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HELENA HENRIQUES VIEIRA

BIODIVERSIDADE E PADRÕES DE COOCORRÊNCIA ENTRE FITOPLÂNCTON E BACTÉRIA NA CASCATA TRÓFICA DOS RESERVATÓRIOS DO RIO TIÊTE: UMA ABORDAGEM COM *HIGH THROUGHPUT SEQUENCING*

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Tese apresentada ao Programa de Pós-Graduação em Ecologia e Recursos Naturais da Universidade Federal de São Carlos, para obtenção do título de Doutora em Ciências, área de concentração: Ecologia e Recursos Naturais

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Certifico que a defesa realizou-se com a participação à distância do(s) membro(s) Sandra Maria Feliciano de Oliveira e Azevedo, Ana Beatriz Furlanetto Pacheco e, depois das arguições e deliberações realizadas, o(s) participante(s) à distância está(ao) de acordo com o conteúdo do parecer da banca examinadora redigido neste relatório de defesa.

Prof. Dr. Armando Augusto Henriques Vieira

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RESUMO

As comunidades microbianas de ambientes aquáticos compõem uma complexa rede ecológica onde organismos interagem entre si e com o ambiente. A maneira como estas comunidades microbianas estão estruturadas está sob influência de fatores abióticos como temperatura, pH, disponibilidade de nutrientes, entre outros, visto que os diversos *taxa* de organismos respondem de maneira diferente aos fatores ambientais. A disposição em cascata de reservatórios gera propriedades limnológicas além das frequentemente relatadas para sistemas lênticos. Maior mistura de água gera maior turbidez da água nos reservatórios a montante, pulsos de nutrientes e introdução de organismos proveniente dos reservatórios anteriores e das adjacências de cada reservatório são característicos de sistemas em cascata. Estações de seca e de chuva devem modular tais atributos através da quantidade de água que conecta os reservatórios, de maneira que a estação chuvosa promove maior transporte de água da montante à jusante, devido ao menor tempo de residência, além de maior introdução de material alóctone. O contrário é esperado na estação de seca, onde o menor fluxo de água entre os reservatórios aumenta o tempo de residência dos reservatórios permitindo que os organismos que ali se encontrem permaneçam por período mais longo nos reservatórios. Neste estudo, exploramos a composição da comunidade microbiana de quatro reservatórios em cascata do médio-baixo Tietê assim como os fatores que regeram a migração dos microrganismos ao longo da cascata nos períodos de seca e de chuva. Por fim, analisamos o efeito do gradiente trófico nas redes ecológicas das comunidades microbianas de cada reservatório. Nós encontramos que a formação em cascata destes reservatórios influenciou a estrutura das comunidades microbianas de maneira que cada subcomunidade (bactérias aderidas a partículas, de vida livre, cianobactérias e fitoplâncton eucariótico) estudada respondeu a diferentes fatores ambientais que variaram ao longo da cascata ou de estações. Da mesma

maneira, a variação nas estruturas das subcomunidade ao longo da cascata foi guiada tanto por fatores locais (condições ambientais local de cada reservatório), que promovem seleção, por fatores regionais (dispersão dos orgaismos), entretanto com predominância dos fatores regionais. Finalmente, observamos a influência do gradiente trófico nas redes ecológicas de interações microbianas: ambientes altamente eutrofizados, por constringir a diversidade especialmente de produtores primários, produz redes com alta modularidade, ou seja, subgrupos interagindo de maneira menos inserida da rede global. Já com a redução da eutrofização observamos redes mais conectadas, com menor modularidade, onde subgrupos encontram-se imersos na rede global da comunidade.

Palavras-chave: Reservatórios. Reservatórios em cascata. Metacomunidade microbiana aquática. Redes ecológicas. Gradiente de estado trófico.

ABSTRACT

Microbial communities of aquatic environments make up a complex ecological network where organisms interact with each other and with the environment. The way these microbial communities are structured is influenced by abiotic factors such as temperature, pH, nutrient availability, etc., since the different taxa of organisms respond differently to environmental factors. Cascade arrangement generates limnological properties beyond those often reported for lentic systems. Greater mixing of water, that generates greater water turbidity in upstream reservoirs, nutrient pulses, and introduction of organisms from the previous reservoirs and the adjacencies of each reservoir are characteristic of cascade systems. Dry and rainy seasons should modulate such attributes through the amount of water that connects the reservoirs, so that the rainy season promotes greater upstream transport of water from the downstream due to the shorter residence time, as well as greater introduction of allochthonous material. The opposite is expected in the dry season, where the lower flow of water between the reservoirs increases the residence time of the reservoirs allowing the organisms to remain for a longer period in the reservoirs. In this study, we explored the composition of the microbial community of four cascading reservoirs of the medium-low Tietê River as well as the factors that governed microorganism's turnover along the cascade in the dry and rainy periods. Finally, we analyzed the effect of the trophic gradient on the ecological networks of the microbial communities of each reservoir. We found that the cascade formation of these reservoirs influenced the structure of the microbial communities so that each subcommunity (bacteria attached to particles or free-living, cyanobacteria and eukaryotic phytoplankton) studied responded to different environmental factors that varied along the cascade or seasons. In the same way, the variation in subcommunity structures along the waterfall was guided by both local factors (local environmental conditions of each reservoir,

promoting selection) and regional (dispersion of organisms), but with regional factors predominating. Finally, we observed the influence of the trophic gradient on the ecological networks of microbial interactions: highly eutrophic environments, by constricting the diversity, especially of primary producers, produce networks with high modularity, that is, subgroups interacting less inserted the global network. With the eutrophication reduction we observed more connected networks, with less modularity, where subgroups are immersed in the global network of the community.

Keywords: Reservoirs. Cascading reservoirs. freshwater bacterioplankton metacomunity. Ecolgical networks. Trophic state gradient

LISTA DE SIGLAS

BB – Barra Bonita

Chla – Chlorophyll-a

DL – Dispersal Limitation

DO – Dissolved Oxigen

D - Drift

DOC – Dissolved Organic Carbon

FL – Free-living

HD – Homogenizing Dispersion

HTS – High throughput sequencing

MO – Matéria Orgânica

MOD – Matéria Orgânica Dissolvida

NA – Nova Avanhandava

OTU – Operational Taxonomic Unit

PA – Particle attached

PCA – Principal Coordinate Analysis

Pr – Promissão

RC – Raup-Crick

rDNA – Ribossomal deoxyribonucleic acid

rRNA – Ribossomal ribonucleic acid

RT – Residence Time

S - Selection

TI – Três Irmãos

TN – Total Nitrogen

TP – Total Phosphorous

TSI – Trophic State Index

βMNTD - beta-mean-nearest taxon distance

βNTI - beta-nearest taxon index

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INTRODUÇÃO GERAL

Comunidades Microbianas aquáticas

O universo microbiano aquático abriga rica biodiversidade que compõe complexas redes de interação responsáveis por importante porção da ciclagem de nutrientes orgânicos e inorgânicos. As bactérias heterotróficas são responsáveis por reintroduzir às teias tróficas grande parte da matéria orgânica dissolvida em matéria orgânica particulada no ambiente através da alça microbiana (AZAM et al., 1983). Microrganismos capazes de realizar fotossíntese, como cianobactérias e microalgas, são importantes fontes de matéria orgânica (MO) que é liberada ao ambiente na forma dissolvida e então preferivelmente consumido por bactérias (SIMON; CHO; AZAM, 1992; THORP; DELONG, 2002). Contudo, bactérias utilizam tanto matéria orgânica lábil como matéria orgânica recalcitrante, principalmente terrestre carreado pela chuva.

Ambientes aquáticos eutrofizados, com grande disponibilidade de nutrientes favorecem a rápida proliferação, principalmente de cianobactérias que formam densas florações e liberam grande quantidade de matéria orgânica dissolvida para o ambiente, constituindo uma rica fonte de matéria orgânica disponível para o bacterioplâncton (GIROLDO; VIEIRA; PAULSEN, 2003; THORP; DELONG, 2002). Estas densas florações geram consequências que podem se estender ao longo da cadeia trófica, como: competição com outras espécies fitoplanctônicas por nutrientes; o sombreamento da camada subsuperficial que impede o desenvolvimento de outros grupos fitoplanctônicos, pois essas florações encontram-se geralmente na superfície; na camada subsuperficial, com a ausência de organismos fotossintizantes, o ambiente se torna anóxico, o que favorece linhagens bacterianas anaeróbicas; e morte da floração, que disponibiliza uma grande carga de matéria orgânica para bactérias e de toxinas, (cianotoxinas)

(SANT'ANNA et al., 2008). Em ambientes aquáticos tropicais, as consequências da eutrofização são intensificadas, pois na região tropical, a alta incidência solar leva a maior produção primária e excreção de compostos pelo fitoplâncton e altas temperaturas elevam o metabolismo bacteriano, levando o sistema à limitação por DOC (Freitas et al., 2018). Desta maneira, as florações fitoplanctônicas têm importante impacto na teia trófica afetando o fluxo de energia através desta (AZAM; MALFATTI, 2007).

Os microrganismos estabelecem associações entre si em uma gama de interações que variam de mutualísticas, facultativas, parasíticas e associações espécie-específica. Nas associações alga-bactéria, as bactérias podem se abrigar na ficosfera, área sob influência da liberação de compostos pela célula algal, como mucilagem excretada pelo fitoplâncton e que envolve a célula algal (BELL; MITCHELL, 1972). A ficosfera é um ambiente rico em nutrientes e matéria orgânica. Bactérias ali alojadas assimilam a MOD liberada pela alga e também estão protegidas de predação (SEYMOUR et al., 2017). Para o fitoplâncton, essa associação é geralmente benéfica, pois bactérias suprem o fitoplâncton com nutrientes orgânicos e inorgânicos mineralizados (PAERL et al., 2001), promotores de crescimento (CROFT et al., 2005) e antibióticos prevenindo ataques de outras bactérias e vírus (MAYALI; AZAM, 2004) .

Para organismos auxotróficos (incapazes de produzir alguns compostos essenciais à sua sobrevivência), estas associações são fundamentais onde a coexistência garante a viabilidade de uma ou ambas as partes envolvidas. Por exemplo, estima-se que 50% do fitoplâncton é auxotrófico para vitamina B12 (CROFT; WARREN; SMITH, 2006). A viabilidade destes fitoplanctons auxotróficos é dependente de associações com linhagens de bactérias capazes de produzir a vitamina ou precursores para sua síntese (GRANT et al., 2014). Entretanto, fitoplâncton e bactéria associados podem competir pela vitamina.

Diferentes espécies fitoplanctônicas excretam diferentes qualidade e quantidades de MO (FOGG, 1983) e o fitoplâncton hospedeiro pode também selecionar bactérias às quais irão se associar (TEELING et al., 2012). Assim, diferentes *taxa* bacterianos são recrutados para associarem-se ao fitoplâncton. Além disso, o estado fisiológico do fitoplâncton também influencia o bacterioplâncton associado, uma vez que algumas linhagens são mais propensas a associações em estágios iniciais ou terminais de florações (BAGATINI et al., 2014; EILER; BERTILSSON, 2007; RUSSO et al., 2016). Desta maneira, espera-se que a diversidade fitoplanctônica tenha impacto na diversidade do bacterioplâncton. Ainda, bactérias podem assumir um comportamento oportunista e induzir a morte da floração para acessar o conteúdo celular algal (SEYEDSAYAMDOST et al., 2011) ou ainda resultar em competição por nutrientes, afetando o crescimento do fitoplâncton. Bactérias de vida livre também interagem com o fitoplâncton através da MO que é liberada no meio. Entretanto, associações entre bactérias de vida livre e fitoplâncton são menos específicas (GROSSART et al., 2005; SIGEE, 2005).

