250 bp chemistry (Laboratório Multiusuário Centralizado para Sequenciamento de DNA em Larga Escala e Análise de Expressão Gênica, Univerdade Estadual Paulista, Jaboticabal, São Paulo, Brazil). All sequences were submitted to the BioSample database hosted by NCBI (https://www.ncbi.nlm.nih.gov/sra/SRP127556).

Quality filtering, denoising and removal of potential chimeras and non-bacterial sequences were performed with UPARSE (Edgar, 2013) following a previously established pipeline (Logares et al., 2014; Logares, 2017). In summary, paired-end reads were merged and all sequences shorter than 100 bp were discarded. Merged sequences were clustered into operational taxonomic units (OTUs) at 97% identity cutoff using UPARSE (Quast et al., 2013), and the taxonomic classification was done with BLASTn against SILVA 119.1 (Zhang & Fang, 2000) with at least 75% similarity threshold. For further analyses, all chloroplasts and Archaea sequences were discarded. To enable comparisons between samples OTU table was randomly subsampled (rarefied) based on the sample with the least number of reads (10,341).

2.6 DOM uptake experiments

To study microbial uptake of DOM we performed a batch culture experiment with two distinct and abundant natural sources of DOM found in lake Janauacá: soil and macrophytes (*Panicum repens*). We chose *P. repens* because this was the most

abundant macrophyte species observed in the study area during the experiment (May 2016). *P. repens* is a floating grass, that converts atmospheric carbon dioxide into biomass through a C_4 metabolic pathway and can cover large areas of Amazon floodplains (Hess et al., 2003; Silva et al., 2013). Macrophyte-derived DOM represents an autochthonous source with high amounts of labile DOM components (Bertilsson & Jones, 2003) and has been suggested as the main source of labile DOM to bacterial communities in Amazon floodplain lakes (Waichman, 1996; Melack & Forsberg, 2001). Concomitantly, soil is also an important source of DOM to the lake, especially during the initial stages of flooding (rising waters), when large amounts of terrestrially derived DOM are leached from surrounding soils and river inflowing (Junk et al., 1989).

We first prepared a slurry with 200g of margin soil collected from the lakeshore and 800 ml of Milli-Q water that was subsequently stirred for 3 hours. For macrophytes, we collected and washed leaves, roots and stems of *P. repens* from the lake and mixed this with 500 ml of Milli-Q water. This extract was kept in an ultrasonic water bath for 3 hours, to ensure that sufficient amounts of DOM were extracted. We then filtered each mixture (macrophytes and soil) sequentially through 20, 3, 0.7 and 0.2 *μ*m pore size filters to remove particulate organic matter and microbial cells. We then diluted DOM solutions with Milli-Q water to a concentration analogous to in situ conditions (<5 mg C L^{-1}) and stored these extracts at 4^{o}C until the beginning of the incubations (3 days).

We used a dilution culture approach to cultivate bacterial cells from lake Janauacá by filtering lake water twice through 0.7 μm filters and adding the filtered inoculum to DOM solutions 1:10 (v:v). The experiment consisted of three different treatments incubated in triplicate with the addition of lake bacteria: (i) soil DOM (S), (ii) macrophyte DOM (M) and (iii) a mixture of soil and macrophyte DOM (50% each) (S+M). Incubations were carried out in amber glass bottles (500 ml) for 124 hours in the dark at 30^{o}C (in situ temperature) with constant shaking (x RPM), to avoid photochemical alteration of the DOM and sedimentation, respectively. We took samples for bacterial production (BP) initially (T0) and after 24, 48, 72, and 124 hours of incubation. BP was estimated by the standard H^3-leucine incorporation method (Kirchman et al., 2004), using a carbon:protein conversion factor of 0.86 (Simon & Azam, 1989).

2.7 Statistical Analyses

In this study we used Canonical Analysis of Principal Coordinates (CAP) (Anderson & Willis, 2003) to investigate the link between PARAFAC components (intensities in Raman units) and BCC (relative abundance of bacterial operational taxonomic units - OTUs) in an Amazon floodplain under natural conditions. We computed dissimilarity matrices based on Bray-Curtis (BCC) and Euclidean distance (PARAFAC) and then, to avoid negative eigenvalues, we took the square root of all dissimilarity matrices (Legendre & Legendre, 1998). We performed a Principal Coordinate Analysis (PCoA) to reduce the number of orthonormal axes to be included in CAP analysis, ensuring that patterns seen on our plots would not be over-parameterized and misleading (Anderson & Willis, 2003). Here, this was done with the aim of choosing the number of axes representing at least 75% of the total variation (DOM quality m=4 and bacterial OTUs m=10, cumulative eigenvalues in table S1) of each data set to ideally lose minimal information and also avoid too many dimensions contributing noise and hampering the analysis (Osterholz et al., 2016).

The next step was to check the significance of canonical correlation (Canonical Correlation Analysis – CCorA) computed by permutation (n = 9999). Finally, we considered correlations of individual PARAFAC components (intensity in Raman units) and OTUs (relative abundance) with the first two canonical axes using Spearman rank correlation followed by a false discovery rate (FDR) test for multiple comparisons. We selected all OTUs with an average relative abundance ≥0.1 and significant relationships (FDR adjusted p-value ≤0.05).

DOM optical properties (coefficients, indexes, and components) were tested for differences among group means (seasons and sites) using two-way ANOVA. To visualize which specific group means were different, we performed *post hoc* Tukey tests. Significant results were plotted in violin plots to visualise the distribution of the data and its probability density (kernel density estimation). Repeated measures ANOVA was performed to test for DOM treatment effects over the time, being followed by a *post hoc* Tukey test. All statistics (sections 2.7 and 2.8) were performed in R (version 3.3.3, R Development Core Team, http://cran.r-project.org/) using the packages “vegan”, “ape” and “psych” (Oksanen et al., 2015; Paradis et al., 2004, Revelle, 2018). To create figures, we used the package ggplot2 (Wickham, 2009).

3. Results

3.1 DOM characterization

DOC concentrations were generally low, ranging between 2.7 and 5.9 mg C L^{-1} (with the exception of one sample where the concentration was 9.0 mg C L^{-1}), with a mean annual average of 4.8 mg L^{-1} (table 1). The specific absorbance ($SUVA_{254}$) index varied between 1.8 and 5.2 L mg C^{-1} m^{-1} and showed a negative and strong correlation to DOC concentrations (R^2=0.59 p<0.001). The S_R ranged between 1.1 and 1.7, and had a strong positive relationship with the Freshness Index, suggesting that recently produced DOM were of overall low molecular weight. The Freshness Index also showed a strong positive relationship with chl-*a* concentrations and water temperature (both were correlated, Fig. II - 2a and II - 2b), indicating that the fresh DOM likely originated from recent primary production (Figs. II - 2b and II - 2c).

Table II - 1: Dissolved organic matter properties and environmental variables of each sample performed in Lake Janauacá during the studied period. Abbreviations: dissolved organic carbon (DOC), Slope ratio (S_r), Specific absorbance at 254 nm ($SUVA_{254}$), Florescence Index (FI), Freshness Index (Fresh), Humification Index (HIX), water temperature (Temp), dissolved oxygen (O_2), electrical conductivity (Condut)

Campaign	Site	Sample	DOC (mg L-1)	SR	SUVA254 (L mgC-1 m-1)	FI	Fresh	HIX	Temp (ºC)	secchi (m)	pH	O2 (mg L-1)	Condut (µS cm-1)	Chlorophyll-*a* (ug L-1)	POC (mg L-1)
High water (HW)	basin	HW_ba	4.93	1.22	4.03	1.67	0.50	13.84	29.2	1.6	6.6	3.2	42.3	6.0	0.4
	lake	HW_la	4.37	1.26	4.12	1.69	0.50	13.68	28.8	1.1	6.2	1.7	40.8	1.3	0.4
	macrophyte	HW_ma	5.24	1.29	3.47	1.69	0.49	18.54	30.7	1.6	6.6	0.8	58.0	13.3	0.3
	channel	HW_ch	5.13	1.34	3.20	1.68	0.50	13.48	29.2	1.2	6.6	1.6	53.5	1.6	0.6
	river	HW_sol	5.13	1.31	3.02	1.71	0.49	11.77	28.6	0.7	6.8	1.8	56.4	0.5	0.7
Falling water (FW)	basin	FW_ba	9.02	1.50	1.83	1.70	0.60	11.27	30.9	2.0	6.4	4.3	23.9	8.3	0.8
	lake	FW_la	3.59	1.44	4.01	1.73	0.58	10.96	30.6	1.0	6.8	5.1	48.1	8.2	1.3
	macrophyte	FW_ma	3.92	1.53	4.14	1.72	0.63	9.00	32.3	1.5	6.8	1.2	39.0	24.1	1.9
	channel	FW_ch	3.91	1.40	4.09	1.73	0.59	11.23	30.9	0.9	6.4	2.6	33.8	15.6	1.9
	river	FW_sol	2.70	1.32	4.44	1.74	0.55	9.97	30.7	0.4	6.4	3.1	35.8	2.1	3.2
Low water (LW)	basin	LW_ba	5.90	1.56	2.91	1.65	0.60	9.15	31.3	0.2	6.3	5.6	20.5	86.0	4.3
	channel	LW_ch	5.88	1.72	2.33	1.74	0.64	10.70	32.4	0.0	5.9	4.1	34.3	137.8	23.2
	river	LW_sol	3.69	1.34	3.36	1.74	0.54	15.74	31.4	0.2	6.9	5.7	69.9	7.2	1.9
Early rising water (ERW)	basin	ERW_ba	4.30	1.36	4.05	1.70	0.60	11.26	31.0	0.5	6.8	7.6	46.9	19.8	2.0
	lake	ERW_la	5.38	1.29	3.35	1.82	0.73	12.39	28.2	0.5	7.3	7.3	86.9	106.7	4.5
	macrophyte	ERW_ma	5.54	1.13	4.02	1.75	0.57	15.20	31.8	0.2	6.3	2.4	62.5	14.8	2.8
	channel	ERW_ch	3.62	1.16	5.17	1.71	0.51	12.45	29.3	0.1	7.3	5.6	72.1	2.2	2.3
	river	ERW_sol	3.55	1.13	5.25	1.71	0.51	15.14	29.3	0.1	7.2	5.5	79.7	1.4	2.5
Late rising water (LRW)	basin	LRW_ba	5.35	1.23	4.60	1.66	0.50	22.35	29.4	1.2	6.6	4.2	46.0	-	-
	lake	LRW_la	4.52	1.19	4.38	1.74	0.56	18.38	29.7	0.7	6.4	4.4	72.0	-	-
	macrophyte	LRW_ma	5.17	1.23	4.18	1.73	0.53	15.90	30.5	1.0	6.4	4.4	49.6	4.0	-
	channel	LRW_ch	4.90	1.11	4.82	1.71	0.48	22.58	28.6	0.2	6.4	3.0	48.7	2.1	0.9
	river	LRW_sol	4.95	1.13	4.71	1.71	0.47	24.69	28.7	0.2	6.5	3.0	49.6	2.1	1.0

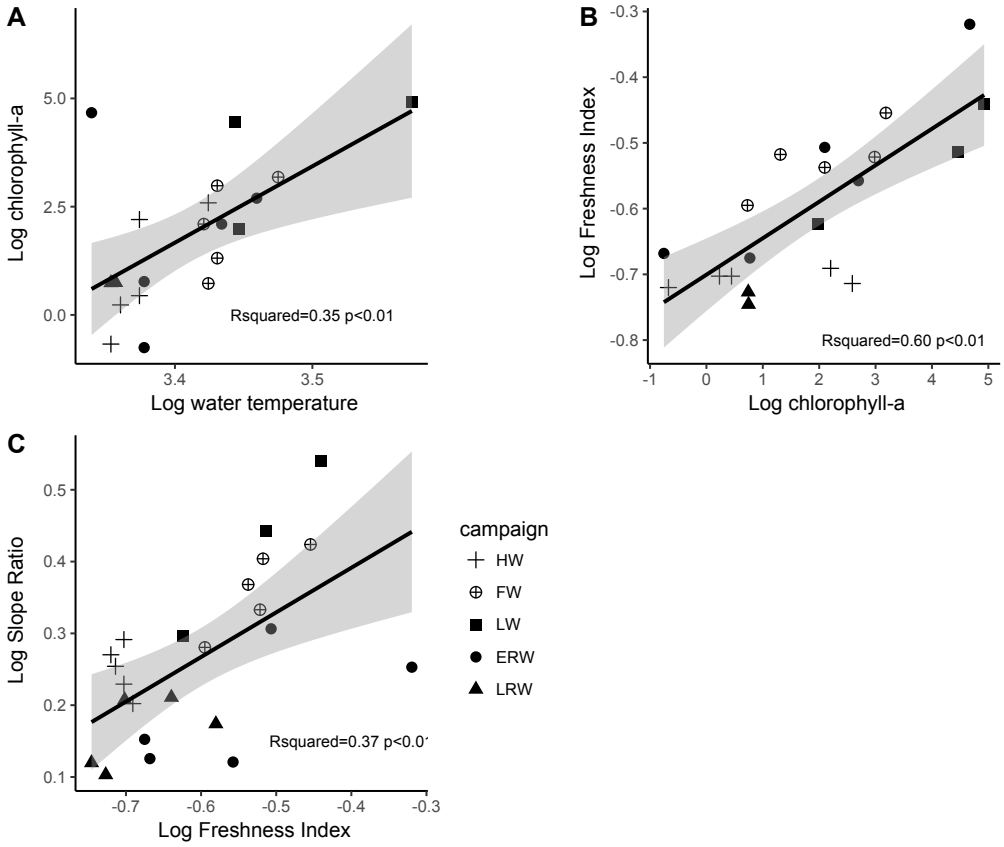


Figure II - 2: Relationships between (A) log water temperature and log chlorophyll-*a* (*n*=20), (B) log chlorophyll-*a* and log Freshness Index (*n*=20) and (C) log Freshness Index and log Slope ratio (*n*=23). Abbreviations: high water (HW), falling water (FW), low water (LW), early rising water (ERW), late rising water (LRW).

3.2 Seasonal patterns in DOM composition

Our results revealed that the contribution of each optical index and PARAFAC component varied strongly across different seasons, but this temporal dynamics was still less evident than the differentiation between sites (Figs. II - 3 and II - 4). Nevertheless, DOC concentration did not vary significantly across either time or space (ANOVA p-value≥ 0.05). DOM spectral slope (S_r; indicator of molecular weight) tended to be higher during the low water (LW) period concomitant to the lowering of the water level (Figs. II - 3a and II - 3b). HIX was significantly higher in

late rising water season (LRW) (Fig. II - 3c), indicating that the inundation of terrestrial surroundings provided humic substances to the lake. Fluorescence Index (FI) did not present a clear seasonal pattern (Fig. II - 3d, ANOVA p-value≥0.05), but a significant difference was seen between open lake and basin sites, being higher at the lake site the farthest away from land (ANOVA p-value≤0.05). C1, C2 and C3 were the most abundant PARAFAC components identified for the studied waters and varied over the year (Figs. II - 4 and II - S3). We found that C1 and C2 responded to water level and showed significantly higher values during high water (HW) and lower values in falling (FW) and low waters (LW) (Figs. II - 4a and 4b). C3 reached maximum values also during high water (HW), but differently from C1 and C2, it continually decreased over the sampling period being at its lowest during the LRW (Fig. II - 4c). C4 was the least abundant component and was significant lower in HW and LRW (Fig. II - 4d, ANOVA p-value≤0.05).

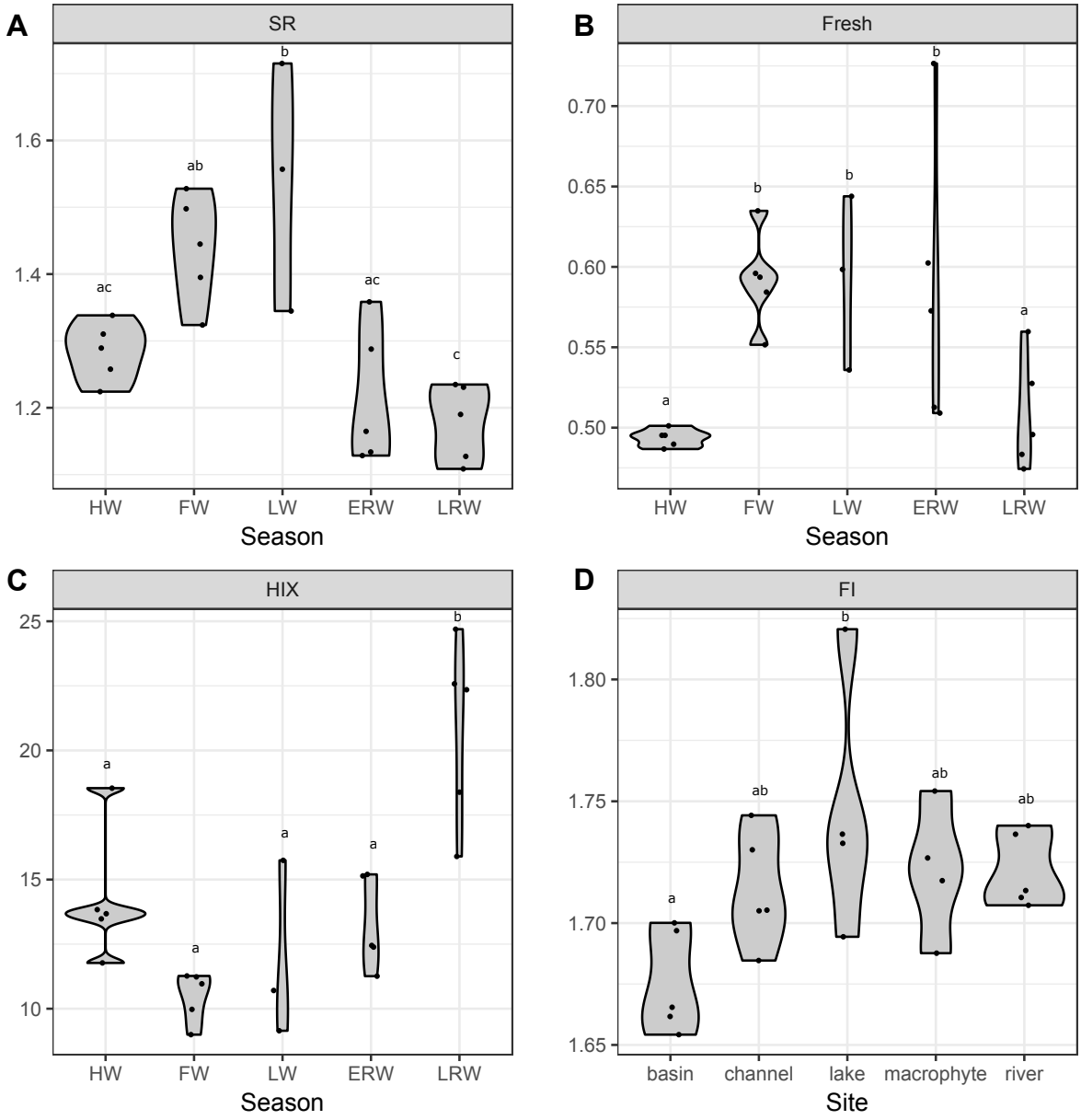


Figure II - 3: Violin plots showing: (A) Slope ratio (SR), (B) Freshness Index (Fresh), (C) Humification Index (HIX) during high water (HW), falling water (FW), low water (LW), early rising water (ERW) and late rising water (LRW) seasons; and (D) Fluorescence Index (FI) in different sampling sites. Significant differences between groups ($p \leq 0.001$, *post hoc* tests) are marked with different letters. Note that the scales of the y-axis and labels of x-axis are variable between graphs.

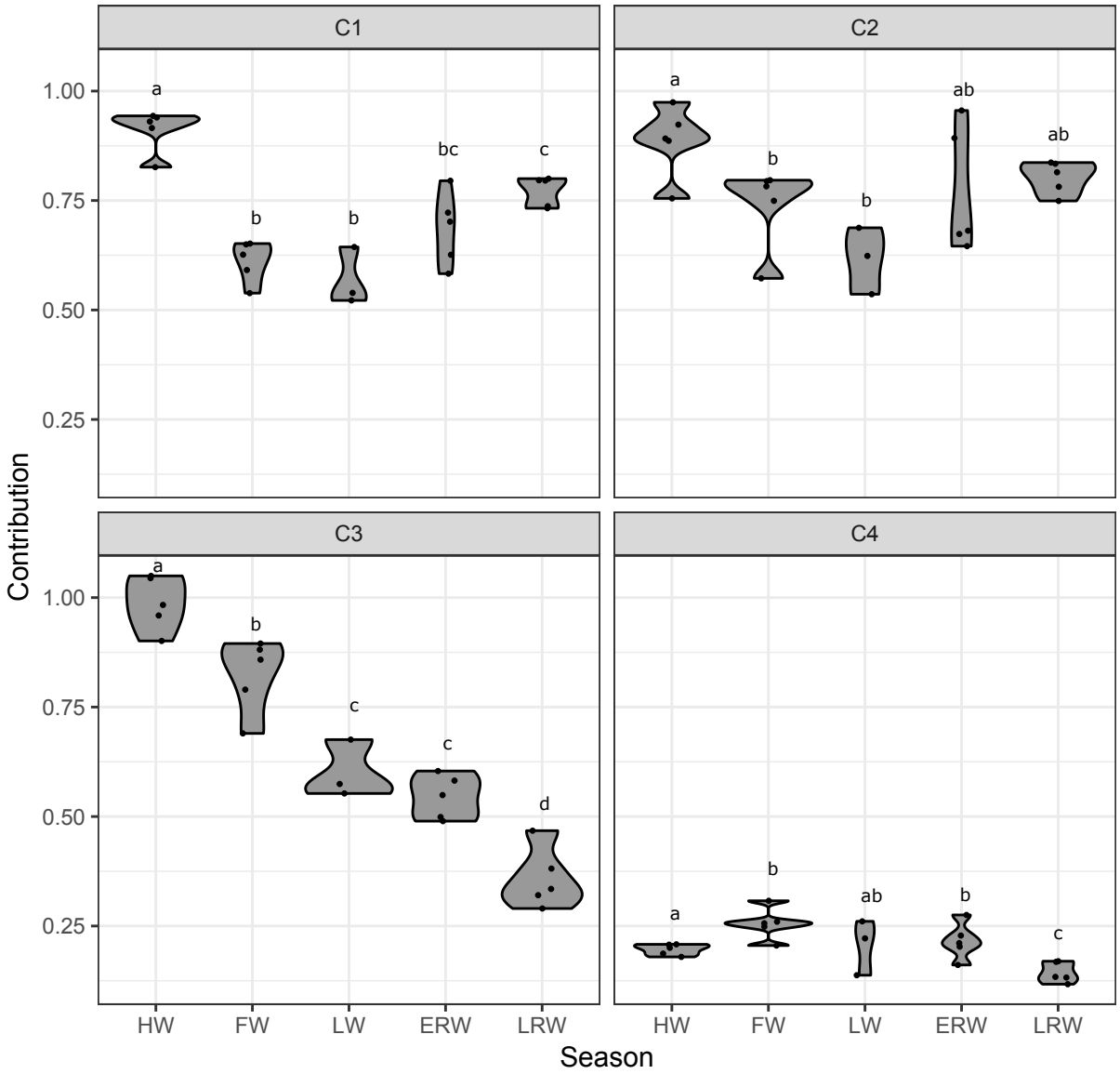
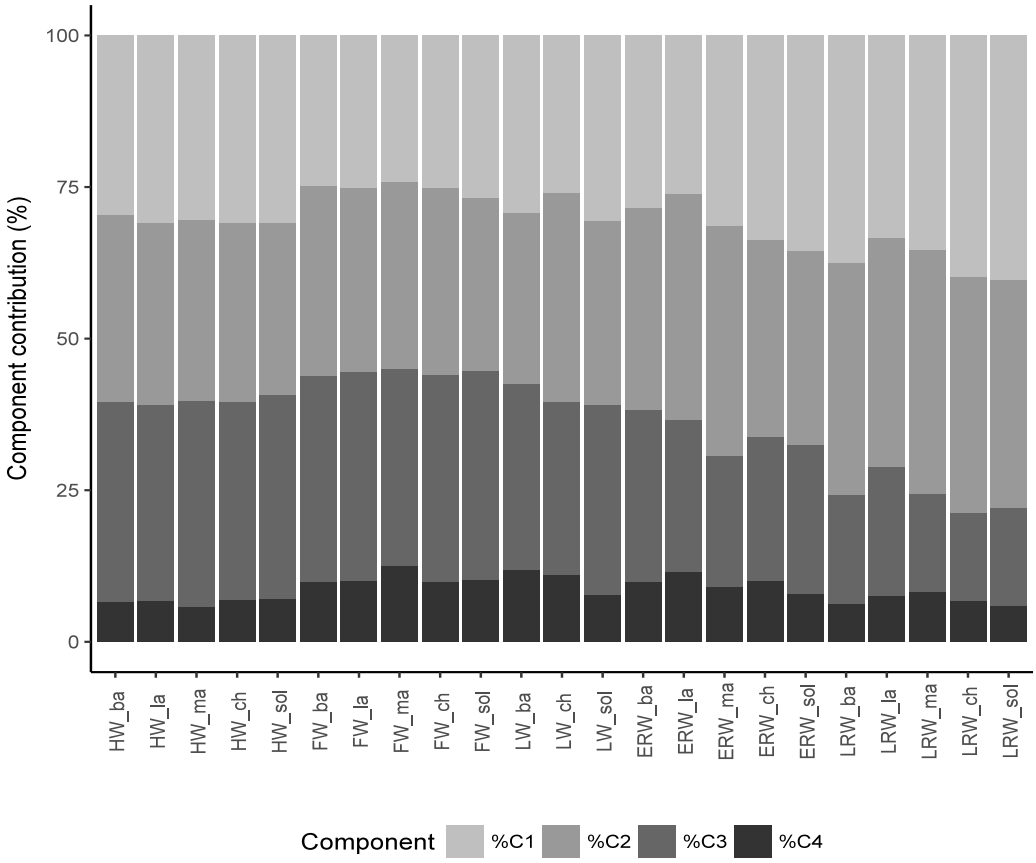


Figure II - 4: Violin plots showing the contribution of each PARAFAC component (C1, C2, C3 and C4 in Raman units) in sampled seasons: high water (HW), falling water (FW), low water (LW), early rising water (ERW) and late rising water (LRW). Significant differences between groups ($p \leq 0.001$, *post hoc* tests) are marked with different letters.



Supplementary Figure II - S3: Histogram with the relative contribution of PARAFAC components in each sample. Abbreviations: high water (HW), falling water (FW), low water (LW), early rising water (ERW), late rising water (LRW), drainage basin (ba), open lake (la), macrophytes (ma), channel (ch), Solimões River (sol).

3.3 Linking bacterioplankton and DOM composition

BP rates from lake Janauacá exposed to distinct sources of DOM typically increased during the course of the incubations for all treatments and varied depending on DOM substrate (Fig. II - 5). The highest BP rates were seen in the mixed treatment (S+M), which reached high values already in the first 24 hours and then stabilized. The behavior of their BP rates over the time was similar to the M treatment (an autochthonous source), but different and higher from the S treatment (allochthonous source) (repeated measures ANOVA $p \leq .05$).

BCC shifted with major differences in the relative abundance of the main bacterial groups over the annual cycle (Figure II – S4). The association between BCC and DOM composition in the form of a pair of canonical axes revealed that the

pattern was driven by differences in hydrology, with distinct components and OTUs being simultaneously more abundant and correlating with a specific hydrological season (Fig. II - 6 and table II - S2). Also, we found that different sampling sites shared more similarities for certain hydrological season, e. g. the channel and Solimões River were very similar at LRW and ERW periods, and the channel was close to the drainage basin and macrophytes bank during FW and HW, respectively (Figure II - 6A). We identified three PARAFAC components, C1, C3 and C4, and 23 OTUs from 6 different phyla (*Proteobacteria* (alpha, beta, gamma), *Actinobacteria, Planctomycetes, Verrucomicrobia, Bacteriodetes* and *Cyanobacteria*) that were significant correlated with this pattern (Fig. II - 6b, Pvalue-FDR≤0.05).

C1 was most strongly correlated with HW while C4 and C3 were associated with the FW period, with C3 being in between (Figs. II - 6a and II - 6b). Regarding OTUs, we identified a similar pattern, indicating that some taxa were correlated with a specific DOM component. For example, the OTU_127 (genus *Synechococcus*) followed the same pattern as component C4, suggesting that they were linked (Fig. II - 6).

All representatives of *Betaproteobacteria* (9 OTUs) seemed to be linked with C1, being more abundant in HW and ERW. In contrast, all representatives of *Alphaproteobacteria* (3 OTUs) were related with FW and HW and negatively correlated with ERW and LRW (Fig. II - 6). The same pattern was seen for C3.

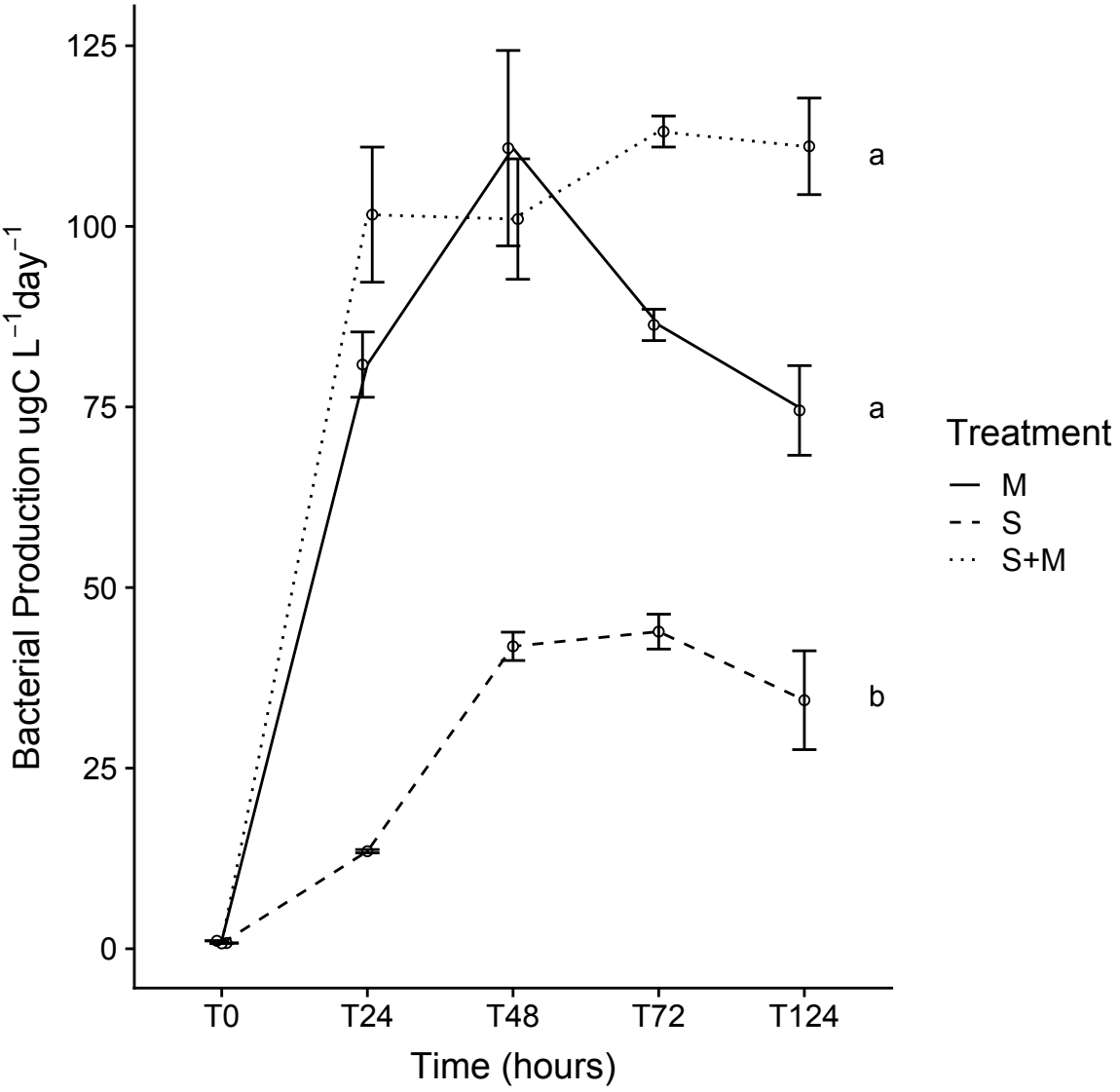


Figure II - 5: Trends of bacterial production (with error bars) in treatments during the incubation of 124 hours: soil DOM (S, dashed line), macrophytes DOM (M, solid line) and a combination of soil+macrophytes DOM (S+M, dotted line_. Letters “a” and “b” denote significant differences between treatments tested by repeated measures ANOVA, followed by *post hoc* Tukey test.