Assim, é fundamental o conhecimento dos organismos presentes num dado ambiente, assim como suas funções desempenhadas nas redes ecológicas. Entretanto sabe-se muito pouco sobre a diversidade microbiana, e o que se tem identificado em bactérias hoje é uma fração ínfima de sua real biodiversidade (PACE, 2009). A maior parte das comunidades de ambientes naturais é composta de micro-organismos ainda não identificados e não cultiváveis em condições laboratoriais (RAPPÉ; GIOVANNONI, 2003), o que impede que se aplique a maneira tradicional de identificação e estudo sobre sua ecologia (ROSZAK; COLWELL, 1987). Esta porção de bactérias não identificadas pode conter possíveis filos ainda não descobertos assim como processos metabólicos ainda não observados para todos os domínios de vida já descritos.

Metagenômica – High Throughput Sequencing (HTS)

A maneira mais eficiente de acessar a biodiversidade microbiana de diversos ambientes é através da metagenômica - *High Throughput Sequencing*, ou sequenciamento massivo de genes alvo de uma comunidade inteira. Esta técnica é um grande avanço no estudo de micro-organismos de ambientes naturais, pois permite a detecção de organismos ainda não identificados, não cultivados ou ainda se quer sequenciados (STEWART, 2012). Para organismos procariotos, o gene 16S rRNA é extensivamente usado e seu sequenciamento recupera grande biodiversidade destes organismos. Entretanto as taxas de evolução deste gene em procariotos podem variar de táxon para táxon e o método de identificação em unidades taxonômicas operacionais (OTUs) pode subestimar ou superestimar a real diversidade existente.

Bactérias podem apresentar sobreposição de grupos funcionais, devido à vasta gama de adaptações ao meio que se encontram, ou seja, a definição de seus papeis ecológicos não é tão bem delineada e muitas podem coexistir particionando os mesmos recursos ou exercer funções similares no ambiente (COMTE; FAUTEUX; DEL GIORGIO, 2013). Assim, o uso de OTU para bactérias pode mascarar, agregando ou separando, grupos funcionais coerentes.

Alguns métodos usam o sinal filogenético do gene 16S rRNA para grupar OTUs em grupos funcionais. A partir da proximidade filogenética de OTUs obtidas com o 16S rDNA que compartilham habitats muito similares, se estabelece grupos funcionais bacterianos aplicável para cada estudo em particular (STEGEN et al., 2013). Por exemplo, em estudos de redes ecológicas é interessante agrupar táxons muito próximos por estes desempenharem papéis muito semelhantes na rede, o que pode resultar em superestimação de correlações positivas (FAUST; RAES, 2016). Fatores ambientais que são seletivos para uma linhagem bacteriana, podem ter efeito igual em linhagens bactérias

filogeneticamente próximas e que respondam igualmente a um determinado fator. De qualquer maneira, o sequenciamento massivo de comunidade microbianas é uma técnica que possibilitou o acesso ao universo microbiano.

Cascata de reservatórios do médio-baixo Tietê

A cascata de reservatórios do médio-baixo Rio Tietê está localizada no Rio Tietê, bacia do Paraná, estado de São Paulo (Fig. 1). O Rio Tietê nasce no município de Salesópolis e tem sua foz no Rio Paraná, a 1.100 km de distância de sua nascente. A cerca de 80 quilômetros distante de sua nascente, no Alto Tietê, está a região metropolitana de São Paulo, altamente urbanizada com cerca de 22 milhões de habitantes. O Rio Tietê atravessa a cidade de São Paulo, onde recebe grande aporte de rejeitos domésticos e industriais provenientes da metrópole e cidades adjacentes. O rio Tietê também é destino de rejeito destas cidades, das indústrias e da agricultura em seu entorno, e por isso, encontra-se em constante eutrofização pela alta descarga de compostos ricos em amônia, fosfato e nitrato (THOMAZ; BINI, 1999). Ao longo do curso, o rio é usado para abastecimento de industrias, irrigação de agricultura e também navegação, pesca e lazer. Devido ao rápido crescimento demográfico e industrial da região, construiu-se reservatórios ao longo do seu leito para geração de energia hidrelétrica (RODGHER, 2001). O primeiro reservatório da cascata do médio-baixo do Rio Tietê, Barra Bonita, com início das operações em 1963, está a aproximadamente 300 quilômetros de distância da cidade de São Paulo. Este reservatório é conhecido por ser hipereutrófico e seu perfil limnológico foi extensamente descrito na literatura (BARBOSA et al., 1999; BUZELLI; DA CUNHA-SANTINO, 2013; COSTA, 2001; RODGHER, 2001; SMITH; ESPÍNDOLA; ROCHA, 2014; TUNDISI et al., 1991; VALENTE; PADILHA; DA SILVA, 2018). Após Barra Bonita, estão as usinas hidrelétricas (UHE) de Ibitinga e Bariri, consideradas usinas fio d'água, o que significa que a vazão de seus reservatórios

é irrelevante na produção de energia. Em seguida, a 180 km de Barra Bonita estão os reservatórios de Promissão, de Nova Avanhandava, distante 55 km de Promissão e finalmente, o reservatório de Três Irmãos, a 170 km de Nova Avanhandava (Fig. 1).

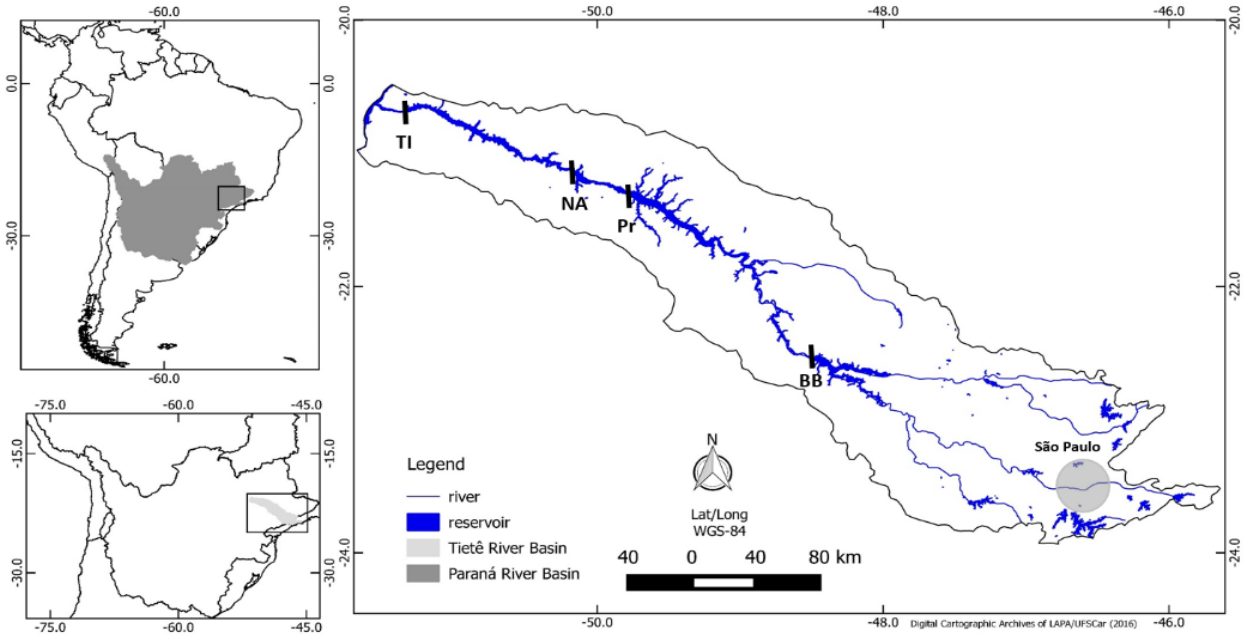

Figura 1 - Mapa da bacia do Paraná (painel à esquerda e acima), Rio Tietê (painéis à esquerda e abaixo e central) e localização dos reservatórios do médio-baixo Rio Tietê destinados à geração de energia hidrelétrica. O círculo em cinza indica a região metropolitana de São Paulo. Barra Bonita (BB), Promissão (Pr), Nova Avanhandava (NA) e Três Irmãos (TI). Modificado de Freitas et al. (2018).

Muitos autores já estudaram os componentes lóticos (trechos do rio) e lênticos (reservatórios) da cascata do médio-baixo Rio Tietê. Nestes estudos, eles investigam as condições limnológicas, bioquímicas e fisiológicas. Estudos sobre os organismos variam desde peixes (MARUYAMA; CASTRO; PAIVA, 2009; STEFANI, 2006),

macroinvertebrados (RODRIGUES, 2003), fitoplâncton (CAVENAGHI et al., 2003; MOURA et al., 2013; PADISÁK et al., 2000), bactérias (BRAMORSKI; VILLELA, 2014; FREITAS et al., 2018). Estudos bioquímicos abordam compostos autóctones, como polímeros exudados pelo fitoplâncton e seu papel ecológico na vida microbiana, assim como processos naturais de degradação de tais compostos (BITTAR, 2005; VIEIRA, 2000), e alóctones, como a concentração de metais pesados que são descarregados no rio (BEVILACQUA et al., 2009; PASCHOAL, 2002).

Características limnológicas de reservatórios

As características limnológicas dos reservatórios são decorrentes de sua morfometria, como profundidade, comprimento, volume, assim como de sua hidrologia, como tempo de residência, e também do entorno (área urbana, rural, tipo e extensão de plantio, etc). Entretanto, reservatórios não naturais possuem características próprias que diferem dos naturais. Como descrito por Straškraba, (1994) os reservatórios artificiais diferem dos naturais em aspectos qualitativos e quantitativos. Em relação aos aspectos qualitativos, os reservatórios artificiais são mais jovens, tem profundidade máxima próxima à barragem, enquanto que nos naturais, a profundidade máxima está localizada centralmente. O sedimento é composto de matéria alóctone e os gradientes longitudinais são determinados pelo fluxo de água (STRAŠKRABA, 1994). Em relação aos quantitativos, reservatórios artificiais tem menor tempo de retenção, maior área de inundação e maior amplitude na flutuação hidrodinâmica (nível da água).

A eutrofização de corpos aquáticos é um processo que pode ocorrer naturalmente pelo carreamento de matéria alóctone para o sistema. Entretanto, a ação antropológica, como descrita acima para a cascata de reservatórios do Médio-baixo Tietê, pode

potencializar o processo de eutrofização (TUNDISI; MATSUMURA-TUNDISI; ABE, 2008).

Reservatórios em cascata

A disposição espacial de reservatórios em cascata gera características limnológicas próprias ao sistema e estão intrinsicamente relacionadas com o tempo de retenção dos reservatórios (STRAŠKRABA, 1994). As características limnológicas do primeiro reservatório da cascata são similares a de um reservatório isolado. Entretanto, reservatórios subsequentes experimentam variações decorrentes do fluxo de água conectando-os. Primeiramente, a cascata de reservatórios funciona como uma armadilha de nutrientes, onde boa parte dos nutrientes fica retida no primeiro reservatório e de maneira decrescente nos reservatórios seguintes. A diminuição dos nutrientes ao longo da cascata se dá por processo de sedimentação de partículas suspensas e assimilação pelo fitoplâncton e, então, observa-se melhoria na qualidade da água, como a crescente transparência da água e oxigenação a jusante da cascata (Fig. 2).

Consequentemente, observa-se também que produtores primários passam a ser compostos por espécies típicas de ambientes oligotróficos com decaimento na produção primária, devido aos nutrientes limitantes que ficam presos e também assimilados pelo fitoplâncton nos reservatórios anteriores (Fig. 2).

Entretanto, a produção primária é compensada a jusante, devido à maior penetração de luz na coluna d'água, devido justamente à sedimentação de partículas nos reservatórios anteriores. Ainda, um curto tempo de residência promove descarga da biomassa dos produtores primários para reservatórios subsequentes, porém promove alta da produtividade, devido à introdução de nutrientes provenientes dos reservatórios acima na cascata (STRAŠKRABA, 1994).

A dinâmica hidrológica particular de reservatórios em cascata deve transferir também consequências aos organismos presentes nestes ambientes. Visto que estes organismos interagem entre si e com o ambiente, é esperado que a estrutura das comunidades, e consequentemente, a maneira como redes ecológicas são estabelecidas nessas comunidades, estejam sob influência das propriedades limnológicas geradas pelo arranjo espacial em cascata dos reservatórios.

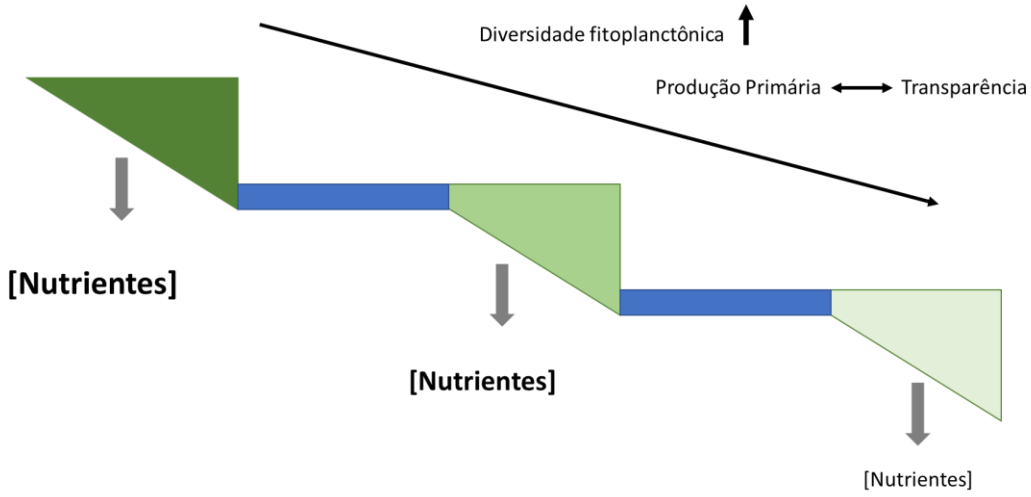

Figura 2 - Representação gráfica dos efeitos da formação em cascata em reservatórios. A jusante da cascata, a concentração de nutrientes tende a diminuir, devido ao consumo pelos produtores primários e também pelo processo de sedimentação de partículas. A transparência da água consequentemente aumenta assim como a oxigenação, o que compensa a diminuição dos nutrientes e sustenta a produção primária nos reservatórios menos eutróficos.

Um fator importante à composição microbiana de ambientes aquáticos é o tempo de residência e o regime de chuvas. Juntos, esses fatores regulam a entrada e saída de organismos do ambiente (dispersão). O regime de chuvas promove a entrada de microrganismos oriundos das adjacências dos reservatórios pelo carreamento de matéria alóctone que será inserida no ambiente enquanto o tempo de residência regula a saída destes organismos. Numa cascata de reservatórios, organismos são expedidos dos

reservatórios a montante para os reservatórios a jusante. Assim, em períodos de chuva, o fluxo de água que conecta os reservatórios é mais intenso e somado ao menor tempo de residência uma maior quantidade de organismos é dispersada ao longo da cascata. Para micro-organismos que são diminutos em tamanho e muito abundantes (FENCHEL; FINLAY, 2004; FINLAY, 2002), essa dispersão é potencializada. Porém, a dispersão pode ser também dependente da distância entre os reservatórios, onde os mais próximos tendem a ter maior dispersão entre si, ou dispersão homogeneizadora das comunidades de organismos. Concordantemente, reservatórios mais distantes podem dificultar a dispersão dos micro-organismos estando estes então em dispersão limitadora. Outro fator que influencia a dispersão de micro-organismos é o filtro ambiental, ou fatores locais de cada reservatório que selecionam organismos e impeça que alguns grupos de organismos sobrevivam ali.

HIPÓTESES

1) Sabendo que parâmetros bióticos e abióticos influenciam a composição das comunidades microbianas e que disposição dos reservatórios em cascata gera condições ambientais além das comumente observadas para reservatórios isolados, como o decaimento da trofia e turbidez, *esperamos que a comunidade microbiana apresente estrutura que covarie com as condições ambientais de cada reservatório*. Ainda, como o regime de precipitação também influencia as condições ambientais geradas pela disposição em cascata dos reservatórios por modular diretamente o fluxo de água conectando-os através do tempo de residência de cada reservatório, *esperamos que as comunidades microbianas da cascata apresentem diferenças em sua estrutura entre os períodos de seca e de chuva.*

2) Micro-organismos têm dispersão facilitada devido à sua alta abundância e tamanho celular. Sabendo que fatores ambientais (locais) promovem a seleção destes micro-organismos, mas que também fatores regionais dos ambientes, como a distância entre reservatórios da cascata interferem em sua dispersão, *esperamos que durante o período de chuva a menor conectividade entre os reservatórios causada pelo menor fluxo de água e, portanto, maior tempo de residência, as comunidades microbianas estariam predominantemente sob influência de seleção. Durante o período chuvoso, o menor tempo de residência homogeneizaria as condições ambientais entre os reservatórios assim como as comunidades, que estariam sob maior dispersão e pouca influência de seleção.*

3) Visto que o estado trófico dos reservatórios em cascata é um gradiente decrescente, e que a produção primária responde diretamente ao estado trófico, *as associações estabelecidas dentro das comunidades microbianas devem igualmente ser influenciadas por esse gradiente de trofia. Desta maneira, esperamos que o decaimento*

da trofia ao longo da cascata influencie na composição e topologia das redes de cada reservatório.

OBJETIVOS

1) Avaliar os efeitos que a disposição em cascata dos reservatórios gera nas condições ambientais e sobre as estruturas das comunidades bacterianas e de fitoplâncton eucariótico ao longo da cascata e nos períodos de seca e de chuva.

2) Identificar os processos ecológicos regionais e locais (deriva, dispersão homogeneizadora, limitação por dispersão e seleção) que estão dirigindo o *turnover* das comunidades microbianas ao longo da cascata nos períodos de seca e de chuva.

3) Avaliar o efeito do gradiente trófico da cascata sobre as redes ecológicas compostas pelas comunidades de bactérias aderidas e fitoplanctônica.

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METODOLOGIA GERAL

Local de estudo

O local estudado localiza-se no sistema do médio-baixo rio Tietê, bacia do rio Paraná, estado de São Paulo, Brasil (Fig. 1). A cascata abriga seis reservatórios artificiais de usinas hidrelétricas: Barra Bonita (BA), Bariri, Ibitinga, Promissão (PR), Nova Avahandava (NA) e Três Irmãos (TI), nesta ordem (Fig. 1). Bariri e Ibitinga não foram amostrados por serem reservatório fio-d'agua de menor volume. O sistema está localizado em uma região cercada por atividades agrícolas que injetam grandes quantidades de nutrientes no sistema, além dos rejeitos domésticos e industriais. Nesta cascata fluvial, o fluxo de água dirige-se para o interior do continente, e o despejo de resíduos de origem antrópica é maior no reservatório de Barra Bonita, devido às grandes cidades a montante, como a região metropolitana de São Paulo. Por causa disso, o reservatório de Barra Bonita é o reservatório mais eutrofizado da cascata, e a produtividade e o estado trófico diminuem ao longo do fluxo do rio devido ao gradual efeito de remoção / diluição de nutrientes (Freitas et al., 2018).

A região onde o sistema está localizado passou por uma grave seca em 2015 que comprometeu o abastecimento de água à bacia (INMET, 2016). Como esses reservatórios foram originalmente construídos para a geração de energia hidrelétrica, a empresa hidrelétrica responsável controla o regime de abertura e fechamento das comportas para manter a quantidade regular de água nos reservatórios, influenciando, portanto, o tempo de residência dos reservatórios. No final de 2015, o evento El Niño levou à intensificação das chuvas na região, criando assim condições anormais de precipitação que resultaram em episódios de inundação nos reservatórios (World Meteorological Organization, 2016).

Coleta das amostras

Amostras dos quatro reservatórios foram coletadas a cada dois meses, com inicio em maio de 2015 e término em março de 2016, totalizando seis eventos de amostragem. Estabelecemos pontos de amostragem usando um GPS (Global Position System) dentro de cada reservatório de modo que as coletas fossem realizadas no ponto de maior profundidade, próximo à barragem (Tabela 1)

Tabela 1 - Localização, volume e distânica dos quatro reservatórios da porção médio-baixo do Rio Tietê. A localização está em coordenadas obtidas com GPS (Global Position system) e a distancia (km) é em relação ao primeiro reservatório (Barra Bonita).

	Barra Bonita	**Promissão**	**Nova Avanhandava**	**Três Irmãos**
Localização (GPS)	22° 32.648' S 048° 27.97' W	21° 19.123' S 049° 44.724' W	21° 06.455' S 050° 10.954 W	20° 40.110' S 051° 16.855' W
Volume (m^3)	$3.14\ 10^9$	$7.42\ 10^9$	$2.72\ 10^9$	$13.8\ 10^9$
Distancia (km)	0	180	235	355

Cerca de 1 litro de água foi coletado das águas sub-superficiais em cada reservatório. Por filtração seqüencial, separamos o fitoplâncton eucariótico e as bactérias aderidas a partículas (particle-attached – PA) em filtros de policarbonato de 3µm (Sartorius Stedim-Biotech). Do volume filtrado, subsequentemente coletamos bactérias de vida livre (free-living - FL) em filtros de membrana de acetato de 0,22um (Sterivex) (Fig. 3). Os filtros contendo as comunidades bacterianas foram imediatamente congelados em nitrogênio líquido e transportados para o laboratório para armazenamento a longo prazo a -80 ° C.

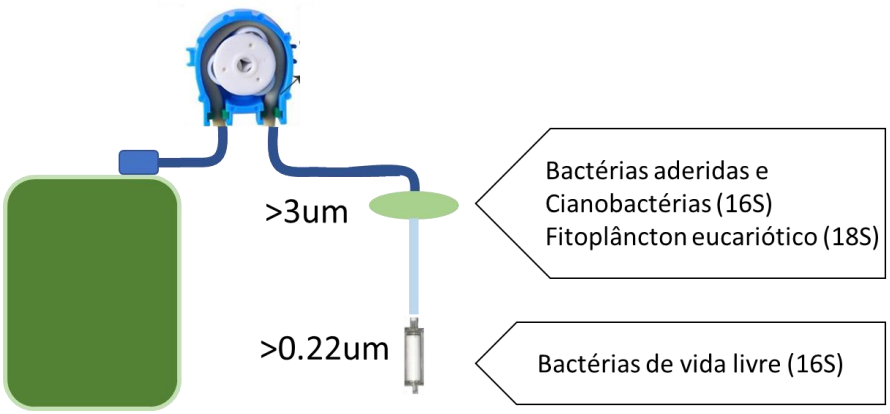

Figura 3 – Representação gráfica do processo de filtragem das amostras coletadas. O conteúdo coletado dentro do galão de 20L é bombeado por uma bomba peristáltca ao filtro de 3 um, onde ficam retidas as comunidades de bactérias aderidas a partículas, cianobactérias e o fitoplancton eucariótico. Em seguida, o que não ficou retido passa por um filtro de 0.22 um, onde fica retida a comunidade de bactérias de vida-livre.