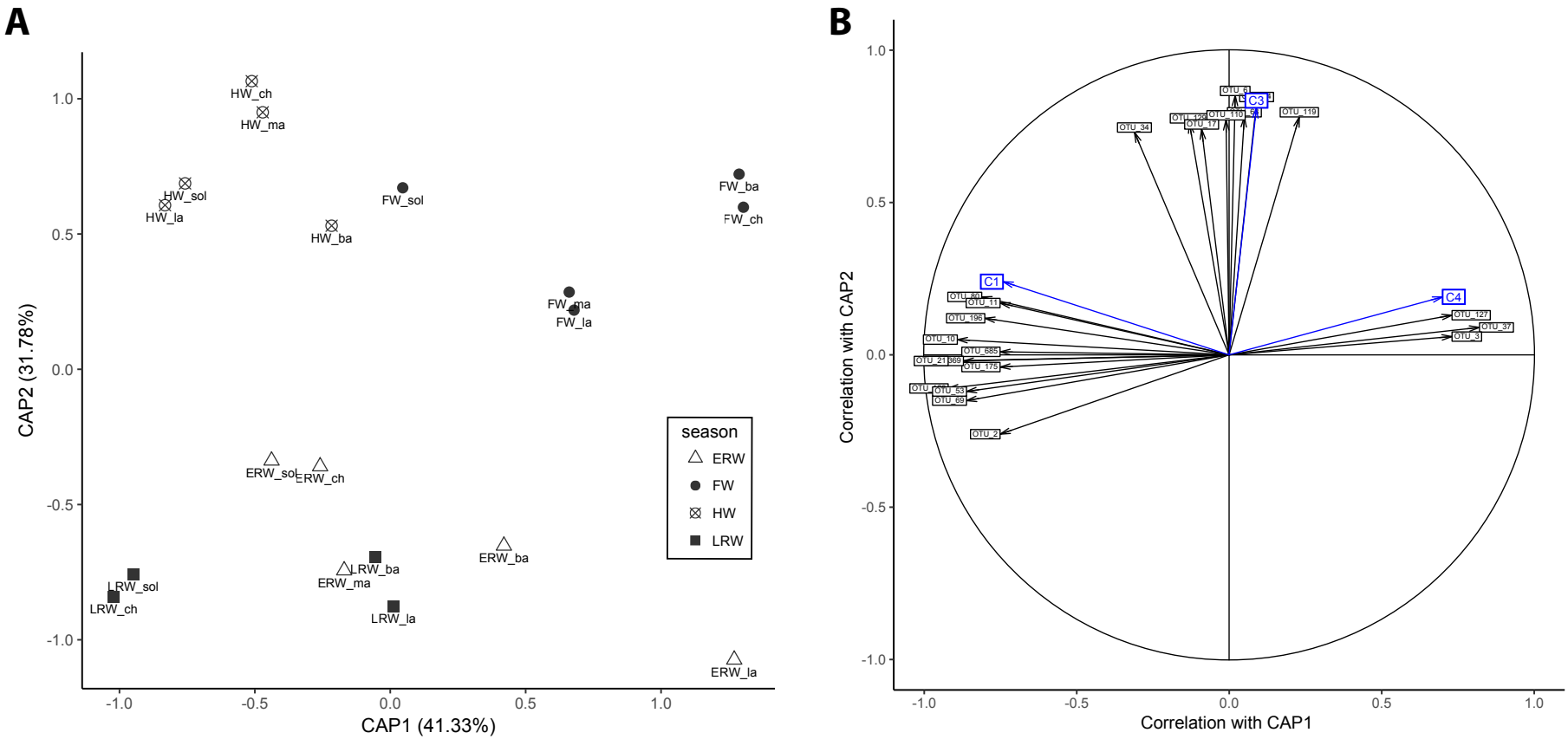


Figure II - 6: (A) Ordination of Canonical Analysis of Principal coordinates axes between bacterial community and all DOM components; (B) the correlated fluorescence components (intensities) and OTUs (relative abundance).

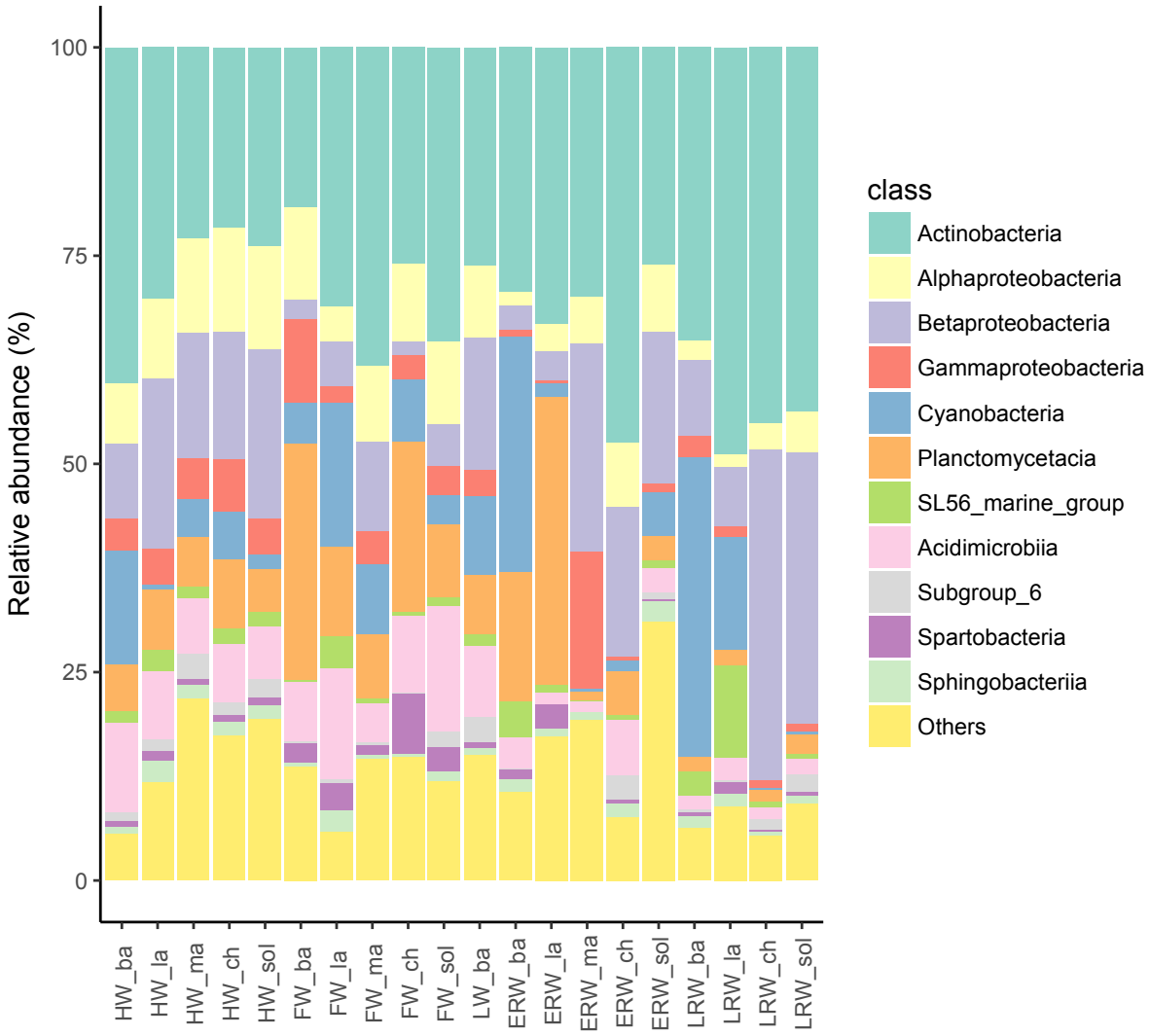


Figure II - S4: Relative abundance (%) of the most abundant classes found in Lake Janauacá in each sample

Supplementary Table II - S1: Number of PCoA axes, eigenvalues, relative and cumulative eigenvalues of each axis. Note that we highlight in grey the cumulative proportion higher than 75% that were 4 axes for DOM components and 10 axes for bacterial community composition (OTU).

	DOM			OTU		
PCoA_Axes (n)	Eigenvalues	Relative_eig	Cumulative_eig (%)	Eigenvalues	Relative_eig	Cumulative_eig (%)
1	0.4289	0.3688	36.8779	0.8482	0.1942	15.2755
2	0.2879	0.2475	61.6317	0.6240	0.1386	26.5131
3	0.1154	0.0993	71.5567	0.5212	0.1108	35.9000
4	0.0908	0.0780	79.3614	0.4263	0.0923	43.5781
5	0.0578	0.0497	84.3326	0.3904	0.0784	50.6098
6	0.0311	0.0267	87.0039	0.3659	0.0673	57.1995
7	0.0280	0.0241	89.4149	0.3084	0.0581	62.7541
8	0.0231	0.0198	91.3998	0.2857	0.0501	67.9002
9	0.0228	0.0196	93.3622	0.2495	0.0432	72.3941
10	0.0171	0.0147	94.8351	0.2300	0.0370	76.5369
11	0.0140	0.0120	96.0399	0.1941	0.0315	80.0324
12	0.0108	0.0093	96.9666	0.1907	0.0264	83.4669
13	0.0096	0.0083	97.7926	0.1831	0.0218	86.7651
14	0.0079	0.0068	98.4745	0.1767	0.0175	89.9483
15	0.0068	0.0058	99.0564	0.1640	0.0135	92.9017
16	0.0048	0.0041	99.4705	0.1455	0.0098	95.5224
17	0.0038	0.0033	99.7960	0.1423	0.0064	98.0858
18	0.0024	0.0020	100.0000	0.1063	0.0031	100.0000

Supplementary Table II - S2: Taxonomic identification of each correlated OTU with an average relative abundance ≥0.1% and the correlation coefficient with canonical axes 1 and 2 (CAP1, CAP2).

OTU_ID	CAP1	CAP2	Phylum	Class	Order	Family	Genus	Species
OTU_3	0.73	0.06	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Pirellula	uncultured_bacterium
OTU_37	0.82	0.09	Verrucomicrobia	Spartobacteria	Chthoniobacterales	Chthoniobacterales_Incertae_Sedis	Terrimicrobium	uncultured_bacterium
OTU_127	0.73	0.13	Cyanobacteria	Cyanobacteria	SubsectionI	FamilyI	Synechococcus	uncultured_bacterium
OTU_6	0.02	0.85	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	uncultured	uncultured_bacterium
OTU_63	0.05	0.78	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	uncultured	uncultured_bacterium
OTU_34	-0.31	0.73	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Planctomyces	uncultured_Planctomyces_sp.
OTU_24	0.09	0.83	Proteobacteria	Gammaproteobacteria	Methylococcales	Methylococcaceae	Methyloparacoccus	uncultured_bacterium
OTU_119	0.23	0.78	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium	uncultured_alpha_proteobacterium
OTU_110	-0.01	0.77	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	uncultured_bacterium
OTU_129	-0.13	0.76	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillales_Incertae_Sedis	Reyranella	uncultured_bacterium
OTU_17	-0.09	0.74	Actinobacteria	Acidimicrobiia	Acidimicrobiales	Acidimicrobiaceae	CL500-29_marine_group	uncultured_bacterium
OTU_80	-0.81	0.19	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Chryseolinea	uncultured_bacterium
OTU_11	-0.75	0.17	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Polynucleobacter	uncultured_bacterium
OTU_196	-0.8	0.12	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Limnohabitans	uncultured_bacterium
OTU_175	-0.75	-0.04	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	12up	uncultured_beta_proteobacterium
OTU_10	-0.89	0.05	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Polynucleobacter	uncultured_beta_proteobacterium
OTU_685	-0.75	0.01	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	uncultured	uncultured_beta_proteobacterium
OTU_369	-0.87	-0.02	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	uncultured	uncultured_bacterium
OTU_21	-0.92	-0.02	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	uncultured	uncultured_bacterium
OTU_123	-0.92	-0.11	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	uncultured	uncultured_bacterium
OTU_53	-0.86	-0.12	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	uncultured	uncultured_bacterium
OTU_69	-0.86	-0.15	Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	Opitutus	uncultured_Verrucomicrobia_bacterium
OTU_2	-0.75	-0.26	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade	uncultured_bacterium

4. Discussion

Once aquatic DOM provides organic carbon and nutrients required for bacterial metabolism and growth, and to tracing the compositional variability within DOM over time and space may help elucidate potential interactions between bacteria and specific DOM components (Kujawinski, et al. 2011).

The strong relationship between primary production (chl-*a*) and DOM age (Freshness Index) appeared to be due to strong seasonal changes. For instance, an increase in water temperature was related to decreased water level (during the falling water season - FW) and a subsequent increase in phytoplankton biomass (chl-*a*). In turn, the freshly produced DOM from primary producers enhanced the Freshness Index and low-weight molecular content (S_R) of bulk DOM. While Amazonian floodplains are among the most productive ecosystems in our biosphere (Melack & Forsberg, 2001), phytoplankton production is generally considered a minor C source, at least at the regional scale (only 8%, compared to 52% of macrophytes and 32% of flooded forest trees) (Junk, 1985; Melack & Forsberg, 2001; Melack et al., 2009). However, in some lakes phytoplankton biomass can still reach high levels (Forsberg et al., 2017), especially during low waters periods as found here (falling, low, and early rising water). Additionally, high phytoplankton excretion rates have been reported in tropical systems, associated with high incident light and rapid nutrient exhaustion, promoting the release of labile DOM that fuels bacterial growth and respiration (Morana et al., 2014; Freitas et al., 2017; Amaral et al 2018).

Our results suggested that this fresh DOM is mainly produced and released by phytoplankton (an autochthonous source). Nonetheless, we highlight the direct role of macrophytes as an alternative source of labile DOM and also propose an indirect effect as a natural fertilizer for Amazon floodplains, where nitrogen and phosphorus are limiting factors (Devol et al., 1984; Forsberg, 1984). The dominant species in Lake Janauacá during this study was *P. repens* (C_4 metabolism), a perennial aquatic grass suggested to host N_2 fixing bacterial symbionts (Martinelli et al., 1992; Junk & Piedade, 1997). When the water level retreats (falling water season), macrophytes begin to decompose, persisting only in the rhizomes, which in turn release large amounts of nutrients and stimulate phytoplankton growth (Rai & Hill, 1984; Junk & Piedade, 1997; Melack & Forsberg, 2001). Concomitantly, we observed increased chl-*a* concentrations in Lake Janauacá.

The increase in the degree of humification (HIX) during rising water (LRW) is due the entering of allochthonous materials (leaves, soil, wood, etc) into the water upon temporary flooding of terrestrial habitats (Junk et al., 1989) or river inflowing (Ertel et al 1986). In addition, large amounts of senescent biomass decay and both inorganic and organic materials (deposited during the terrestrial phase) are

hydrologically mobilized at the aquatic-terrestrial transition zones during rising water seasons. The increase in water level and terrestrial organic matter inputs will then affect environmental conditions and influence on aquatic biota (Junk et al., 1989; Thomaz et al., 2007).