Parâmetros e análises ambientais

As variáveis ambientais pH, temperatura e oxigênio dissolvido foram medidas com uma sonda multiparamétrica YSI 6600 V2 (YSI, Yellow Springs, OH, EUA) no momento das coletas. A clorofila-a (Chla) foi extraída segundo Marker et al. (1980) e Nusch (1980) e quantificada em espectrofotômetro como descrito por Lorenzen, (1967). O carbono orgânico dissolvido (dissolved organic carbono – DOC) e o nitrogênio total (Total nitrogen – TN) foram quantificados utilizando o analisador Shimadzu TOC-V cph, equipado com o Total Nitrogen Module. Fosfato e sulfato livres foram analisados por cromatografia iônica usando um sistema Dionex ICS - 1100 (Thermo Scientific). O fósforo total foi calculado a partir da soma da quantidade de fósforo soluvel reativo e fósforo particulado orgânico. O fósforo orgânico, foi quantificado por método colorimétrico após digestão com persulfato de potássio, como descrito em (Mackerth, 1978).

O tempo de residencia foi calculado a partir da seguinte equação:

$$RT = V / q \quad (1)$$

Onde V é o volume (m^3) e q é o influxo de água dos tributários ($m^3\ d^{-1}$). Os dados hidrológicos foram fornecidos pela Agência Nacional de Águas (Ana, 2016) e Instituto Nacional de Meteorologia (INMET, 2016).

Extração de material genético, amplificação e sequenciamento

O material genético das comunidades microbianas foi extraído usando o kit PowerSoil DNA Isolation (MoBio). Para acessar as comunidades procarióticas (PA, FL e cianobactérias) fizemos amplificação da região V3-V4 do gene 16S rDNA usando primers 341F (5'CCTACGGGNGGCWGCAG-3') e 805R (5'-GACT ACHVGGGTATCTAATCC-3') (HERLEMANN et al., 2011). Para acessar a comunidade fitoplanctônica eucariótica, a região V4 do gene 18S foi amplificada usando os primers TAReuk454FWD1 (5'-CCAGCASCYGCGGTAATTCC-3 ') e TAReukREV3 (5'-ACTTTCGTTCTTGATYRA-3') (STOECK et al., 2010). Os produtos de PCR foram marcados com o kit NEXTERA XT V2 (Illumina).

O sequenciamento de alto rendimento foi realizado usando a plataforma Illumina MiSeq. As sequências foram processadas usando UPARSE (EDGAR, 2013), conforme descrito em (LOGARES, 2017; LOGARES et al., 2014) para controle de qualidade sequencial e agrupamento em unidades taxonômicas operacionais (operational taxonomic unity – OTU) em uma similaridade de seqüência ≥ 97%. A classificação taxonômica foi obtida com o BLASTn contra o banco de dados SILVA 119.1 (ZHANG et al., 2000). A tabela de dados contendo as OTUs foi rarefeita e, em seguida, as comunidades de bactérias aderidas a partículas e de vida livre foram separadas, assim como as cianobacterias.

Cada capítulo da tese explora as metacomunidades microbianas ao longo da cascata de reservatórios e dos períodos de seca e chuva da seguinte maneira:

Capitulo 1 - explora a composição das subcomunidades microbianas (PA, FL, cianobactérias e fitoplankton eucariótico) ao longo da cascata e nos períodos de seca e chuva;

Capitulo 2 - aborda os padrões de dispersão das subcomunidades de bactérias PA, FL e cianobactérias ao longo da cascata e nos períodos de seca e chuva;

Capitulo 3- aborda as redes ecológicas estabelecidades entre as subcomunidades PA, cianobactérias e fitoplancton eucariótico de cada reservatório usando todas as seis amostragens.

CAPÍTULO 1 - MICROBIAL COMMUNITY COMPOSITION FROM A TROPICAL CASCADE OF RESERVOIRS

Abstract

Freshwater natural systems hold limnological features that differ from damned reservoir, and the cascade arrangement has intrinsic factors that causes extra limnological variability towards downstream reservoirs. Microbial community's composition is influenced by local conditions, such as environmental physical and chemical factors from the habitat, but also by trophic interaction with other organisms. Consequently, pulses of nutrients and organisms caused by the cascade formation is an important factor driving microbial communities' composition from these systems. In this study, we explored the microbial community from a tropical cascade of four reservoirs in two marked seasons of dry and rainy period to evaluate effects that cascade arrangement generates on environmental conditions and communities' composition. In our results, cascade arrangement yielded particular environmental conditions in reservoirs, such as decrease in nutrients and in primary production, due to sedimentation of organic matter along the cascade. Communities had its composition explained by distinct factors at different taxonomic levels. Primary producers (cyanobacteria and eukaryotic phytoplankton) and particle-attached bacteria were responding to variables linked to the trophic state, with variations on their composition better explained by reservoir. Frequent cyanobacterial blooms of few species observed in all four reservoirs reflected the constant input of nutrients into the system. In turn, free-living bacteria had greater variation in its composition along season rather than along the cascade, with temperature and pH as the major factors influencing this shift.

Keywords: Cascade of reservoir. Tropical reservoirs. Metacommunity. Microbial community composition. Trophic gradient.

Introduction

Freshwater natural systems hold limnological features that differ from damned reservoir, and the cascade arrangement, *i.e*. reservoirs in series within a riverbed, has intrinsic factors that causes extra limnological variability towards downstream reservoirs (STRAŠKRABA, 1990a). As Straskraba (1990a) described, cascade morphology imparts consequences to chemical and physical properties in the system: towards downstream, mixing depth and bottom temperature tends to increase; turbidity tends to decrease and, consequently, light intensity in the upper layer increases. Higher phosphorous uptake by phytoplankton upstream and lower turbidity downstream results in lower primary production along the cascade and is not surpassed by the rising light intensity downstream (STRAŠKRABA, 1990a)**.** In tropical regions, features linked to productivity are potentialized by the higher solar radiation and temperature along the year. Stratification events are common in tropical habitats in deep waters during the summer (LEWIS, 1987), however, in cascade reservoirs it is unlikely due to the constantly mixing of water column. The typical nutrient limitation in tropical aquatic reservoirs due to high primary production is surpassed by the frequent pulses of nutrients caused by the water flushed due to dam operation, which also regulates the expedition of organisms, with further impact in the communities' structure (LINDSTRÖM et al., 2006; LINDSTRÖM; BERGSTRÖM, 2004). Microbial community's composition is influenced by local conditions, such as environmental physical and chemical factors from the habitat**,** but also by trophic interaction with other organisms, such as grazing by zooplankton and mixotrophic phytoplankton, competition, viral attacks (Reynolds, 1997, Calijuri 2002). Consequently, pulses of nutrients and organisms caused by the cascade formation is an important factor driving microbial communities' composition from these systems.

Freshwater systems hold the greatest part of organic matter and microbial loop is responsible for remineralize important portion of dissolved organic matter. Hence, aquatic microbial communities are important players in biogeochemical processes and the structure of microbial communities has direct impact on biogeochemical cycles from a given habitat, since groups of organisms plays different roles in the global process.

The tropical cascade of reservoirs from the medium-low Tietê River (Brazil) has been object of many studies regarding different groups of organisms, as zooplankton (TUNDISI; MATSUMURA-TUNDISI; ABE, 2008), micro crustaceans (SANTOS-WISNIEWSKI; ROCHA, 2007), phytoplankton, (CALIJURI; DOS SANTOS, 1996) as well as water quality (RODGHER, [s.d.]; SMITH; ESPÍNDOLA; ROCHA, 2014; SOTERO-SANTOS et al., 2006; TUNDISI; MATSUMURA-TUNDISI; ABE, 2008). Few studies investigated the cascade, and those found in the literature addressed water and sediment quality (RODGHER et al., 2005), phytoplankton communities (CALIJURI, 2002), macroinvertebrates (CALLISTO et al., 2005), fishes (PETESSE; PETRERE, 2012), and a recent study addressed the effects of cascade arrangement in bacterial metabolism within the system (FREITAS et al., 2018). Despite the great importance of studies above shedding light of limnological and ecological aspects of the cascade, there are no studies exploring microbial communities' compositions along reservoirs within the cascade.

The use of techniques to unravel environmental samples, such as high throughput sequencing, recovers a range of organisms where great portion is not yet known or even cultivated. This helps to better reconstruct phylogeny and evolution of bacterial groups, with the discovery of new organisms and also, may undercover important players in the biogeochemical cycle. In this study, we explored microbial communities spatially from four reservoirs from the cascade and temporally in two marked seasons of dry and rainy

period. To build a better resolution of microbial communities' structure, we analyzed separately eukaryotic phytoplankton, Cyanobacteria, free living and particle-attached bacterial communities. Our aims were to 1) identify the effects that cascade arrangement generates on environmental conditions; 2) evaluate the effects of cascade arrangement on microbial communities during the dry and rainy period.

Methodology

Environmental analysis

We used all the six sampling times to explore environmental conditions and communities of each reservoir. Seasonality was represented by samplings of the whole cascade divided in three groups: May (D1) and July (D2) 2015 as dry season; September (I1) and November (I2) 2015 as intermediary season, and January (R1) and March (R2) 2016 as rainy season. For seasonality effects on communities, we conducted analysis with samplings from dry and wet season.

First, we calculated the trophic state index (TSI) according to Cunha et al., (2013) using values of total phosphorous and chlorophyll-a. We calculated the mean of TSI for each reservoir and from dry and rainy period for the cascade to check if there were significative differences between reservoirs and rainfall seasons. With environmental variables we performed a Principal component analysis (PCA) and a paired Student t-test in order to explore variables that were variating significantly seasonally and spatially.

Communities analysis

In order to investigate environmental variables influencing communities' distribution, we performed Mantel test of correlation between communities' Bray-Curtis distance matrices and Euclidean distance matrices of environmental parameters. Mantel

correlation test was performed with different taxonomic levels: Bacterial communities were analyzed by Phyla, Class and OTU levels and Phytoplankton by Class and OTU levels. To access whether spatial or temporal factors explained better communities' distribution, we performed PERMANOVA analysis communities by reservoir and seasons (dry and rainy) with the same taxonomic levels used in Mantel correlation test. For PERMANOVA analysis, seasonality included the intermediary season (July and September 2015), referent to sampling between dry and rainy seasons to avoid bias regard rainfall regime, because analysis of communities' composition by reservoir were made with all six sampling times. PERMADISPER analysis was performed previously to PERMANOVA to check if variation in our data would not interfere at PERMANOVA. We then built heatmaps for each community with the taxonomic level which had the better explanation with PERMANOVA analysis using the "complete" method (UPGMA) for cluster analysis. For this analysis, we also used all six samplings.

All the statistical analyses were performed in computing environment *R* (*R* Development Core Team, 2017) using the packages *vegan* (OKSANEN et al., 2013) and *heatplus* (Ploner, 2017).