Concerning the fluorescence components (PARAFAC), we observed that C1 and C2 (both are terrestrially-derived humic components), dominated during HW, when the water level is maximum and the lake receives a considerable input of allochthonous materials from the inundated forest and river inflowing. Despite C3 has been also an abundant terrestrial-humic like component, it presented a seasonal pattern very different from C1 and C2, being a indicative of its different origin. Our findings are in agreement with previous studies that demonstrated the predominance of terrestrial and largely refractory DOM in the Amazon River and its tributaries (Ertel et al., 1986; Hedges et al., 1986) and in another Brazilian wetlands (Pantanal, Dalmagro et al., 2018).

In contrast, C4 provided a signature of autochthonous protein-like DOM and was less abundant compared to the other components, which is typical as this protein-like peak is only a small fraction of the total DOM fluorescence. We believe that compounds represented by C4 are continuously produced by primary producers but, due to their reactive nature, they are rapidly consumed by bacteria and, thus, do not accumulate appreciably in the water column. Primary producers, phytoplankton and macrophytes could be responsible for producing this DOM component. It is well known that a wide range of bacterial taxa have the capacity to assimilate and metabolize low-weight labile molecules (such as those represented by C4), but the capacity to cleave the larger molecules in the supposedly “recalcitrant” DOM requires a set of specialized hydrolytic enzymes that are absent in many taxa (Kritzberg et al., 2006). An investigation of the autotrophic carbon sources of bacterioplankon in a similar floodplain lake, based on the isotopic analysis of plants, DOC and respired CO_2, demonstrated that the DOC consumed by bacteria was derived predominantly from herbaceous macrophytes, while the DOC that accumulated on the lake was predominantly refractory carbon derived from terrestrial sources, largely consistent with our interpretation (Waichman,1996).

Our result aligned with the hypothesis of co-occurrence of a small fraction of labile and freshly produced DOM, which is rapidly consumed, with a larger pool of

recalcitrant DOM (mostly humic) at the Amazon Basin (Mayorga et al., 2005) and in other aquatic ecosystems (Bertilsson & Jones, 2003). Small seasonal variations in DOC concentrations were previously reported at Lake Janauacá (Albéric et al., 2018). Similar results for another Amazon floodplain lake were reported by Waichman (1996). However, in this study there was no significant variation in DOM quality as measured by isotopic composition of DOC, suggesting relatively constant and homogeneous sources of DOM from upland soils and flooded forests (Albéric et al., 2018). Other study in Amazon (lake and stream) pointed out that both, quantity and quality of DOM, vary depending on the photochemical and bacterial degradation processes that happen differentially between types of systems and seasons (Amado et al., 2006). Here, we demonstrated that optical properties of DOM is a useful and practical approach for revealing seasonal and spatial patterns in DOM quality and could be very useful to cover the heterogeneity and complexity of Amazonian aquatic systems functioning and elsewhere.

A widespread view in aquatic biogeochemical studies is that the bacterial communities who preferentially consume autochthonously produced DOM, also obtain energy more efficiently than they would from allochthonous DOM, essentially due to greater accessibility and higher nutritional value (Kitzberg et al., 2006; Guillemette et al., 2013). Here, we also found higher rates of BP in macrophyte leachates (more labile DOM) than soil treatments (humic source). However, the mixed treatment had the highest BP rates, indicating that a synergistic effect of allochthonous and autochthonous sources could contribute to even more effective uptake of DOM. Possible explanations for this synergistic effect could be microbial niche diversification and substrate specialization in the microbial communities or resource/nutrient complementarity. Niche diversification concerns the differential response in growth rates of different groups of bacteria on distinct types of DOM. Such differences in BCC explain differential uptake and utilization of autochthonous and allochthonous C (Judd et al., 2006). Another explanation could be resource complementarity, where soil leachates may provide some limiting nutrients and trace elements while macrophyte leachates supply readily available energy sources in form of organic substances. Another speculation is that there could be a priming effect, where labile DOM substrates enhance the overall utilization of less reactive compounds by promoting the activity of bacterial communities (Guenet et al., 2010).

Despite being previously reported in incubations experiments in the Amazon (Ward et al., 2016) as well as in other aquatic ecosystems (Bianchi, 2011; Kuehn et al., 2014), further studies would be necessary to evaluate if there is any such priming effect in bacterial DOM degradation in these ecosystems.

Identifying the links between DOM and BCC has been a subject of great interest, triggering research efforts at multiple scales and in different experimental settings. Still, these interactions have remained less explored under natural conditions in aquatic systems (Ruiz-González et al., 2015; Osterholz et al., 2016; Amaral et al., 2016), and to our knowledge this is the first attempt in a tropical floodplain system. Although the link between BCC and DOM does not always come out clearly (Langenheder et al., 2005), most studies have reinforced the central role of bacteria for DOM transformation and the importance of DOM availability and quality as drivers of bacterial metabolism and composition (Kritzberg et al., 2006; Judd et al., 2006). Experimental approaches are fundamental because they allow the manipulation of community composition and tight control of environmental conditions, enabling researchers to isolate the effects of specific environment factors on composition and functioning of microbial communities (Reed et al., 2007). However, these approaches also have limitations since the so-called “bottle effect” promotes growth of specific fast-growing taxa that are typically rare in natural ecosystems, and this may accordingly have a great effect on the outcome of the experiments (Krammer et al., 2008; Hammes et al., 2010). Additionally, studies under laboratory conditions often focus on a narrow range of organic substrates of known compositions, and do not fully consider the interplay between the complex DOM mixtures seen in natural habitats and the equally complex indigenous microbial communities.

Overall, we found that BCC shifts was not correlated with DOM quantity but tightly coupled to the wide natural variation we observed in DOM composition (chla and PARAFAC), which broadly followed a seasonal pattern and that some specific bacterial taxa were correlated with a specific component within the DOM pool. For example, we found an interesting link between C4 and the OTU_137 (*Synechococcus*) during FW. This may indicate a role of these pico-cyanobacteria in producing and releasing labile DOM during this period, and this was paralleled by an increase in phytoplankton biomass (chl-*a*) and Freshness Index of the DOM.

Concerning members of *Betaproteobacteria* and *Alphaproteobacteria,* these results imply specialization of these groups in using contrasting sets of substrates and relates to the availability and concentration of such resources during specific seasons. In agreement with this, a preference for fulvic acid-rich compounds by *Alphaproteobacteria* has already been suggested in a previous study (Amaral et al., 2016). In general, a major factor that distinguish these two classes is that members of freshwater *Alphaproteobacteria* are commonly described as oligotrophs, i.e. good competitors at lower-nutrient availability and able to degrade a variety of organic compounds (Salcher et al., 2013). In contrast, *Betaproteobacteria* require higher amounts of organic nutrients to be competitive.

While the optical measurements used may not have captured the full complexity of DOM composition and dynamics (i.e. detailed patterns in chemodiversity; optics analysis is limited to characterized the DOM portion that have some degree of affinity with light), the approach providing considerable insight into the seasonal patterns in DOM composition, reactivity and aromaticity, and revealed clear correlations between BCC and DOM quality. Moreover, the optical characteristics of DOM have previously been demonstrated to accurately reflect high-resolution molecular-level data at wide geographic and temporal scales (Kellerman et al., 2015).

In conclusion, we found that the flood pulse modulated the source and aromaticity of DOM, and that there were strong links between these characteristics and bacterial community composition. We reiterate that the CAP approach did not resolve causal interactions (i.e. when and at what rate DOM was being consumed or produced by bacteria) but it was very effective in revealing bacterial-DOM relationships under natural conditions. Overall, our results support the hypothesis that a large pool of terrestrial-humic DOM and a smaller pool of freshly and labile autochthonous DOM that is preferentially and rapidly recycled by the bacterioplankton. We believe that the concomitant use of between experimental and *in situ* approaches could help researchers better understand when bacterial degradation is a source or a sink for DOM and uncover interaction across contrasting scales, as needed for a better understanding of the global carbon cycle.

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Conflict of interest

The authors declare no conflict of interest.

Authors Contribution

MM, JHM, BF and HS designed the field approach. MM and JHM collected environmental samples and did laboratory measurements. MM performed molecular analyses and statistical processing. MM and DK performed DOM and PARAFAC analyses. MM wrote the first draft of the manuscript and all authors contributed to data interpretation and analysis and subsequent revisions of the manuscript.

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CAPÍTULO 3

“Microbial keystone taxa in Amazon waters”

Microbial keystone taxa in Amazon waters

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Abstract

Living organisms form complex interaction networks, which ultimately drive the structure of ecological communities and the ecosystem functioning. Recently, microbial keystone taxa have been proposed to describe highly connected taxa, which play a crucial role in microbiome structure and functioning. Here, using 16S rRNA amplicon data, we compared the structure of complex microbial interaction networks and searched for keystone taxa of two distant Amazon floodplain lakes, Lake Janauacá and Lake Curuaí. Additionally, we proposed a replicable coefficient (k-value) to identify putative keystone taxa using node-based scores (degree, betweenness and closeness centrality). We found a non-random structure of bacterial communitiesnetworks and identified several keystones (from phyla Proteobacteria, Actinobacteria, Chloroflexi, Planctomycetes, and Cyanobacteria) some of them were the same for both distant lakes, indicating that they are probably also ubiquitous keystone in Amazon aquatic systems. We found a positive relationship between the k-value and the relative abundance of interact bacterial taxa, indicating that keystones were abundant in their systems. After recovered metagenomes in databases of some keystone, we found that they shared similar patterns concerning the relative abundance of genes involved in several metabolic pathways, but more differences in terms of potential to have functions related to biogeochemical cycles. Overall, the identification of keystones may be crucial for a better understanding of the functioning of floodplain ecosystems and their role in global biogeochemical cycles.

1. Introduction

All species of our biosphere interact with other species forming complex ecological networks that comprise trophic, parasitic and mutualistic interactions (Prouxl et al., 2005; Pocock et al., 2012). The network approach brought valuable insights into ecology, providing a holistic perspective on biodiversity dynamics and biological processes (Prouxl et al., 2005), having enabled us to explore the role of keystone species.

Introduced by Paine (1969), the keystone species concept has been widely applied in ecology, but its definition has constantly evolved ever since (Cotte-Jones & Whittaker, 2012). In most cases, the keystone species concept has been applied to describe an animal or plant species that plays a critical role in maintaining the stability of an ecological community, and if removed from that community affects number of other species and, as a consequence the whole ecosystem structure (Paine, 1969). These species have also an important role in ecosystems robustness and resilience responding to environmental changes or perturbations. In a study using simulations of random and selective primary species loss in 16 food webs from terrestrial and aquatic ecosystems, it was demonstrated that highly connected communities tended to be more robust to species losses, and that the removal of species with few trophic connections generally had little effect though there are exceptions (Dunne et al., 2002).

Microbes dominate ecological processes and account for most of the biomass in aquatic systems (Cotner & Biddanda, 2002). The advent of high-throughput sequencing and the development of bioinformatic tools in the last two decades allowed us to access the huge diversity of microbes from different environments, providing valuable insights into temporal and spatial patterns of microbial assemblies (Sogin et al., 2006; Newton et al., 2011; Sunagawa et al., 2016). Furthermore, these data offer an unprecedented opportunity to reveal interactions among microbes, and to identify keystone species, which have crucial role to ecosystem functioning.

Microbial keystone taxa have been recently proposed to describe highly connected taxa (regardless their abundance across time and space), which play a crucial role in microbiome structure and functioning (Banerjee et al., 2018). Human microbiome studies provided most of the empirical evidence of microbial keystone taxa (Hajishengallis et al., 2011, MORE). However, probably due to the difficulty to

detect and quantify complex ecological relationships in microbial systems, most studies have used computational tools especially network analyses, to identify putative keystone microbes (Steele et al., 2011; Vick-Majors et al., 2014; Lupatini et al., 2014; Fisher & Mehta, 2014).

Although network analyses stands out as a powerful computational tool, there is no consensus about which correlation-based approach (e.g. Pearson, Shannon, SparCC), algorithm, metrics, and threshold should be used to achieve good estimations, and to be comparable (Faust & Raes, 2012; Banerjee et al., 2018). Despite that the use of collective node-based scores can identify a keystone taxon with 85% accuracy (Berry & Widder, 2014), no studies have defined a quantifiable threshold for identifying keystone taxa in microbial systems (Banerjee et al, 2018).

The Amazon is good example of an ecosystem relevant for global carbon budgets where aquatic microorganisms play a central role degrading (mainly terrestrial) organic carbon (Richey et al., 2002), but few information is available on the dynamics of microbial communities in Amazon (but see de Melo et al. 2019), and even less about the interactions, the identity and role of microbial species in ecosystem functioning.

Here, we compared the structure of complex microbial interaction networks and searched for keystone taxa of two distant Amazon floodplain lakes, Lake Janauacá and Lake Curuaí. To do so, we propose a replicable method to identify keystone taxa using node-based scores: the K-value. We believe that if there are common keystone taxa in these two distant floodplain systems, they probably play crucial roles in most Amazon floodplain systems.

Our questions, applying the K-value proposed here, were: I) Are interaction patterns among microbial communities the same among ecosystems with similar characteristics? II) Are there ubiquitous keystone taxa in these systems, even if they are distant of each other? III) Are those keystones the most abundant taxa in microbial communities? IV) Which ecological functions are they involved?

2. Methodology

2.1 Study area and Sampling

We compiled 16S rRNA data from two Amazon floodplain lakes, Lake Janauacá and Lake Curuaí (Figure III - 1). Lake Janauacá (3°23’ S; 60°18’ W) is

located along the south margin of the Solimões River in the Central Amazon basin near to Manaus (Amazonas, Brazil). The lake watershed extends for an area of 770 km^2 and includes a floodable area ranging between 23 km^2 at low water to 390 km^2 at high water period (Pinel et al., 2015). Lake Grande de Curuaí (or Curuaí) (2°10’ S, 55°28’ W) is located along the southern margin of the lower reach of the Amazon River, near to Óbidos city (2°10'S, 55°28'W, Pará, Brazil). The floodplain is composed by several interconnected lakes temporally or permanently connected to the Amazon River by channels, and covered a floodable area varying between 1340 and 2000 km^2 (Kosuth, 2002), according to the hydrological period. The two lakes shared similar characteristics such as both are permanent connected with a white-water river and are regulated by the annual hydrological cycle – flood pulse. However, they are far for each other by a straight distance of approximately 600 km.

Ideally, to detect robust associations between microorganisms within and between habitats using network analysis is recommended to have large sample sets covering spatial and temporal gradients in order to include a sufficient variability in taxon abundances (Barberán et al., 2011). Therefore, for each lake, we sampled a total of 18 samples, including different habitats, hydrological seasons and size-fractions (totalizing 36 samples). The three habitats were: aquatic-terrestrial transition zone (TR), open lake (OP) and main river-lake transition zone (RL) (Fig. III - 1). We sampled in high (HW), falling (FW) and rising waters (RW) in both, free-living (FL) and particle-attached (PA) size-fractions.

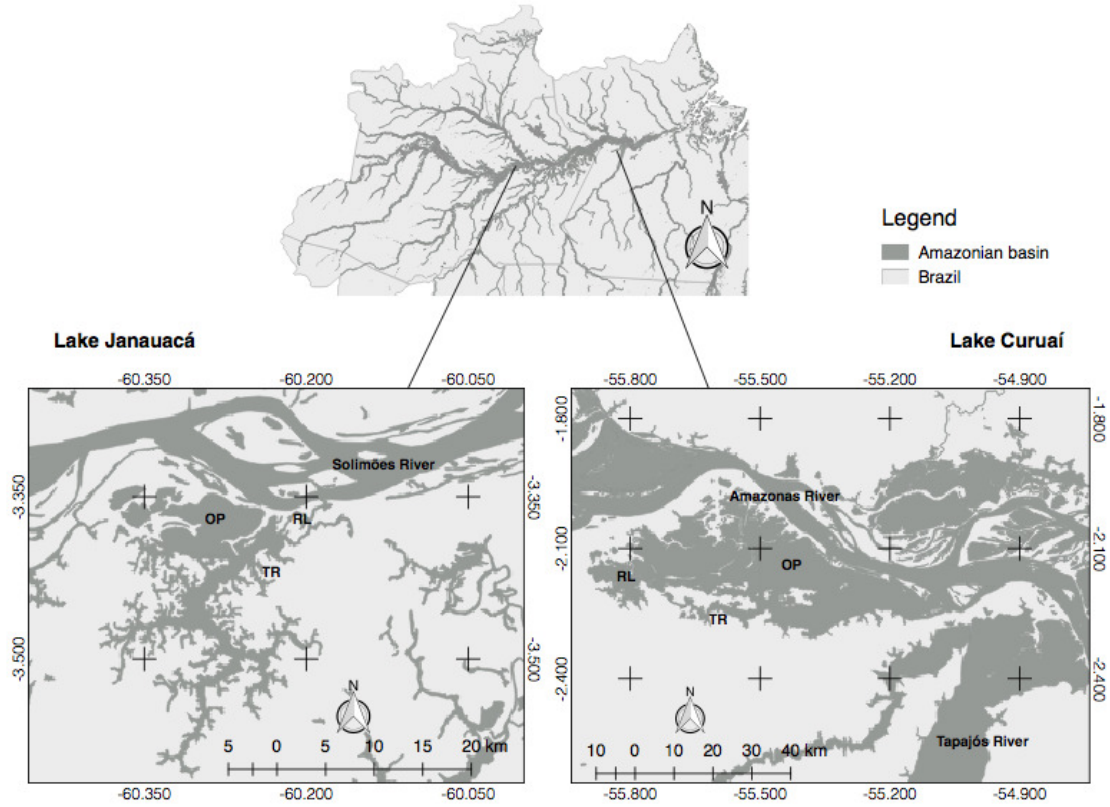

Figure III - 1: Map of the study area Lake Janauacá and Lake Curuaí showing sampling sites: open lake (OP), terrestrial-aquatic transition zone (TR), river-lake transition zone (RL).

2.2 Bacterial Community Composition (BCC)

All sequences used in the present work can be found in the BioSample database hosted by NCBI (https://www.ncbi.nlm.nih.gov/sra/SRP127556). Briefly, samples for determination of BCC were prefiltered through 3 µm polycarbonate membranes (Whatman® Nucleopore, Maidstone, UK) to collect the PA size-fraction and filtrates were subsequently passed through 0.2 µm polycarbonate membranes (Millipore® Isopore, Burligton, MA, USA) to collect the FL size-fraction. Membranes were stored at -80 ºC until the extraction procedure. DNA from the filters (0.2 and 3 µm) was extract using a phenol-chloroform protocol and amplified using the primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACT ACHVGGGTATCTAATCC-3') for the V3/V4 region of the 16rRNA gene (Herlemann et al., 2011). Finally, the high-throughput sequencing was performed on an Illumina Miseq2000 instrument (for more details see de Melo et al., 2019).

All sequences were processed together using UPARSE (Edgar, 2013)

following a previously established pipeline. After quality filtering, denoising and removal of potential chimeras and non-bacterial, we merged paired-end reads and removed all sequences shorter than 100 bp. Then, we clustered the merged reads into operational taxonomic units (OTUs), considering 97% identity cutoff (Quast et al., 2013). The taxonomic classification was done with BLASTn against SILVA 119.1 (Zhang & Fang, 2000) with at least 75% similarity threshold. For further analyses, we discarded all chloroplasts and Archaea sequences. Finally, using the function rarefy from the package vegan in R (Oksanen et al., 2015), we randomly sub sampled the OTU table based on the sample with the least number of reads (18 061).

2.3 Interaction network analysis

To construct the interaction networks, we removed rare and poorly prevalent OTUs, and kept only those that were detected in at least 50% of our samples in each floodplain lake (the filtering as done is a common procedure in microbial networks – see Comte et al., 2016; Geng et al 2016). Interaction networks were inferred based on sparse correlations for compositional data (SparCC) (Friedman & Alm, 2012). SparCC estimates the linear Pearson correlations between the log-transformed components and uses a permutation-based approach to generate (pseudo) p-values. Here it was done comparing the correlations obtained for the real data to the ones obtained from the shuffled data (bootstrapping procedure with 100 iterations). All these steps were performed in Python environment using the package SparCC (https://bitbucket.org/yonatanf/sparcc).

After removing autocorrelations, we considered that two OTUs were linked if their absolute correlation coefficient was greater than 0.6 or less than -0.6 with a strong evidence for the association ($P \leq 0.001$). We used these correlations to construct interaction networks using the igraph package version 1.0.1 (Csardi & Nepusz, 2006) in R. For the purpose of this study we did not differentiate positive and negative correlations. The nodes in this network represent OTUs and the edges that connect these nodes represent the correlation coefficient between OTUs.

Various network-based metrics and indexes, including number of edges and nodes, modularity (M), clustering coefficient (CC), average path length (APL), network diameter (ND), average degree (AD) and graph density, were used to describe and compare the properties of networks (Table III - 1). We also compared

our networks with random networks generated using the Erdös-Rényi model with equal number of nodes and edges (Erdös and Rényi, 1959). We compared the metrics calculated for the observed networks to the confidence interval (using a threshold for C.I.=95%) of the same metrics of 10 random networks.

Table III - 1: Glossary with the description of the metrics used in this study.

Metric	Level	Description	Reference
Nodes	network	the connection point	
Edges	network	number of interactions between nodes	
Density	network	proportion of possible edges that are connecting nodes	Newman et al., 2001
Diameter	network	the longest path between any two nodes in a network	Urban & Keitt, 2001
Modularity (M)	network	measures the strength of division of a network into modules	Albert & Barabási, 2002
Average degree (AD)	network	the average number of edges that connected nodes	Watts & Strogatz, 1998
Average path lenght (APL)	network	the average number of shortest paths between a pair of nodes in the network	Watts & Strogatz, 1998
Clustering Coefficient (CC)	network	measures the degree to which nodes in a graph tend to cluster together	Koschützki et al., 2005
Degree	node	Number of nodes which a node is connected to	Koschützki et al., 2005
Closeness Centrality	node	measures the mean distance from a node to other nodes	Koschützki et al., 2005
Betweenness	node	measures the shortest paths going through a given node to other	Koschützki et al., 2005

2.4 Identification of keystone taxa

We used node-based scores to identify putative keystone taxa in bacterial communities of each lake by combining scores of high mean degree, high closeness centrality and low betweenness, as previously suggested (Berry & Widder, 2014; Banerjee et al., 2018). We proposed a formula to calculate a coefficient (K-value) for each node (OTU), considering equal weight for the three scores: degree (D), closeness centrality (C) and betweenness (B). First, we standardized the scores to range between 0 and 1:

$$D_z = D - D_{min} / D_{max} - D_{min}$$

$$C_z = C - C_{min} / C_{max} - C_{min}$$

$$B_z = B - B_{min} / B_{max} - B_{min}$$

Considering the standardized scores of each node we proposed two scenarios: a) the best scenario (perfect keystone), when D and C were maximum (equal to 1) and B minimum (equals to 0); b) the worst scenario, when D and C were minimum (equal to 0) and B maximum (equals to 1). Using standardized scores, we calculated the K for each node and normalized K to range between 0 and 1 as follow:

$$K = (D_z+1)*(C_z+1) / (B_z+1)$$

$$K_z = K - K_{min} / K_{max} - K_{min}$$

Finally, to identify keystone taxa we calculated the confidence interval of 95% and considered all OTUs with $K_z > CI_{95\%}$ as a keystone:

$$CI_{95\%} = \mu_{Kz} + 1.96 * \partial_{Kz}$$

Where μ_{Kz} is the K_z mean and and ∂_{Kz} is the K_z standard deviation.

2.5 Keystone taxa functional analysis

In order to identify the functions that keystone taxa are involved we searched for correspondent genomes in NCBI database. Then, using the software MEBS (Multigenomic Entropy Based score, De Anda et al., 2017), we produced a list of scores by biogeochemical cycle (carbon, nitrogen, oxygen, iron, and sulfur) with p-FDR ≤ 0.001.

We used the FOAM - Functional Ontology Assignments for Metagenomes database (Prestat et al., 2014) to perform genomes annotation. This database was developed for screening of environmental metagenomic and metatranscriptomic sequence datasets for functional genes, allowing the characterizations and comparison of functions and metabolic pathways important in environmental microbial ecology (Prestat et al., 2014). The annotation procedure was made over the predicted genes with HMMer v.3.2.1 (Eddy, 2011; hmmer.org). The parsing was made as it follows: e-value < $1e^{-5}$; minimum coverage of 50%; minimum alignment of 80 amino acids, and no overlap of domains.

3. Results and discussion

3.1 Bacterial community composition (BCC)

The number of high-quality sequences recovered after sequence processing of the total of 36 samples were 3,850,361 sequences, 2,092,541 for Lake Janauacá and 1 757,820 for Lake Curuaí, which were clustered in 4,340 and 3,478 OTUs, respectively. After rarefaction (18,061 reads/sample), we retained 3,769 OTUs for Janauacá and 2,660 OTUs for Curuaí. We found 2,063 OTUs shared for both ecosystems. The diversity indexes (Chao 1, Shannon's, and Simpson's indexes) demonstrated higher values for Lake Janauacá than Lake Curuaí (Table III - S1).

In general, there was variation within and between systems (Figures III - S1 and III - S2), but both lakes were dominated by the same groups, mainly members of the classes Actinobacteria (relative abundance of 23 and 25% for Janauacá and Curuaí, respectively), followed by Gammaproteobacteria (16 and 15%), Planctomycetacia (12 and 8%), Alphaproteobacteria (11 and 4%), Oxyphotobacteria

(7 and 16%), Acidicrobiia (6% for both), and Verrucomicrobiae (4 and 5%). Members of Actinobacteria and Proteobacteria (Gamma and Alpha) are common and often numerically important component in a variety of freshwater habitats, including the Amazon basin (Satinsky et al., 2015; Doherty et al., 2017). The abundant classes Alphaproteobacteria, Planctomycetacia and Oxyphotobacteria, showed different patterns between systems, Alphaproteobacteria and Plancotmycetacia were more abundant in Lake Janauacá than Curuaí, and Oxyphotobacteria in Curuaí than in Janauacá.

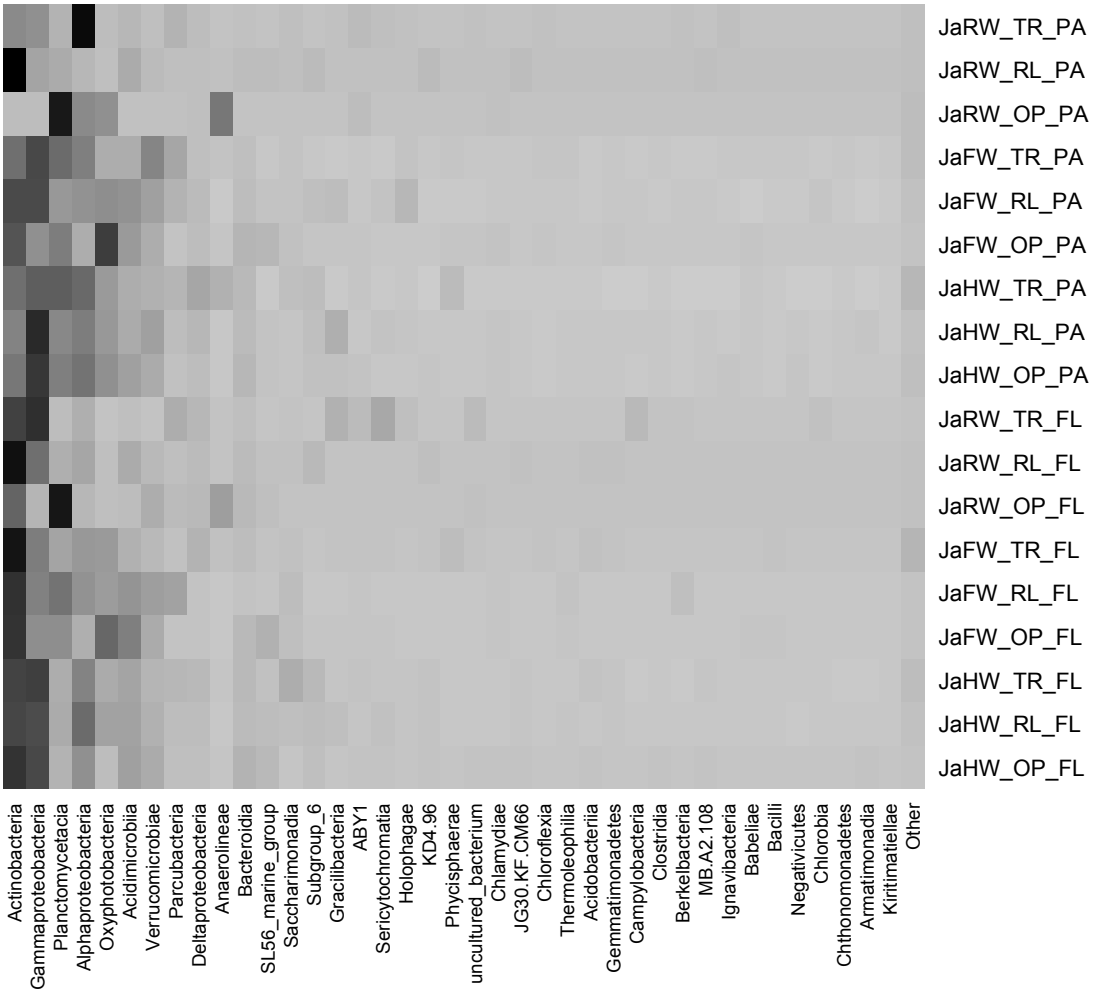

Figure III - S1: Heatmap showing the relative abundance of the main bacterial classes found in Lake Janauacá. The scale of colours varies between black and light grey, where black squares represent very abundant classes and light grey non-abundant. Abbreviations: Lake Janauacá (Ja), aquatic-terrestrial transition zone (TR), open lake (OP), main river-lake transition zone (RL), high (HW), falling (FW) and rising waters (RW), free-living (FL) and

particle-attached (PA) size-fractions.