Results

Environment

The studied cascade showed decrease in turbidity, as Secchi disk values increased downstream (Table 1.1). Total nitrogen and chlorophyll-a decreased along the cascade, as result of lower primary production, as well as decrease in DOC (Table. 1.1).

According to trophic state index and thresholds proposed by Cunha et al., (2013), all reservoirs sampled from the cascade are hypereutrophic (SM Table 1.1) and the trophic state between all reservoirs was significantly different during the dry period, but not

during the rainy period. However, trophic state gradient on the cascade between dry and rainy period was not significantly different. (Fig. 1.1)

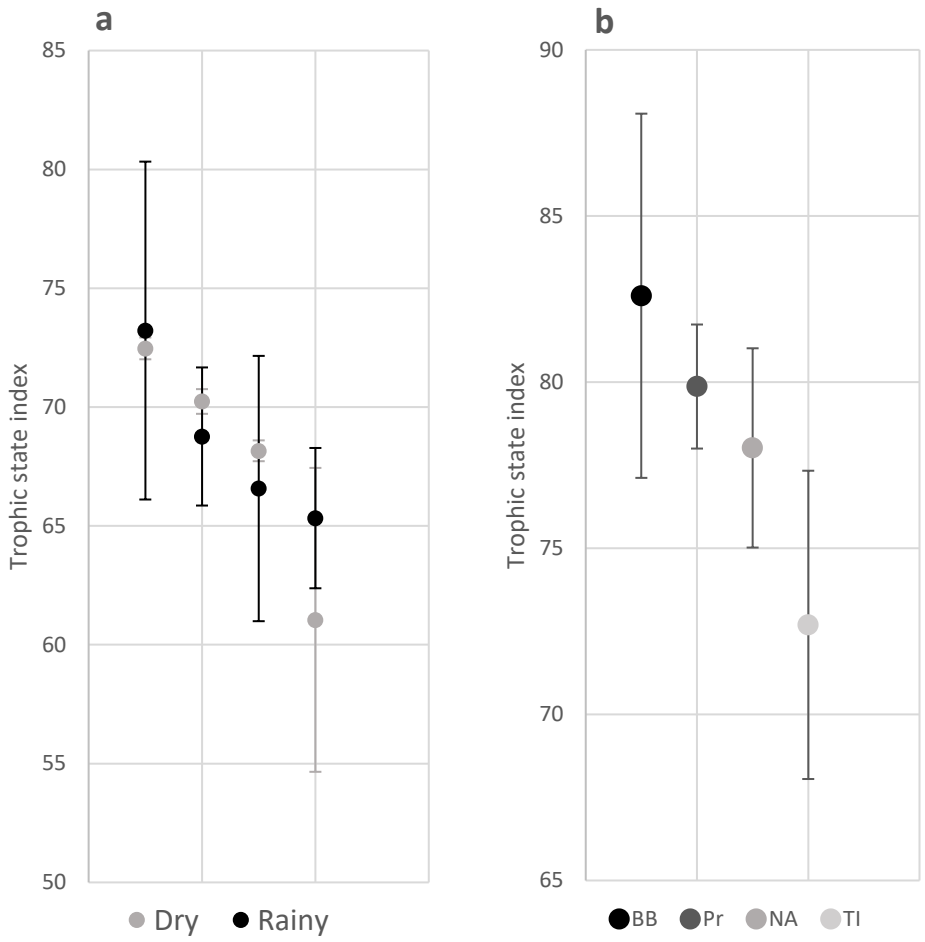

Figure 1. 1 - Dispersion plot of trophic state index and standard deviation calculated for the cascade during dry and rainy period (a) and by reservoir (b). (BB) Barra Bonita, (Pr) Promissão, (NA) Nova Avanhandava and (TI) Três Irmãos.

Environmental variables that had significant variation Between reservoirs in arrangement they are on the cascade were temperature, $P\text{-}PO_4$ and sulphate between BB and Pr and chlorophyll-a, sulphate and Secchi value between NA and TI. DOC, pH, $P\text{-}PO_4$, phosphate and Secchi had significant variation between pairs of reservoirs non-

consecutives on the cascade (Table 1.1). Between dry and rainy period, variables that varied significantly were temperature, pH, phosphorous and residence time (Table 1.2).

PCA analysis with environmental variables showed that sampling points relative to BB reservoir were separated from others reservoir (Fig 1.2). Along the first axis, sampling points were positioned reflecting the spatial arrangement, whereas along the second axis, seasonality. All samplings from dry period were positioned above the first axis, with exception of NA_D2 (Fig 1.2). The first axis explained 43% of total phosphorous variation (score -1.16), total nitrogen (score -1.11), phosphate (score -0.92), Chlorophyll-a (score -0.91), DOC (score -0.86), sulphate (score -0.83) (Fig 1.2). The second axis explained 21% of temperature variation (score -0.98), residence time (score 0.79) and Secchi (score 0.71) (Fig 1.2).

Table 1. 1 Minimum, maximum and average values of environmental variables measured by reservoir. Barra Bonita (BB), Promissão (Pr), Nova Avanhandava (NA) and Três Irmãos (TI). Letters correspond to pairs of reservoirs where: BB-Pr (a), BB-NA (b), BB-TI (c), P-TI (d), NA-TI (e) and significant ($p<0.05$) t-test values obtained between consecutive reservoirs are represented by an asterisk.

	BB			Pr			NA			TI			t-test ($p>0.05$)
	min	**avg**	max	min	**avg**	max	min	**avg**	max	min	**avg**	max	
Chla ($\mu g\ L^{-1}$)	1.7	**33.2**	76.3	8.0	**18.2**	43.6	4.6	**12.3**	26.4	0.8	**4.3**	11.4	d, e*
Temp (°C)	20.2	**24.3**	27.8	22.0	**25.6**	29.8	22.6	**25.9**	29.0	23.0	**26.5**	29.5	-
pH	6.8	**7.4**	8.0	7.5	**8.2**	9.0	7.0	**8.3**	9.5	7.3	**7.9**	9.2	a*, b
DO (%)	20.9	**80.5**	116.8	60.8	**102.3**	155.3	61.5	**99.1**	155.4	71.3	**91.3**	132.0	-
TN ($mgN\ L^{-1}$)	2.4	**3.7**	6.2	0.5	**0.7**	1.1	0.7	**0.8**	1.2	0.4	**0.4**	0.6	-
DOC ($mgC\ L^{-1}$)	7.8	**10.4**	21.7	5.0	**5.7**	7.4	5.1	**6.1**	8.6	4.1	**4.7**	5.5	c,d
P-PO4 ($mgP\ L^{-1}$)	8.45	**49.18**	109.21	1.21	**4.86**	13.73	1.24	**4.04**	9.04	1.57	**9.02**	28.90	a*, b, c
SO4 ($\mu g\ L^{-1}$)	15.52	**22.0**	29.89	8.92	**14.5**	20.36	12.21	**14.7**	17.95	11.39	**12.6**	13.8	a*, b, c, e
Secchi (m)	0.3	**1.2**	3.8	1.0	**1.4**	1.9	0.9	**1.2**	1.6	1.8	**3.9**	7.6	c, d, e*
RT (days)	31.5	**99.8**	179.6	35.7	**143.3**	283.6	13.1	**48.8**	104.8	63.6	**357.7**	1000.5	-

Table 1. 2 - Minimum, maximum and average values of environmental variables measured by rainfall period. Chlorophyll-a (Chla), Temperature, pH, Dissolved oxygen (DO in percentage of atmospheric values, right above the water surface), total nitrogen (TN), soluble reactive phosphorous (P-PO4), depth of Secchi disc, and Retention time (RT). T test is the result of paired T-test between rainfall regime (* $p<0.05$).

	Dry			Rainy			
	min	avg	max	min	avg	max	T test
Chla (µg L^{-1})	0.8	**12.2**	23.9	1.7	**20.7**	76.3	
Temp (°C)	20.2	**235**	26.7	26.0	**28.2**	29.8	*
pH	7.3	**8.4**	9.1	6.8	**7.4**	8.1	*
DO (%)	74.8	**106.8**	133.6	20.9	**83.9**	117.1	
TN (mgN L^{-1})	0.5	**1.3**	3.6	0.4	**1.3**	3.4	
DOC (mgC L^{-1})	4.1	**6.4**	8.9	4.1	**8.4**	21.7	
P-PO4 (mgP L^{-1})	0	**0.04**	0.13	0	**0.03**	0.1	*
SO4 (µg L^{-1})	12.86	**19.1**	29.89	8.92	**12.7**	16.31	
Secchi (m)	0.9	**3.1**	7.6	0.3	**1.4**	3.0	
RT (days)	63.1	**314.3**	1000.5	13.3	**45**	94.9	*

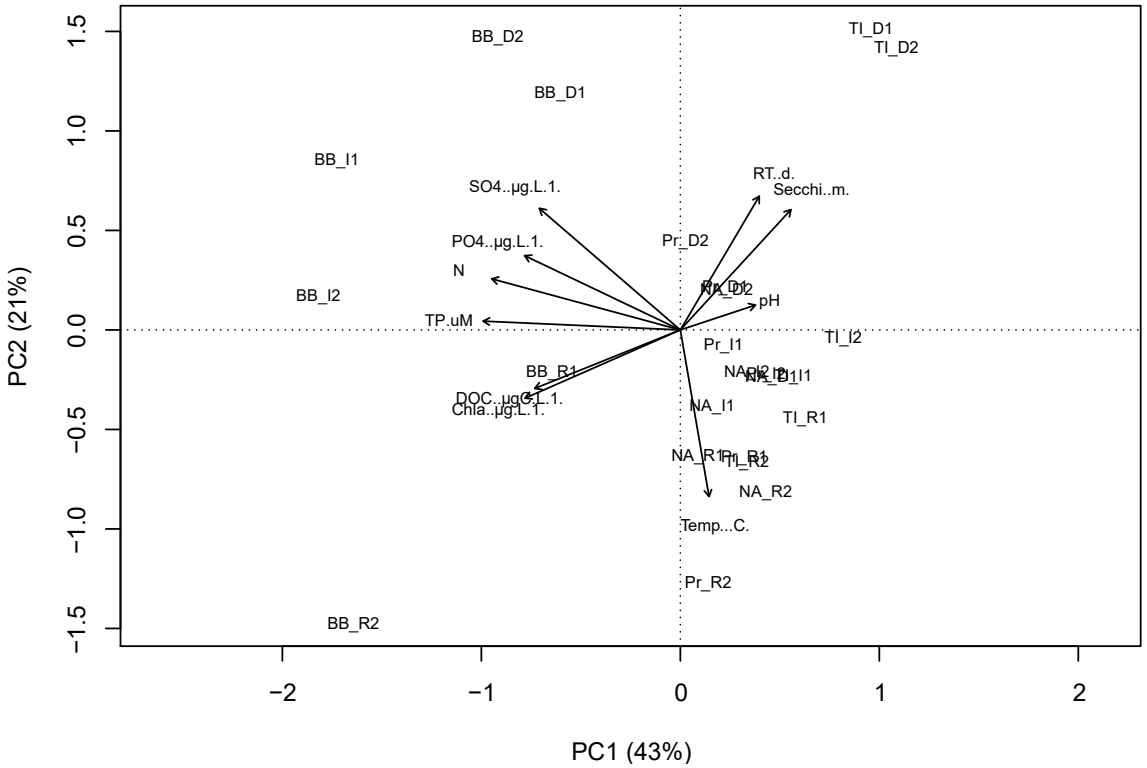

Figure 1.2 - Principal component analysis of environmental variables measured by sampling points. Barra Bonita (BB), Promissão (Pr), Nova Avanhandava (NA) and Três Irmãos (TI). Samplings from dry period are represented by D1 (May/2015) and D2 (July/2015), from intermediary period by I1 (September/2015) and I2 (November/2015) and from rainy period by R1 (January/2016) and R2 (March/2016). Environmental variables: Chlorophyll-a (Chla), Dissolved organic carbon (DOC), Temperature (Temp), pH, Total phosphorous (TP), Total nitrogen (TN), Phosphate (PO_4), Sulphate (SO_4), Residence time (RT) and Secchi.