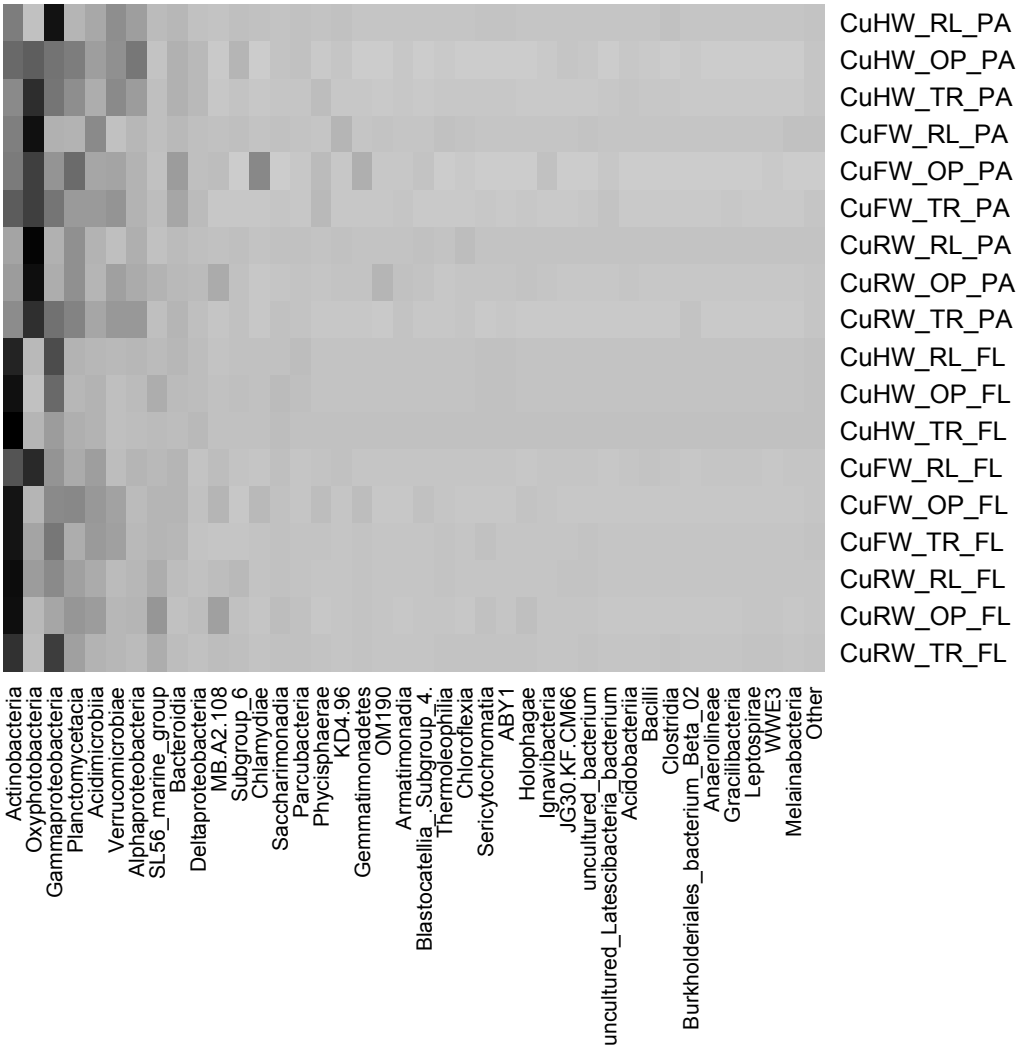

Figure III - S2: Heatmap showing the relative abundance of the main bacterial classes found in Lake Curuaí. The scale of colours varies between black and light grey, where black squares represent very abundant classes and light grey non-abundant. Abbreviations: Lake Curuaí (Cu), aquatic-terrestrial transition zone (TR), open lake (OP), main river-lake transition zone (RL), high (HW), falling (FW) and rising waters (RW), free-living (FL) and particle-attached (PA) size-fractions.

Table III - S1: Diversity indexes and number of observed OTUs for each sample of Lake Janauacá and Lake Curuaí. We performed parametric (Student's t-test) and non-parametric (Mann-Whitney U-test) depending on the data normality and homogeneity of variance.

	Sample	Chao 1 index	Simpson's index	Shannon's index	Observed OTUs
Janauacá	JaHW_OP_FL	1186.60	0.97	6.70	794
	JaHW_RL_FL	1332.44	0.98	7.60	1053
	JaHW_TR_FL	1541.40	0.99	8.06	1223
	JaFW_OP_FL	819.79	0.96	6.14	579
	JaFW_RL_FL	721.24	0.97	6.26	558
	JaFW_TR_FL	574.64	0.98	7.24	483
	JaRW_OP_FL	692.27	0.86	4.42	428
	JaRW_RL_FL	834.01	0.96	6.48	649
	JaRW_TR_FL	1028.51	0.95	5.83	681
	JaHW_OP_PA	1026.11	0.98	7.39	896
	JaHW_RL_PA	1154.05	0.99	7.58	929
	JaHW_TR_PA	1864.90	0.99	8.78	1522
	JaFW_OP_PA	909.00	0.97	6.76	712
	JaFW_RL_PA	1126.46	0.99	7.61	929
	JaFW_TR_PA	1163.35	0.99	7.77	927
	JaRW_OP_PA	613.88	0.81	3.77	362
	JaRW_RL_PA	784.00	0.92	5.39	539
	JaRW_TR_PA	1080.40	0.91	5.73	754
	Average	**1025.17**	**0.95**	**6.64***	**778.78^{+}**
Curuaí	CuRW_TR_FL	728.37	0.96	5.80	514
	CuRW_OP_FL	630.20	0.95	5.54	443
	CuRW_RL_FL	682.11	0.96	6.02	518
	CuFW_TR_FL	869.04	0.96	6.03	563
	CuFW_OP_FL	647.16	0.96	6.04	508
	CuFW_RL_FL	1037.00	0.90	5.60	644
	CuHW_TR_FL	562.14	0.92	4.94	411
	CuHW_OP_FL	992.17	0.94	5.72	669
	CuHW_RL_FL	846.37	0.94	5.82	632
	CuRW_TR_PA	1233.54	0.98	7.15	887
	CuRW_OP_PA	697.79	0.93	5.92	552
	CuRW_RL_PA	919.84	0.82	5.05	598
	CuFW_TR_PA	978.75	0.98	6.98	761
	CuFW_OP_PA	857.92	0.97	6.50	589
	CuFW_RL_PA	509.79	0.86	5.21	392
	CuHW_TR_PA	1290.59	0.94	6.54	827
	CuHW_OP_PA	1032.39	0.97	6.96	773
	CuHW_RL_PA	1162.72	0.98	6.90	850
	Average	**870.99**	**0.94**	**6.04**	**618.39**

Mann-Whitney U-Test *$p \leq 0.05$

T-Test $^{+}p \leq 0.05$

3.2 Interaction Networks

Despite that the same thresholds were used to construct interaction networks for the two lakes, the network size and metrics were different (Figure III - 2, Table III - 2). The Janauacá network had 332 nodes and 1327 edges, and Curuaí had 310 nodes and 2677 edges. Most linked OTUs belonged to the most abundant classes Gammaproteobacteria (70 and 53 nodes to Janauacá and Curuaí, respectively), Alphaproteobacteria (40 and 32 nodes), Actinobacteria (38 and 29 nodes), Planctomycetacia (25 and 23 nodes), Verrucomicrobiae (24 and 26 nodes), Acidimicrobiia (22 and 24 nodes).

Although having more nodes, Janauacá network had fewer connections between them, resulting in a lower average degree (AD), 7.994 compared to 17.270 for Curuaí (Table III - 2). These results suggest that there were likely more pronounced associations between bacterial OTUs in Curuaí than in Janauacá. However, Janauacá was more diverse, with a greater number of OTUs and higher Shannon's diversity index (Table III - S1).

Both had a similar network diameter (ND), 6.2 and 6.4 for Janauacá and Curuaí, respectively. We observed clustering coefficients (CC) of 0.360 and 0.459, and modularity (M) of 0.522 and 0.387, to Janauacá and Curuaí, respectively. The average path length (APL) was higher in Janauacá than in Curuaí, 3.577 and 2.897, respectively. For both systems, M, APL, and CC were higher in the observed networks than in the random networks, indicating a non-random community assembly (Table III - 2). Higher CC indicates that the network had "small world" properties, i.e. nodes were highly interconnected (clustered), more than expected by chance in random networks. These patterns indicate that there is a dominance of deterministic processes such as environmental filtering in shaping community composition (Horner-Devine et al., 2007). Considering that these systems have similar properties regarding to seasonal conditions and landscape, environmental filters could be similar, culminating in a similar general pattern in BCC as observed here.

Despite the higher number of edges in Lake Curuaí's network (M=0.387), Lake Janauacá appears to have a more modular community structure (M=0.522). Usually, modularity values higher than 0.4 suggest that the network has a modular structure and that in the module edges are more tightly connected with each other

than in a random combination of the same number of edges (Newman, 2006; Fortunato & Barthélemy, 2007). Detecting modules in biological networks is a way to identify highly connected groups of nodes (OTUs) that could have different ecological functions with some degree of independence between them. Other important idea is the relationship between modular and local stability of ecological communities. Several studies discuss that environmental changes or small perturbations could be more rapid damped in ecological communities with modular structure because they could retain the impacts of a perturbation within a single module and minimizing impacts on other modules (May, 1972; Krausse et al., 2003; Grilli et al., 2012). In this sense, microbial community in Lake Janauacá could be more resistance to perturbations than in Lake Curuaí.

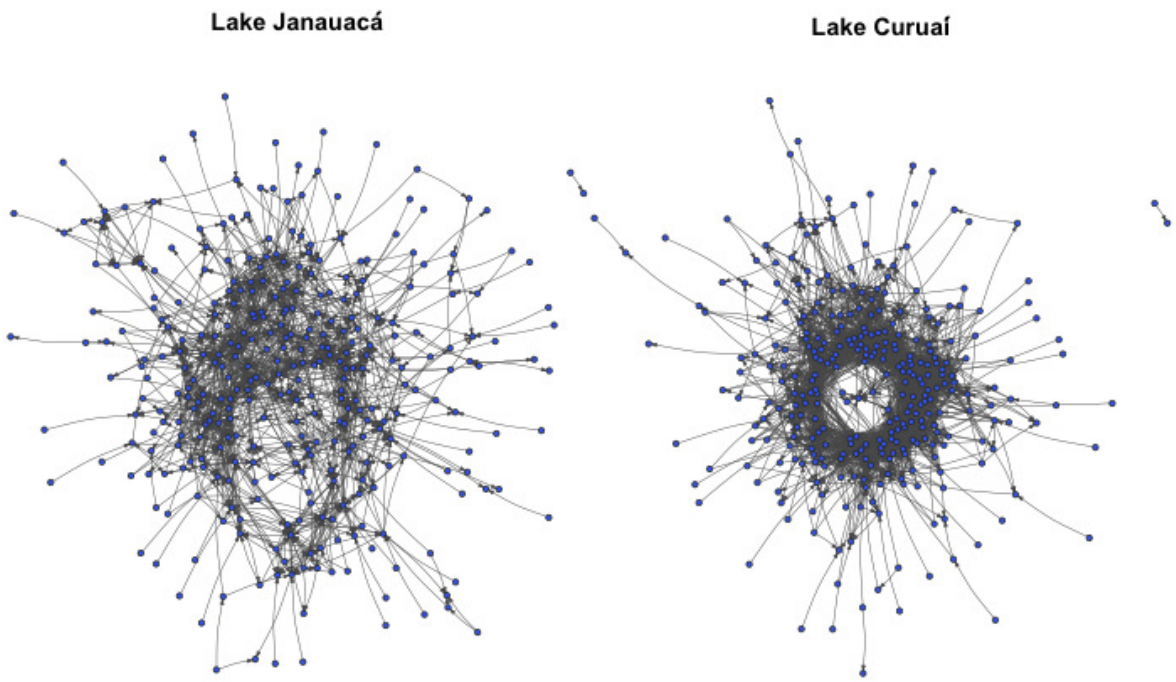

Figure III - 2: Microbial interaction networks of two Amazonian floodplain lakes (Lake Januacá and Lake Curuaí) inferred based on sparse correlations for compositional data (SparCC). Blue circles represent nodes and black arrow edges that connect nodes.

Table III – 2: Network metrics for Lake Janauacá and Lake Curuaí (C.I. stands for 95% confidence interval).

	Janauacá		Curuaí	
Network properties	*Empirical*	*Random (C.I.)*	*Empirical*	*Random (C.I.)*
Nodes	332	332	310	310
Edges	1327	1327	2677	2677
Modularity	0.522	[0.314-0.320]	0.387	[0.195-0.199]
Graph density	0.012	0.024	0.026	0.056
Diameter	6.2	[4.904-5.296]	6.40	[3.539-4.061]
Average Path Lenght	3.577	[3.011-3.018]	2.897	[2.303-2.303]
Average Degree	7.994	7.994	17.27	17.271
Clustering Coefficient	0.360	[0.023-0.027]	0.459	[0.054-0.057]

3.3 K-value: a new tool to identify keystone taxa

Here we proposed a K-value, an easy and reproducible method to identify putative microbial keystone taxa, to compare them between different ecosystems, and to decipher seasonal and spatial patterns. Our K-value combined scores of high mean degree, high closeness centrality and low betweenness, as recommended recently by Banerjee et al. (2018) in a review.

Using the K-value, we identified 11 keystone OTUs for Janauacá and 12 for Curuaí, 4 were shared by both systems (OTU_2, OTU_3, OTU_29, OTU_33, Table III - 3). Shared OTUs belong to the phyla Actinobacteria, Proteobacteria (class Gammaproteobacteria) and Chloroflexi (Table III - 3). Despite do not share all the same OTUs as keystones, we observed that the other keystones (particular of each lake) belonged to the same phyla, Actinobacteria, Planctomycetes, Proteobacteria, Chloroflexi and Cyanobacteria, indicating coherence in higher taxonomic levels than OTU.

In a recent study, keystone microbial taxa across several types of ecosystems (e.g. soils, grasslands, forests) were reviewed. For aquatic systems in particular, it was found keystones belonging to the genus *Pelagibacter*, *Chlorobium* and *Nitrospira*, Burkholderiales and Rhizobiales orders, and Chloroflexi and Verrucomicrobia phyla (to further information refer to Banerjee et al., 2018). Other study with long-term aquatic datasets revealed that the highly connected microbial taxa belonged to the phyla Actinoacteria, Chloroflexi, Planctomycetes, and Proteobacteria (Herren & McMahon, 2018). Although we used different

methodologies and thresholds, our results also demonstrated similar patterns (Table III - 3), for example, OTU_33 (order Burkholderiaceae) and OTU_29 (phylum Chloroflexi) were keystones in both systems. These results indicate that our K-value is an efficient and replicable approach to identify and compare putative keystones and could be used in other context and ecosystems.

Concerning the abundance patterns of our OTU keystones (Table III - 3), we found keystone taxa abundances were quite variable, ranging between 0.16 and 7.70% in relative abundance. We observed a positive and significant relationship between the relative abundance and the k-value (Fig. III - 3). A premise of the concept is that keystones exert their influence on microbiome functioning irrespective of abundance, i.e. keystone species are not always the most abundant species in an ecosystem. However, in the present study, we found that in general, keystones taxa were abundant in both lakes, and also well distributed in many other freshwater systems. Indeed, other studies also suggested that abundant and easily detectable organisms might have a high impact on microbiome structure (Lupatini et al, 2014; Ma et al., 2016). As a future perspective, more studies are needed to confirm if this pattern is not a computational artefact due to their high and broad relative abundance (Banerjee et al., 2018).

We also investigated the general patterns of abundance *versus* nodes-based scores, plotting the relative abundance (%) of OTUs included in interaction network against their correspondent normalized scores (D, C, B and K) (Fig. III - S3). We observed that higher K-value, degree and closeness centrality tended to be higher in the most abundant microbial taxa (Figs. III - 3A, 3B and 3C). However, we observed an opposite pattern for betweenness (Fig. III - 3D). The relative abundance seems to be an important parameter that defines a putative keystone species, and although it was consistent between systems, there was no strong relationship between OTUs abundance and node-based scores, other than K-value.

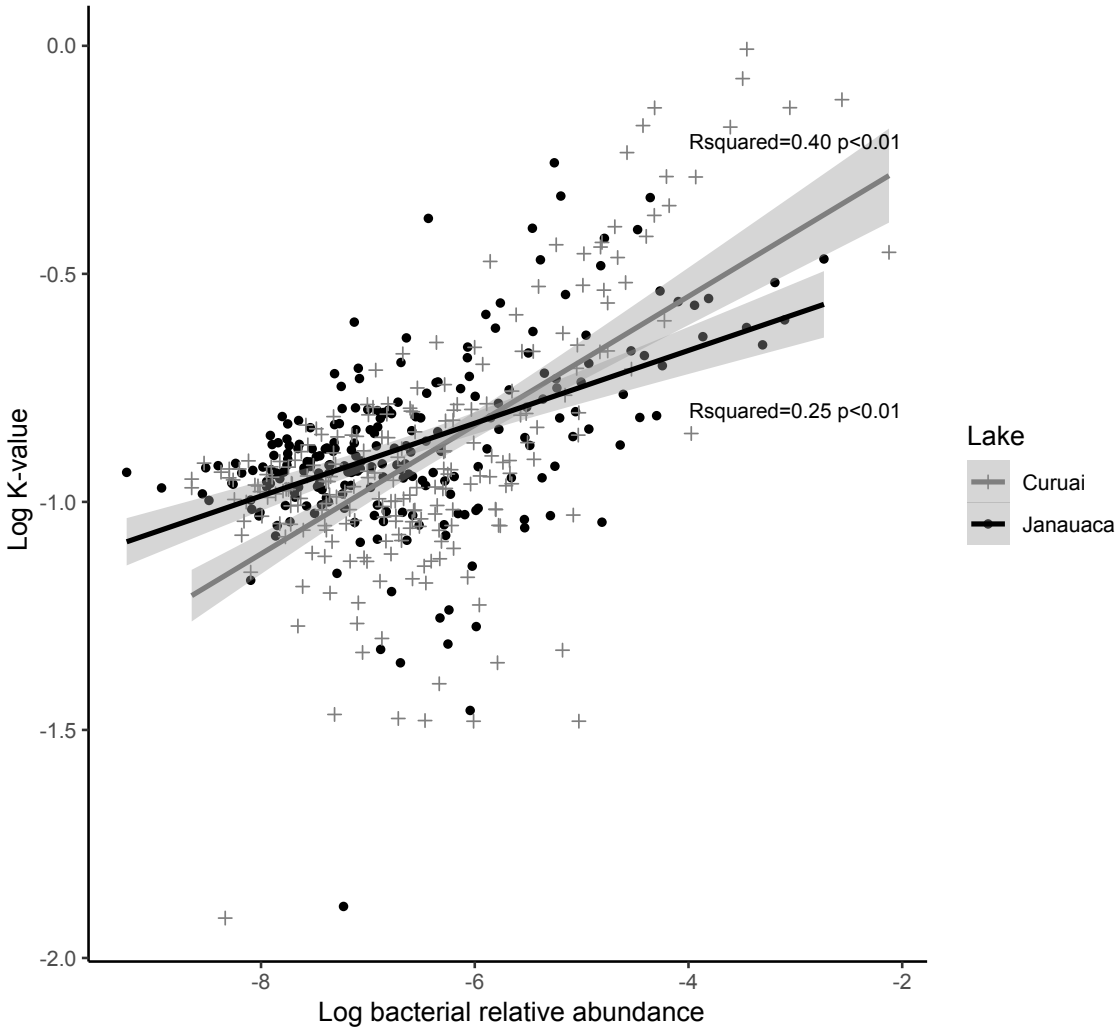

Figure III - 3: Linear regression between relative abundance of OTUs and the K-value found in both systems, Lake Janauacá and Lake Curuaí.

Table III - 3: K-value, relative abundance (%) and taxonomic classification of the microbial keystone OTUs identified in this study in each lake.

	OTU_ID	K	Relative ab (%)	Phylum	Class	Order	Family	Genus
Janauacá	OTU_33	0.77	0.52	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Limnohabitans
	OTU_13	0.72	0.55	Planctomycetes	Planctomycetacia	Gemmatales	Gemmataceae	
	OTU_6	0.72	1.28	Actinobacteria	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	CL500-29_marine_group
	OTU_117	0.68	0.16	Actinobacteria	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	CL500-29_marine_group
	OTU_29	0.67	0.43	Chloroflexi	SL56_marine_group			
	OTU_21	0.67	1.14	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade
	OTU_9	0.66	0.83	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	
	OTU_2	0.63	6.51	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade
	OTU_139	0.63	0.46	Cyanobacteria	Oxyphotobacteria	Synechococcales	Cyanobiaceae	Cyanobium_PCC-6307
	OTU_15	0.62	0.81	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Polynucleobacter
	OTU_3	0.60	4.11	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade
Curuaí	OTU_8	0.99	3.16	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade
	OTU_12	0.93	3.04	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Polynucleobacter
	OTU_2	0.89	7.70	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade
	OTU_3	0.87	4.73	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade
	OTU_33	0.87	1.33	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Limnohabitans
	OTU_29	0.84	1.20	Chloroflexi	SL56_marine_group			
	OTU_5	0.84	2.71	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	
	OTU_23	0.79	1.03	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	Candidatus_Methylopumilus
	OTU_3377	0.75	1.49	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade
	OTU_10	0.75	1.96	Chloroflexi	SL56_marine_group			
	OTU_37	0.70	1.53	Actinobacteria	MB-A2-108			
	OTU_119	0.69	1.33	Cyanobacteria	Oxyphotobacteria	Synechococcales	Cyanobiaceae	Cyanobium_PCC-6307

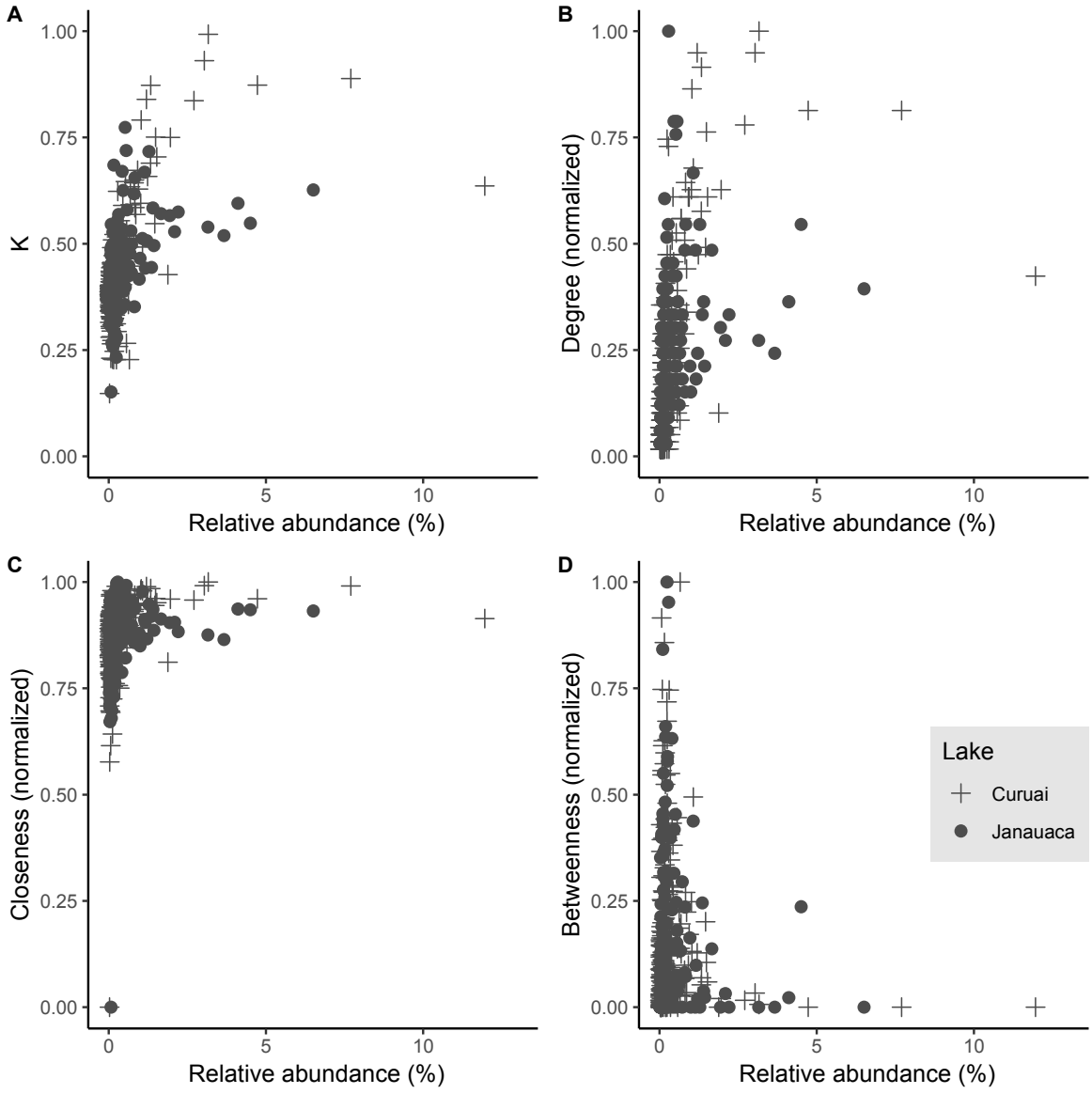

Figure III - S3: Relationship between relative abundance of OTUs (%) and nodes-metrics (normalized for 0 to 1) found in both systems, Lake Janauacá and Lake Curuaí. A) K-value; B) Degree; C) Closeness Centrality; D) Betweenness.

3.4 Featuring functions in keystone taxa

We identified several keystone OTUs in both lakes, Janauacá and Curuaí, four of them were shared, two belonging to the clade hgcI (OTU_2 and OTU_3), a *Limnohabitans* species (OTU_33), and a member of the group SL56 (OTU_29). Members belonging to the clade hgcI (also known as acI lineage) are small (<0.1 μm^3), free-living, and very abundant in a wide range of freshwater ecosystems

(Newton et al., 2011). Concerning the metabolic potential, it was demonstrated that they have genetic abilities to take carbohydrate and N-rich organic compounds (Ghylin et al., 2014). Additionally, members of this clade have the potential to utilize sunlight via actinorhodopsin, which might promote anaplerotic carbon fixation (Ghylin et al., 2014), indicating the potential capacity to use light for energy.

Limnohabitans sp. has been recognized as an abundant group in many freshwater systems (Simek et al., 2010). A study that recovered the whole genome of two strains of this genus found that both have capabilities as photoautotrophs and ammonia oxidizers, which indicates a great metabolic versatility (Zeng et al., 2012). SL56 is a group of Chloroflexi quite abundant in the eplimnion of freshwater systems (Mehrshad et al., 2018). Metabolic insights into the reconstructed metagenomic assembled genomes (Chloroflexi MAG's completeness ≥ 30%) suggested a primarily heterotrophic life style, and light-driven energy generation via rhodopsins (Mehrshad et al., 2018).

Limnohabitans sp., members of the clade hgcI and group SL56 could be pointed as Amazon keystones because they were found as keystone taxa in two distant Amazon systems. As a consequence, it is highly probable that the taxa are keystone taxa in most Amazonian floodplain lakes. They are usually abundant taxa in freshwater systems and have a great metabolic versatility. These features could be useful to maintain them in such dynamic and complex aquatic systems as Amazonian floodplains that includes a deep seasonal variation, and huge differences in water level, dissolved oxygen, nutrients supply and turbidity among seasons (Junk et al., 1989; de Melo et al., 2019).

We were able to recover whole genomes of 3 keystone taxa, *Limnohabitans* sp. (shared keystone - OTU_33), *Polynucleobacter cosmopolitanus* (OTU_15; and OTU_12 which is a *Polynucleobacter* sp.) and *Cyanobium gracile* PCC-6307 (OTU_119 and OTU__139) and accessed the scores of the functional categories of each microorganism. The bar chart summarizes the relative abundance of genes involved in several microbial metabolic processes (Fig. III - 4). Although we featured some differences between them herunder, there is some functional redundancy between these taxa, implying that keystones have similar metabolic capacities. A growing number of studies suggest that microbial diversity enhances ecosystem functioning (Peter et al., 2011; Langeheder et al., 2010), but there is an increasing

idea that microbial communities have a degree of functional redundancy (Allison & Martiny, 2008; Comte et al., 2013).

Cyanobium gracile PCC-6307 is a cyanobacteria, therefore has fewer genes involved in fermentation, and more genes involved in amino acids utilization and biosynthesis, in carbohydrates metabolism, and in the synthesis of saccharides and derivates. On the other hand, *Polynucleobacter cosmopolitanus* has more resistance to environmental stress (more genes involved in cellular response to stress) that could be an indicative of the potential to complex fermentation (more genes involved in fermentation). Finally, *Limnohabitants* sp. has a higher acetate production potential among all the evaluated organisms, and a greater number of genes associated to the use and synthesis of amino acids, possessing a more oxidative metabolism that it was evidenced by the greater number of genes associated to the tricarboxylic acid (TCA) cycle. This could indicate an advantage of these organisms in Amazon waters, which are rich in aromatic-terrestrial organic matter such as lignin and cellulose-derived molecules (Ward et al., 2013; de Melo et al., 2019), once it was observed a increase in the enzymes of the TCA cycle and glucogenesis in some bacterial taxa upon use of aromatic compounds (Zhao et al., 2005; Navarro-Llorens et al., 2005).

Analyzing and comparing the scores of the biogeochemical cycles (carbon, nitrogen, oxygen, iron, and sulfur), it was possible to identify some differences between them (Fig. III - S4). *Cyanobium gracille* had the greatest potential to perform the biogeochemical cycles of all the analyzed elements. *Limnohabitants* sp. also demonstrated a great potential, whereas it does not seem to be very relevant for the sulfur cycle, while *Polynucleobacter cosmopolitanus* had the lowest scores, and seems to be not associated with the carbon and sulfur cycles. The highest scores for the three taxa were found for the nitrogen cycle. Some studies pointed out that nitrogen is a limiting factor for primary production in the Amazon waters (Forsberg 1984), indicating the importance of nitrogen cycling as a key mechanism in oligotrophic waters. Also, these results could explain why nitrogen-cycling microorganisms are keystone taxa in Amazon systems.

Overall, we found similar patterns concerning the relative abundance of genes involved in several metabolic pathways, but more differences in terms of potential to have functions related to biogeochemical cycles. These results imply that major functions could be responsible for a taxa whether or not a keystone, but to explain

why keystones are different between distinct ecosystems would be necessary further investigation in deeper levels, e.g. complete metabolic pathways, genes expression.

In conclusion, our study found a similar bacterial community structure in two distant Amazon floodplain lakes and a non-random interaction network structure. We proposed a simple and replicable method to identify putative keystones (K-value), which is easily extendable to comparisons across different ecosystems. Using this node-based score we identified several keystones (from phyla Proteobacteria, Actinobacteria, Chloroflexi, Planctomycetes, and Cyanobacteria) some of them were the same for both distant lakes, indicating that they are probably also ubiquitous keystone in Amazon aquatic systems. We highlight that concomitant effort between computational inferences and empirical studies is necessary to uncover the real identity and role of microbial keystone species in their environments (Banerjee et al., 2018). Understanding how microbes interact is crucial to elucidate their community assembly patterns and to help to target the link between microbial community structure and ecosystem functioning.