Microbial communities

The sequencing of 16S rDNA gene yielded a total of 8.141.098 reads (minimum of 9.868 reads/sample) representing 2367 OTUs and after rarefaction and exclusion of chloroplastidal referent sequences (37), 2112 OTUs remained, where 36 were relative to Cyanobacteria from particle-attached fraction (>3 um) and 31 from cyanobacteria from free-living fraction (> 0.22 um). Free living bacteria community was composed by 1758 OTUs and particle attached by 1728 OTUs. Uncultured OTUs at some taxonomic level

accounted with ~90% of total OTUs obtained. Bacterial community (particle-attached + free-living) was composed by 33 phyla.

The sequencing of 18S rRNA gene yielded a total of 6.075.608 reads (minimum of 63.554 reads/sample) representing 2571 OTUs and after rarefaction step 1607 OTUs remained, where 550 were relative to photosynthetic phytoplankton, which were used in this study. Eukaryotic phytoplankton was represented by 550 OTUs distributed in 19 Classes. OTUs classified to species level accounted for 321, 213 at genus level (with 139 uncultured), 27 up to order (with 9 uncultured). 139 OTUs were not identified further than class level and among those, 112 were uncultured. 7 OTUs were not identified even at class level, however, these OTUs accounted with very low frequency and were included among "others".

Most abundant Phyla (relative abundance >1%) of Free-living and Particle attached communities

The most abundant phyla (relative abundance >1%) of free living and particle attached bacterial communities were Actinobacteria, Bacteroidetes, Cyanobacteria, Chloroflexi, Chlorobi, Planctomycetes, Proteobacteria, Verrucomicrobia. When analyzed by reservoir, Armatimonadetes phyla figured among most abundant phyla of particle attached community at Três Irmãos reservoir, however with 1% of total community (Fig. 1.3).

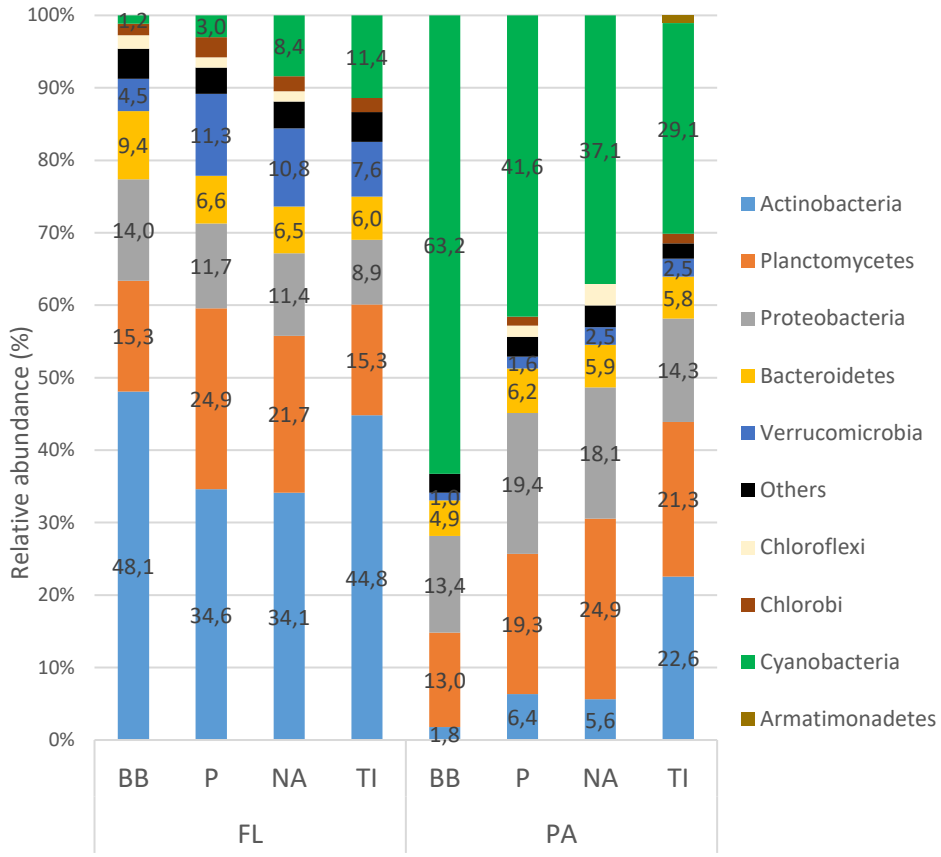

Figure 1. 3 - Relative abundance of most abundant (relative abundance >1%) Phyla of free-living (FL) and particle attached (PA) bacterial community by reservoir. Barra Bonita (BB), Promissão (Pr), Nova Avanhadava (NA) and Três Irmãos (TI). Values of relative abundance of Classes are indicated on barplot, on the correspondent section.

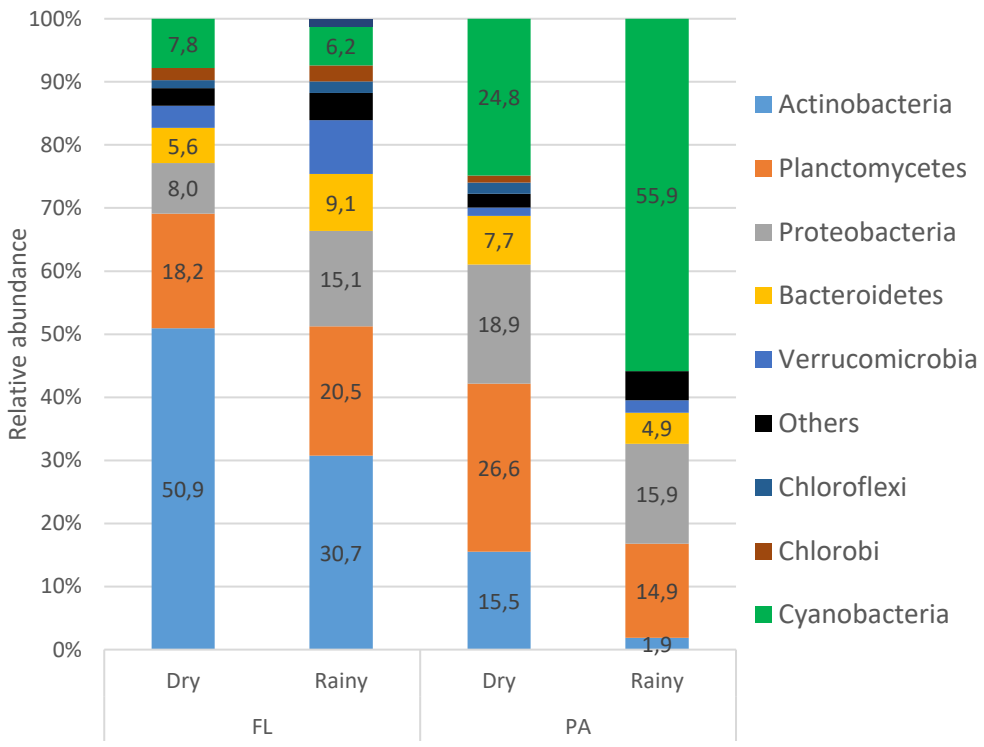

Figure 1. 4 - Relative abundance of most abundant (>1%) Phyla of free-living (FL) and particle attached (PA) bacterial community by rainfall regime. Values of relative abundance of Classes are indicated on bar plot, on the correspondent section.

Most abundant OTUs (relative abundance >1%) of free-living and particle-attached communities

At OTU level, free-living had 17 most abundant OTUs and particle attached 12 OTUs (SM Table 1.2) and 4 of them were shared by both fractions: an uncultured member of *hgcl_clade* (OTU_2); an uncultured member of the family Planctomycetaceae (OTU _6), an uncultured *Planctomyces* (OTU _4) and a Cyanobacteria *Synechoccocus* (OTU _14) (SM Table 1.2, SM Fig. 1.1 a-b).

The three most abundant OTUs exclusively from Particle attached fraction corresponded to an uncultured *Microcystis sp., Planktothrix pseudagardhii HAB414* and an uncultured *Cylindrospermopsis,* all members of Cyanobacteria phylum (SM Table 1.2,

SM Fig. 1.1 a-b). Most abundant (>1%) heterotrophic bacteria exclusive to particle-attached were *Roseomonas* (Proteobacteria) uncultured (OTU_42), Cytophagaceae, Bacteroidetes, (OTU_24) and a member of hgcL_clade (OTU_5) (SM Table 1.2).

In Free-living community, the three exclusives most abundant OTUs, corresponded to uncultured members of *hgcl*_clade, Actinobacteria (OUT_5), a member of LD29, Verrucomicrobia (OTU_3), and a member of CL500-3, Verrucomicrobia (OTU_7) (SM Table 1.2).

Most abundant (relative abundance >1%) Classes of Eukaryotic Phytoplankton

Eukaryotic phytoplankton community composition showed predominance of Class Cryptophyceae in all reservoirs (Fig. 1.5). The second and third most abundant classes varied among reservoirs: Chlorophyceae and Mediophyceae at Barra Bonita, Bacillariophyceae and Chlorophyceae at Promissão; Bacillariophyceae and Dinophyceae at Nova Avanhandava, Dinophyceae and Chlorophyceae at Três Irmãos. Only at Três Irmãos Class Trebouxiophyceae was included among "others" due to the low frequency (relative abundance <1%) (Fig. 1.5).

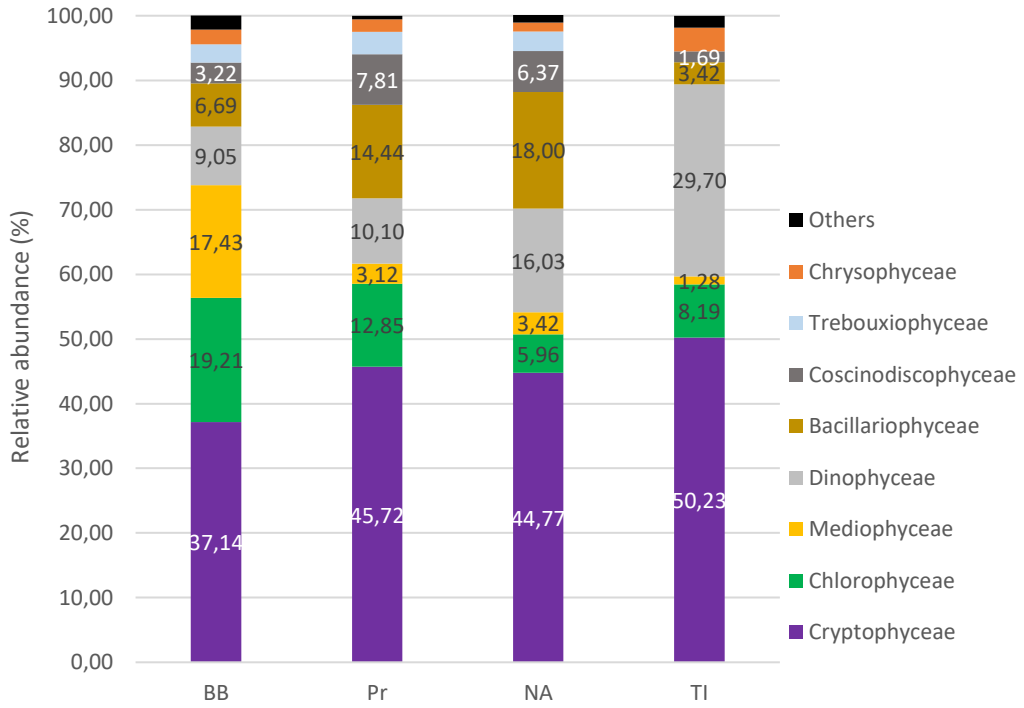

Figure 1. 5 - Relative abundance of most abundant (>1%) classes of phytoplankton community by reservoir Barra Bonita (BB), Promissão (P), Nova Avanhadava (NA) and Três Irmãos (TI). Values of relative abundance of Classes are indicated on barplot, above the correspondent section.