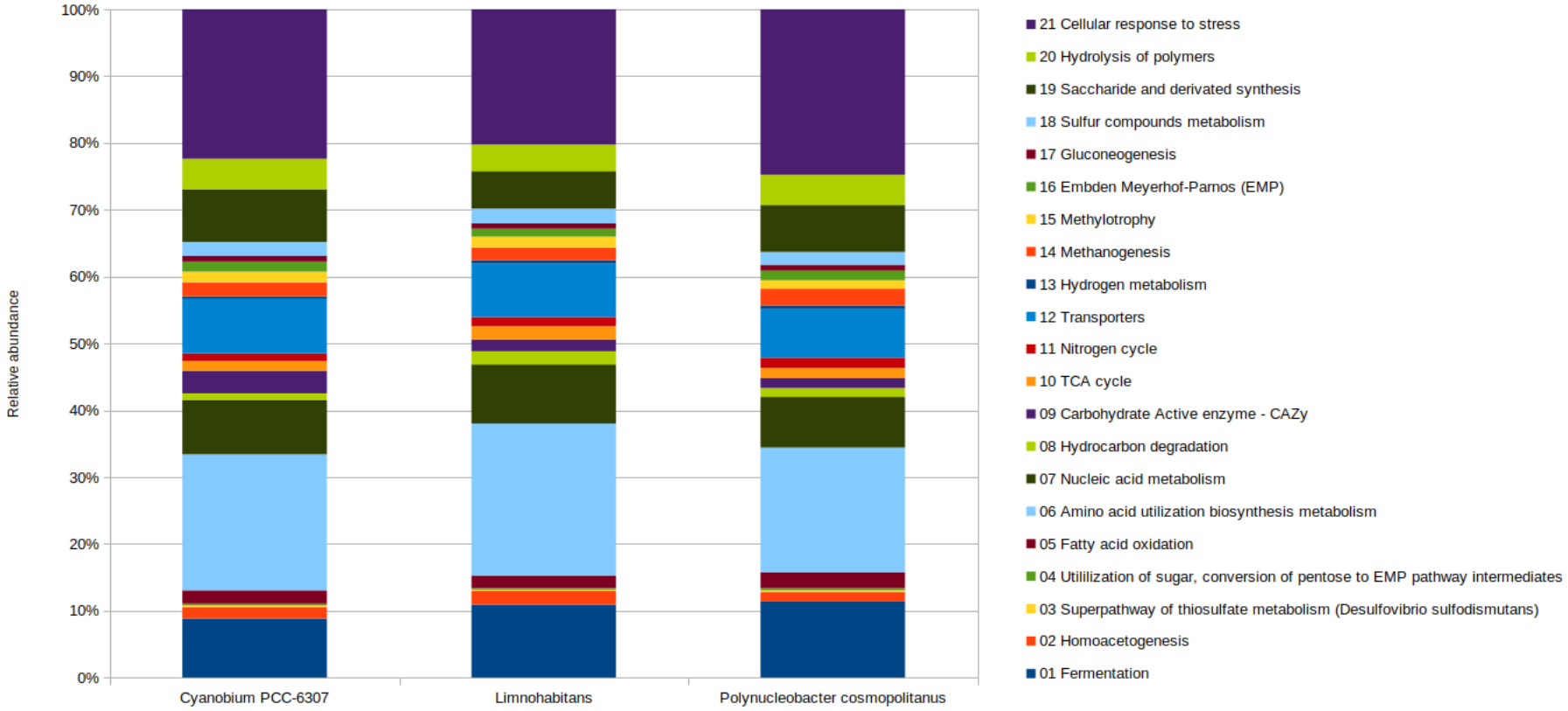

Figure III - 4: Relative abundance of functional genes identified for the three recovered genomes: *Cyanobium gracile* PCC-6307, *Limnohabitans sp., and Polynucleobacter cosmopolitanus*

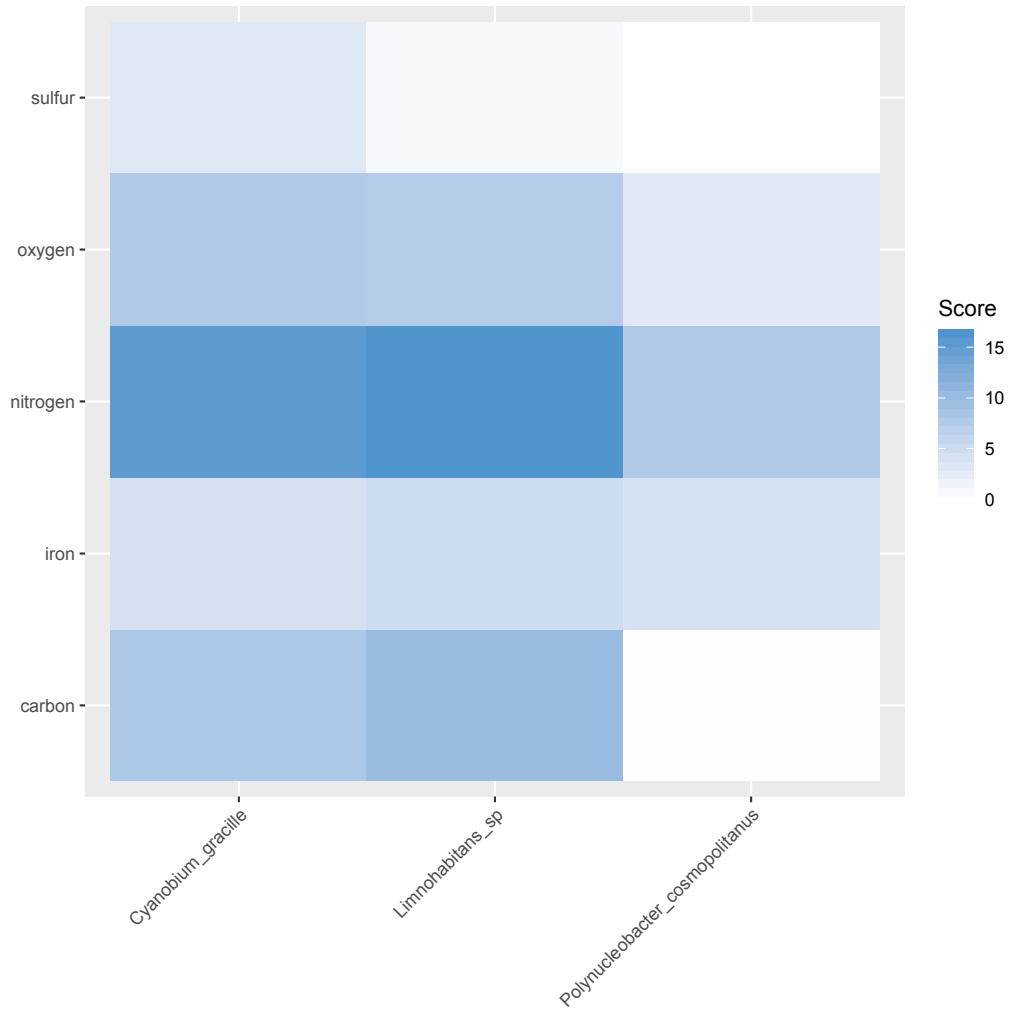

Figure III- S4: Heatmap of the scores of the main biogeochemical cycles for each keystone taxa (recovered metagenomes).

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Conflict of interest

The authors declare no conflict of interest.

Authors Contribution

MLM and HS designed the research approach. MLM, CSJ and HS performed network analyses and developed the K-value calculation. MLM wrote the first draft of the manuscript and all authors contributed to data interpretation and analysis and subsequent revisions of the manuscript.

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CONCLUSÕES GERAIS

Neste trabalho, nós investigamos a composição, regulação e dinâmica sazonal das comunidades bacterianas de sistemas de várzea amazônicos (diagrama da tese abaixo). Nossos resultados apontaram o importante papel do ciclo hidrológico (pulso de inundação) como força moduladora dos fluxos de água e materiais entre o rio principal e suas planícies de inundação (e sistemas associados). Usando uma abordagem de metacomunidade, revelamos que esses fluxos controlaram a dispersão de microrganismos e também as condições ambientais, que consequentemente filtraram o estabelecimento de bactérias dispersas. Observamos que diferentes fontes de bactérias contribuíram para a CCB do lago ao longo do ano e que a importância de cada fonte estava relacionada à um estágio específico do ciclo hidrológico. Por exemplo, durante a estação cheia, devido à alta vazão e nível de água, o rio Solimões foi a principal fonte de bactérias para a comunidade do lago Janauacá.

Em relação à composição das comunidades bacterianas, observamos padrões semelhantes aos encontrados anteriormente para outros sistemas amazônicos e de água doce em geral. Membros dos filos Actinobacteria, Proteobacteria, Planctomycetes e Cyanobacteria, foram os mais abundantes e variaram sazonalmente em abundância relativa. Como conclusão do Capítulo 1 destacamos que os processos de dispersão desempenham um importante papel em redes hidrológicas altamente conectadas e complexas, como os sistemas fluviais amazônicos.

No Capítulo 2, realizamos uma caracterização da MOD utilizando análises de suas propriedades ópticas. Nossos resultados apontaram que a origem, peso molecular e aromaticidade da MOD também foram afetadas por mudanças na hidrologia, indicando que a hidrodinâmica molda os intercâmbios da planície de inundação, rio principal e paisagem terrestre adjacente, e consequentemente afeta a contribuição relativa de MOD alóctone e autóctone. Nossos resultados apontam para um forte acoplamento entre MOD, composição e atividade do bacterioplâncton, e ainda revelou o importante papel de uma pequena fração de MOD lábil autóctone e sua rápida remineralização pelo bacterioplâncton como um processo importante que mantém a quantidade de MOD relativa baixa e estável ao longo do ano.

No último capítulo, analisamos as redes de interação microbiana do lago Janauacá e de mais um sistema com características semelhantes, localizado a jusante do rio Amazonas, o lago Curuaí. Vimos que ambos apresentaram característica semelhantes em relação à composição de táxons, e à organização não-randômica de suas redes de interação. Adicionalmente, usando as métricas das redes, propomos uma abordagem replicável e comparável para se identificar espécies-chave microbianas. Utilizando esse método, identificamos diversas espécies-chave para ambos os lagos e constatamos que algumas eram compartilhadas por ambos os sistemas. Essas pertenciam aos abundantes filos Actinobacteria, Proteobacteria e Chloroflexi, os quais são táxons dominantes e cosmopolitas em vários outros ecossistemas de água doce. Esses resultados indicam que essas espécies-chave podem também exercer papel chave no funcionamento de outros ecossistemas aquáticos amazônicos e sugerimos que mais estudos deveriam ser realizados para confirmar esse padrão, os quais devem considerar o uso de métodos adicionais, como experimentos, entre outras abordagens empíricas.

Como conclusão geral, essa tese disponibiliza importantes informações em relação à composição e fatores estruturadores de comunidades microbianas aquáticas em várzeas amazônicas. O pulso de inundação parece ser o principal fator regulador dessas comunidades, uma vez que controla os fluxos de água, materiais e microrganismos entre o rio principal e suas planícies de inundação, e ainda afeta os parâmetros ambientais que atuam na filtragem de bactérias. Portanto, estudos futuros sobre o bacterioplâncton na Bacia devem considerar ambas as escalas, espaciais e temporais, com ênfase no pulso hidrológico. Também revelamos que a interação entre bactérias parecem ter papel crucial na estrutura e equilíbrio de suas comunidades biológicas e existem espécies-chave na Amazônia, e que podem ser peças cruciais para um melhor entendimento sobre o funcionamento de ecossistemas de várzea e seu papel nos ciclos biogeoquímicos globais.

APÊNDICE A

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STANDARD PAPER

WILEY Freshwater Biology

Flood pulse regulation of bacterioplankton community composition in an Amazonian floodplain lake

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Abstract

1. Understanding spatial and temporal dynamics of microbial communities is a central challenge in microbial ecology since microorganisms play a key role in ecosystem functioning and biogeochemical cycles. Amazonian aquatic systems comprise a dynamic mosaic of heterogeneous habits but are understudied and there is limited information about the mechanisms that shape bacterial community composition (BCC).
2. There is a consensus that environmental selection (species sorting) and dispersal processes (source–sink dynamics) act in concert to shape the composition of these communities, but the relative importance of each mechanism may vary dramatically through time and between systems.
3. Applying 16S rRNA gene amplicon high-throughput sequencing, we studied factors and processes that modulate BCC in an Amazonian floodplain lake and used source-tracking models to trace the main dispersal sources of microorganisms in the whole floodplain system during a full hydrological cycle.
4. Our source-tracking models indicated that dispersal processes were predominant, explaining most of the BCC variability throughout the study period. We observed more sources contributing to the sink community during the falling water than rising water period, when contributions from the Solimões River dominated.
5. There was a clear seasonal pattern in BCC, closely related to environmental variables, suggesting that the successful establishment of dispersing bacteria also depends on environmental filtering that is linked to water flow.
6. In summary, source–sink dynamics and species sorting were strongly affected by water exchange and connectivity with the main river that varied throughout the flood pulse cycle. Our results demonstrated the influence of lateral transport and temporal dynamics on BCC in Amazonian floodplain lakes that could ultimately impact regional carbon budgets and biogeochemical cycles.

KEYWORDS
16S rRNA gene, high-throughput sequencing, metacommunity, source–sink dynamics, spatiotemporal dynamics

APÊNDICE B

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Influence of plankton metabolism and mixing depth on CO_2 dynamics in an Amazon floodplain lake

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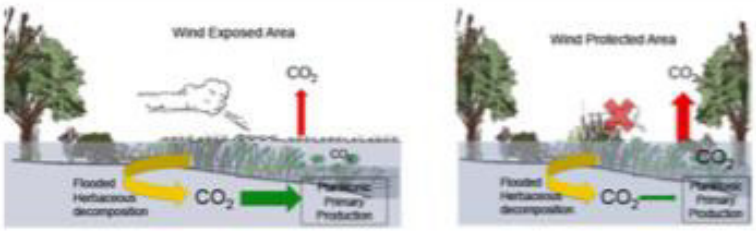
HIGHLIGHTS

- Large diel variations on CO_2 dynamics
- Higher plankton metabolism associated with wind mediated mixing.
- Phytoplankton consumption of CO_2 derived from macrophyte decomposition.
- Planktonic respiration is greater in solar exposed chambers.
- CO_2 emissions enhanced during exceptional drought.

GRAPHICAL ABSTRACT



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ABSTRACT

We investigated plankton metabolism and its influence on carbon dioxide (CO_2) dynamics in a central Amazon floodplain lake (Janauacá, 3°23′ S, 60°18′ W) from September 2015 to May 2016, including a period with exceptional drought. We made diel measurements of CO_2 emissions to the atmosphere with floating chambers and depth profiles of temperature and CO_2 partial pressure (pCO_2) at two sites with differing wind exposure and proximity to vegetated habitats. Dissolved oxygen (DO) concentrations were monitored continuously during day and night in clear and dark chambers with autonomous optical sensors to evaluate plankton metabolism. Overnight community respiration (CR), and gross primary production (GPP) rates were higher in clear chambers and positively correlated with chlorophyll-*a* (Chl-*a*). CO_2 air-water fluxes varied over 24-h periods with changes in thermal structure and metabolism. Most net daily CO_2 fluxes during low water and mid-rising water at the wind exposed site were into the lake as a result of high rates of photosynthesis. All other measurements indicated net daily release to the atmosphere. Average GPP rates (6.8 gC m^{-2} d^{-1}) were high compared with other studies in Amazon floodplain lakes. The growth of herbaceous plants on exposed sediment during an exceptional drought led to large carbon inputs when these areas were flooded, enhancing CR, pCO_2, and CO_2 fluxes. During the period when the submerged herbaceous vegetation decayed phytoplankton abundance increased and photosynthetic uptake of CO_2 occurred. While planktonic metabolism was often autotrophic (GPP:CR > 1), CO_2 out-gassing occurred during most periods investigated indicating other inputs of carbon such as sediments or soils and wetland plants.

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APÊNDICE C

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PRIMARY RESEARCH PAPER

Productivity and rainfall drive bacterial metabolism in tropical cascading reservoirs

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Abstract Tropical reservoirs are main carbon sources to the atmosphere, and bacterial metabolism is a key process in these emissions. Here, we explored the drivers of bacterial metabolism in four tropical cascading reservoirs forming a trophic state gradient, and compared them with those found in the literature (mainly from temperate regions). Bacterial production (BP) and growth efficiency (BGE) responded to trophic state-related variables, while bacterial respiration (BR) was weakly and negatively correlated to dissolved organic carbon (DOC). BP and BGE were higher in reservoirs with higher primary production, while BR (high throughout the whole study period) was greater in less productive reservoirs, where planktonic communities were often limited by phosphorus. The high BR and low BGE observed in less productive downstream reservoirs (i.e., less nutrients and organic matter availability) may be explained by increasing nutrient limitation and proportion of recalcitrant DOC along the cascade. Despite the lower productivity, oligotrophic reservoirs may be more important in terms of carbon biogeochemistry, considering that microbes in those systems mineralize more carbon than upstream productive reservoirs. Moreover, the drivers of bacterial metabolism may act differently according to latitude, as seasonality in the tropics is determined mainly by rainfall rather than temperature.

Keywords Bacterial respiration · Bacterial production · Bacterial growth efficiency · Trophic state gradient

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Introduction

In order to supply the increasing demands in energy and water supply, series of reservoirs have been built throughout the world. The potential of energy production is maximized when dams are disposed sequentially along the river course, and form a cascade of reservoirs that, in turn, have major impacts on ecosystem's matter cycle and energy flows (Straskraba, 1990). Taken as step-like continuous systems,

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