In both periods of rainfall regime, the most abundant microalgal classes were Cryptophyceae, Chlorophyceae, Dinophyceae, Coscinodiscophyceae, Mediophyceae, Trebouxiophyceae and Chrysophyceae. The Class Bacillariophyceae was among the most abundant only during the rainy period (Fig. 1.6).

Microalgae community from the cascade of reservoirs was predominated by Cryptophyceae in both periods, followed by Chorophyceae in the dry period and Bacillariophyceae in the rainy period (Fig. 1.6). The third most abundant class in dry period was Dinophyceae and Chlorophyceae in the rainy period (Fig. 1.6).

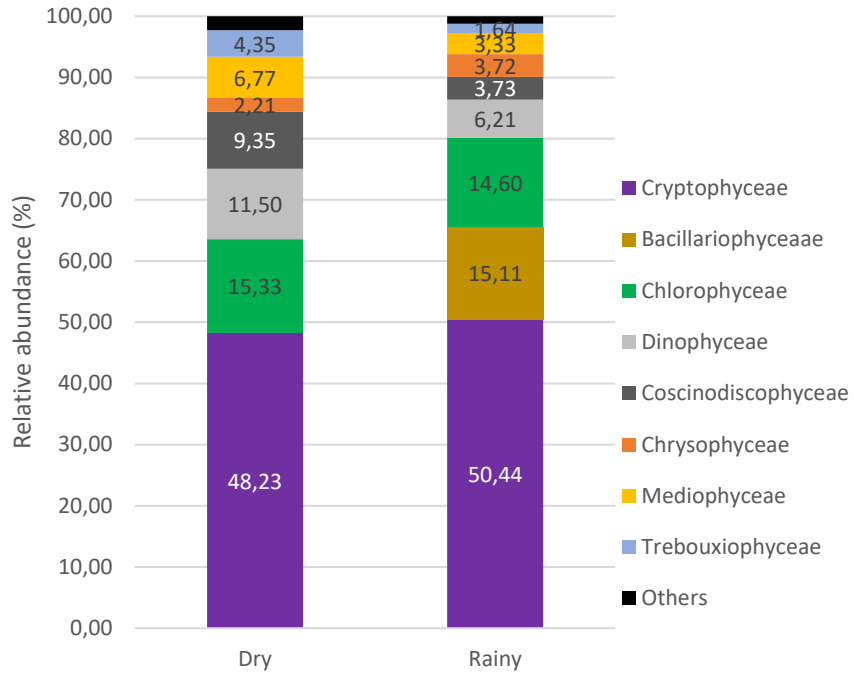

Figure 1. 6 - Abundance relative of most abundant (<1%) classes of eukaryotic phytoplankton community by rainfall regime. Values of relative abundance of Classes are indicated on barplot on the correspondent section.

In order to check for correlations between communities' distribution and environmental variables we performed a Mantel test of correlation between each environmental variable and communities' distances matrices (Table 1.3). All communities presented significant correlation in some taxonomic level with variables linked to trophic state: nitrogen, phosphate and sulphate (Table 1.3). However, chlorophyll-a was correlated only with PA subcommunity. Other significant correlations were found between free living community and temperature, pH, dissolved oxygen, DOC and Secchi; particle attached community and pH, DOC and Secchi and cyanobacteria community and DOC and temperature (Table 1.3). Cyanobacteria present in free-living fraction was mainly represented by *Synechococcus*, further analyses were done only with cyanobacteria from PA (> 3um) community by OTUs and genus levels.

Table 1. 3 - Mantel product-moment of correlation between environmental variables Euclidean distance and communities Bray-Curtis distance matrices at different taxonomic levels. Free living (FL), Particle attached (PA), Chlorophyll-a(Chla), Temperature (Temp), Dissolved oxygen (DO), Total Nitrogen (TN), Phosphate (PO_4), Sulphate (SO_4), Dissolved organic carbon (DOC) and Water Transparency (Secchi).

		Chla (um L-1)	Temp (°C)	pH	DO (%)	TN (µg L-1)	PO4 (µg L-1)	SO4 (µg L-1)	DOC (ug L-1)	Secchi (m)
FL	OTU		0.22**	0.26***	0.27**	0.54***	0.46***	0.35**	0.43***	
	Class		0.30***	0.18***	0.24**	0.15*			0.15	0.17*
	Phylum		0.16*			0.18*				
PA	OTU	0.37**		0.18*		0.52***	0.50**	0.26*	0.37*	0.27*
	Class	0.48***				0.51***	0.54**		0.22	
	Phylum	0.29**	0.20**				0.22*		0.22	
Euk Phyto	OTU					0.47**	0.47**	0.42**		
	Class					0.21*		0.26**		
Cyano	OTUs		0.26**			0.31**	0.32**	0.31**	0.43***	
	Genera								0.31**	

*(p<0.05), **(p<0.01,) ***(p<0.001)*

Regarding to PERMANOVA analysis at OTU level, reservoir explained 27% of free-living community variation ($p<0.001$), 19% of particle attached community ($p=0.008$), 20% of Cyanobacteria community ($p=0.03$), and 23% of microalgae community ($p=0.001$) (Table 1.4). Rainfall regime explained 21% of OTUs variation in free living community ($p<0.001$), 15% of Particle attached community ($p=0.0009$, 14% of cyanobacterial community ($p=0.03$), and 17% of microalgae community ($p=0.004$) (Table 1.4).

Table 1.4 - PERMANOVA values obtained with communities Bray-Curtis distances at different taxonomic levels by reservoir and season. Particle-attached (PA) and Free-living (FL) were analyzed by OTUs, Class and Phyla; Cyanobacteria by OTUs and Genera and eukaryotic phytoplankton by OTUs and Genera. Betadisper values were not significative to any analysis.

			FL	**PA**	**Cyano**	**Euk. phyto**
OTUs	~reserv		r^2=0.27***	r^2=0.19**	r^2=0.20*	r^2=0.23***
	~season		r^2=0.21***	r^2=0.1***	r^2=0.14*	r^2=0.17**
Class/Gen	~reserv		r^2=0.19*	r^2=0.21*	r^2=0.22(PA)*	r^2=0.20*
	~season		r^2=0.29*	r^2=0.14*	r^2=0.14(PA)*	r^2=0.19*
Phyla	~reserv		r^2=0.20*	r^2=0.23**		
	~season		r^2=0.28**	r^2=0.20**		

(p<0.05), **(p<0.01), ***(p<0.001)

At Genera level, reservoirs explained 19% of free-living variation, 21% of particle attached, 22% of Cyanobacteria and 20% of eukaryotic phytoplankton, all with $p=0.04$ (Table 1.4). Seasonality explained 29% of free-living, 14% of particle-attached, 14% of cyanobacteria and 19% of eukryotic phytoplankton (Table 1.4). Finally, when communities were analyzed at Phylum level, reservoir explained 20% of FL ($p=0.03$),

and 23% of PA community variation (p=0.007); and seasonality explained 28% of FL community (p=0.002) and 20% of PA (p=0.01) and distributions (Table 1.4).

The better explanation of free-living community by seasonality also grouped sampling points from dry period separately from rainy period at heatmap representation (Fig. 1.7). Samplings from the first intermediate samplings (I1) were placed with dry period samplings and from the second intermediate period (I2) with rainy period (Fig 1.7) This seaparation was not observable with other communities by reservoir or rainfall regime (SM Fig 1.2a-c).

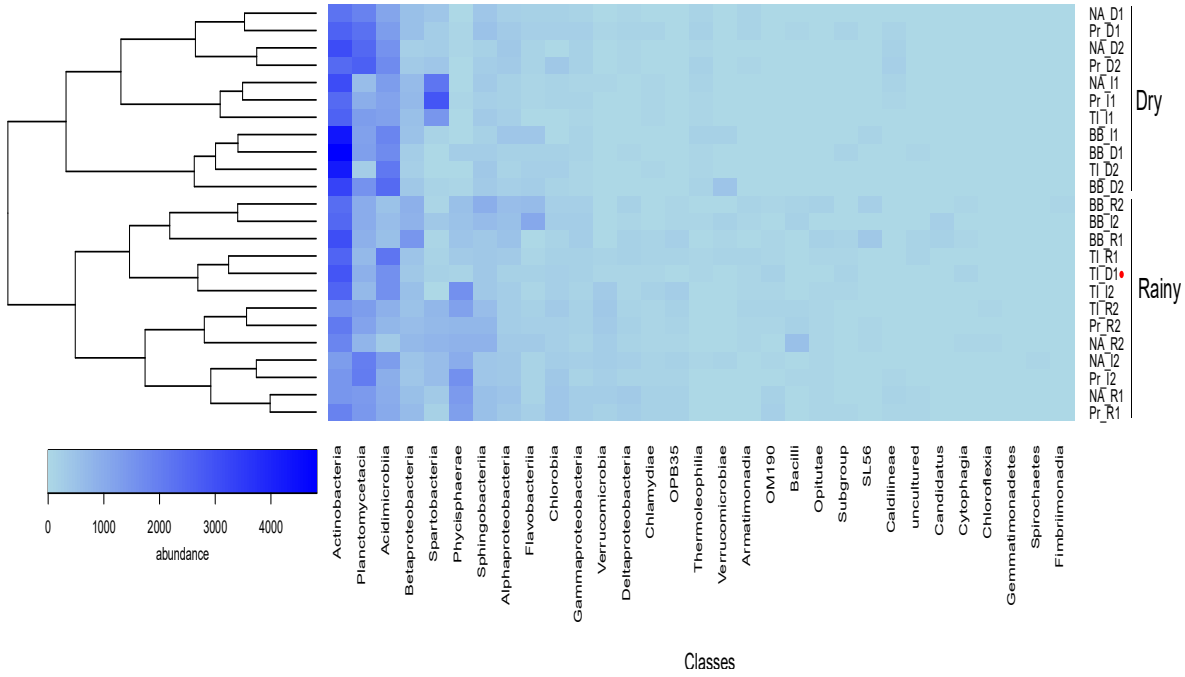

Figure 1. 7 - Heatmap representation by clustering analyses of Free-living by 30 most abundant Classes. Barra Bonita (BB), Promissão (Pr), Nova Avanhandava (NA) and Três Irmãos (TI). Samplings from dry period are represented by D1 (May/2015) and D2 (July/2015), from intermediary period by I1 (September/2015) and I2 (November/2015) and from rainy period by R1 (January/2016) and R2 (March/2016).

Discussion

Environmental conditions

Our aim in this study was to evaluated effects of the cascade arrangement on environmental conditions and explore bacterial and phytoplankton communities across the cascade of reservoirs, as well as in two marked seasons of dry and rainy. Our study provided detailed composition of three fractions of microbial communities obtained with 16S rDNA and microalgae community obtained with 18S rDNA.

We observed along the cascade environmental conditions as predicted by Straškraba, (1990b). Water transparency increased downstream with higher values during the dry period. The higher water transparency at samplings downstream from dry period is due to sedimentation of suspended matter along the cascade. The amount of water flowing along the cascade during the dry period is lower and thus, mixing is also lower. During the rainy period, is expected that greater amount of matter would be resuspended (WETZEL, 1993), due to the higher water intensity flowing, but also due to the catchment area, which input allochthonous matter into the system. The decrease downstream of variables linked to trophic state such as chlorophyll-a, DOC and nutrients, is caused by phytoplankton consumption upstream and decrease in turbidity, with less matter available at the subsurface (STRAŠKRABA, 1990b).

Particle-attached and free-living Communities

Regarding prokaryotic communities, the most abundant phyla found in this study were frequently reported as the most abundant typical phyla in freshwater systems: Actinobacteria, Bacteroidetes, Cyanobacteria, Chloroflexi, Chlorobi, Proteobacteria, Verrucomicrobia (DE MELO et al., 2019; DE OLIVEIRA; MARGIS, 2015; EILER; BERTILSSON, 2004; LIU et al., 2015; LLIRÓS et al., 2014; NEWTON et al., 2011;

ZWART et al., 2002)**.** The Phylum Planctomycetes was also recovered among the most abundant phyla in communities from the cascade of reservoir studied here.

Actinobacteria was the most abundant phylum in Free-living fraction, and particle attached fraction, but with lower relative abundance. This group is characterized by their small size (SALCHER, 2013), what could have resulted in the higher proportion in free-living fraction. One of two most abundant OTUs found in free-living community also among the most abundant in particle attached fraction belongs to acI linage, numerous in freshwater, with particular abundance in free-living fraction (ALLGAIER; GROSSART, 2006; TANG et al., 2009). Actinobacteria is a ubiquitous group found widely in freshwater systems (GLÖCKNER et al., 2000; SEKAR et al., 2003) ranging from temperate to tropical habitats (HAHN, 2009) with greater abundance in surface waters (GLÖCKNER et al., 2000)**.** This successful persistence in variable habitats may be due to an alternative energy generation that some lineages of Actinobacteria have, such as acI lineage, (SHARMA et al., 2008); their possible resistance to Ultraviolet rays (WARNECKE et al., 2005), what explains their high abundance in the epilimnion, especially in a tropical environment, such as the studied system, where solar radiation is high all over the year; and also due to their small size and cell wall composition as adaptations to planktonic lifestyle and resistance to predation (HAHN, 2009; PERNTHALER et al., 2001). Despite previously reported that Actinobacteria shows low changes in its abundance across seasons in freshwater lakes (GLÖCKNER et al., 2000)**,** in this study we found that towards the wet season, this phylum showed decrease in its relative abundance in both fractions.

Bacteroidetes were amongst the most abundant bacterial phyla in both fractions and its abundance remained constant across space and time in both fractions, without any clear pattern as also observed by Eiler and Bertilsson, (2007)**,** but fitting in the average of

1-12%, (GLÖCKNER; FUCHS; AMANN, 1999)**.** This phylum tends to occur in high loads of allochthonous and autochthonous DOC (EILER; BERTILSSON, 2004, 2007), such as cyanobacterial blooms demise, due to its versatile usage of organic matter (TANG et al., 2009). Sphingobacteria, a Class of Bacteroidetes in high abundance in this study (SM. Fig. 1.1e-f), was also found in high abundance at the senescence phase of *Aulacoseira, Cylindrospermopsis* and *Mycrocystis* by Bagatini et al., (2014). Since we observed in this study OTUs of *Mycrocistis* and *Cylindrospermopsis* among the most abundant OTUs from particle attached, it is possible that Sphingobacteria was favored by these cyanobacterial strains, and even more, Sphingobacteria abundance was signaling for a Cyanobacterial bloom demise. Sphingobacteria abundance was higher in free-living fraction and could be profiting of phytoplanktonic compounds released to the medium. Bacteria that is commonly found in association with phytoplankton or attached to other particles are also found detached from particles.

Within the Bacteroidetes phylum, the Class Cytophagia was the most abundant in particle attached fraction (SM. Fig. 1.1e-f). Members of Class Cytophagia class are commonly found attached to particles (DELONG; FRANKS; ALLDREDGE, 1993) and are also among the most abundant freshwater bacterial groups (GLÖCKNER; FUCHS; AMANN, 1999; PERNTHALER et al., 1998). This group comprises mainly aerobic bacteria (despite few anaerobic strains are known), characterized by degradation of complex compounds from the DOM, such as cellulose and chitin (YE et al., 2016). An uncultured member from this Class, (Family Cytophagacea, uncultured bacteria, OTU_24) was among the 11 most abundant OTUs from the particle attached community (Relative abundance of 2.3%). Roselló-Mora et al (1999) conducted an experiment were *Cytophaga* abundance increased in response to addition of cyanobacterial biomass. In our results, Class Cytophagia abundance in particle attached decreased towards the last

reservoir following the decrease of cyanobacteria abundance. Although Class Cytophagia members are also favored when allochthnous matter is carried into the environment (BATTIN et al., 2001)**,** their abundance was lower during the rainy period, when the allochthonous matter was carried into the system (FREITAS et al., 2018), suggesting that they are mainly linked to autochthonous (phytoplankton) matter. Moreover, we also found one low abundant uncultured lineage of *Cytophaga*, (OTU_1139) which shows algicide activity (MAYALI; AZAM, 2004). From the 41 OTUs representing Cytophagia Class, 25 were uncultured without classification further than order, what could encompass more strains of *Cytophaga*.

Yet within Bacteroidetes, Flavobacteria, showed high abundances in free-living fraction (SM. Fig. 1.1e-f). Flavobacteria distributions are known to be correlated with cyanobacterial demise and phytoplankton density (EILER; BERTILSSON, 2007) due to ability of feed on several sources of energy, by converting high molecular weight compounds into low molecular weight (TANG et al., 2009).

Planctomycetes phylum is usually recovered as minor phyla in freshwater systems, around ~5% (EILER; BERTILSSON, 2004; GADE et al., 2004; NEWTON et al., 2011; TADONLÉKÉ, 2007), possibly due to underrepresentation in 16S rRNA gene clone libraries (NEWTON et al., 2011) and unsuitable primers used to recover environmental diversity (VERGIN et al., 1998)**.** In our results, Planctomycetes was frequently among the three most abundant Phylum by reservoir and rainfall regime. Two OTUs (OTU_4 and OTU_6, Planctomycetacia) from this phylum were found amongst the most abundant (relative abundance >1%) in both fractions of free-living and particle attached (SM Table 1.2). The same primers we used here recovered Planctomycetes from Amazonian flood plains (tropical freshwater) among the most abundant phyla in bacterial community (DE MELO et al., 2019)(Tessler et al., 2017), and in artic waters

(HAUPTMANN et al., 2016). Conversely, other studies using different primers found Planctomycetes as an abundant Phyla in a temperate reservoir (CHENG et al., 2011; ZHONG et al., 2016). Planctomycetes members seems to be enrolled in degradation of recalcitrant and complex compounds, changes in DOM size by decomposition and algal polysaccharides (GLÖCKNER et al., 2003; TADONLÉKÉ, 2007) and play an important role as providers of smaller compounds to other groups (COTTRELL; KIRCHMAN, 2000). Planctomycetes members are widely dispersed along diverse trophic levels, including very polluted habitats, due to their ability to oxidize ammonium anaerobically (JETTEN et al., 2005; OP DEN CAMP et al., 2006)**.** Despite the trophic gradient, frequent cyanobacterial blooms were observed in this study and are common at the four reservoirs during the whole year (MINILLO, 2005)**,** what could have sustained the high abundance of Planctomycetes across space and time, since members of this group are associated with cyanobacterial bloom (WOODHOUSE et al., 2016).

Proteobacteria Phylum was among the four the most abundant Phyla in both fractions and seasons. This Phylum is widely dispersed across a range of habitats types due to their wide plasticity in life-style. Members of alpha and betaproteobacteria Classes show fast response to nutrients pulses, characteristic of the cascade, frequently associated with phytoplankton and coexisting due to the partition of the substrate (RUSSO et al., 2016)**.** In our results, Alphaproteobacteria was the most abundant Class in particle attached (SM. Fig. 1.1i-j). Besides the resistance to predation, some members from this Class can process recalcitrant compounds and feed on organic and inorganic substrates (HUTALLE-SCHMELZER et al., 2010) and is frequently found in close interaction with primary producers promoting or inhibiting growth of the associated phytoplankton, suggesting strong functional interaction (BERG et al., 2009). Betaproteobacteria was also abundant in particle attached and the most abundant in free-living fraction (SM. Fig. 1.1i-

j). Members of Betaproteobacteria are efficient in catabolize riverine DOM (GROSSART; PLOUG, 2001) and their high abundance may be due to their high growth rate, overpassing grazing (SALCHER et al., 2008).

Among the most abundant OTUs, *Synechoccocus* (OTU_14, Cyanobacteria) was found mainly in free-living fraction, (SM Table 1.2) (SM. Fig. 1.1a-b). The high abundance of *Synechoccocus* may be due to their small size, which increases their surface area, being more efficient in nutrient uptake (MOUTIN et al., 2002; REYNOLDS, 1984a). This is in accordance with the high occurrence in free-living fraction and the higher abundance of *Synechococcus* towards downstream, where the trophic level is lower. As observed by Sarmento (2012) and in this study, phototrophic picoplankton, such as *Synechoccocus*, are found not only in high abundance, but are frequent in tropical waters, which tend to show nutrient limitation (AMADO et al., 2013; CALLIERI, 2008; FREITAS et al., 2018; SARMENTO, 2012).

We also observed high abundance of species from *Microcystis, Planktothrix* and *Cylindrospermopsis* genera along reservoirs and seasons, as a response to the high degree of eutrophication (BARBOSA et al., 1999; DOMINGOS et al., 1999) (SM. Fig. 1.1a-b). Freitas et al., (2018) observed for these reservoirs at the same sampling period low concentrations of dissolved inorganic nutrients, what could be result of rapidly uptake by primary producers (SARMENTO, 2012), such as Cyanobacteria blooms.

Cylindrospermopsis blooms, in this study, were observed in Promissão, Nova Avahandava and Três Irmãos (SM. Fig. 1.1a-b). Barbosa et al (1999), observed blooms of *Cylindrospermopsis* (a nitrogen fixing cyanobacteria) in Nova Avanhandava and attributed it to the scarcity of nitrogen as result of biological processes occurring in upper reservoirs. *Cylindrospermopsis*, besides atmospheric nitrogen fixation ability, shows adaptations to planktonic life-style, such as low light intensity tolerance, resistance to

grazers and buoyant adaptations, allowing them to travel along the water column to better explore resources (PADISÁK, 1997).

Cyanobacterial strains found here in bloom conditions are worrisome, because they are toxins producers (*Cylindrospermopsis, Mycrocistis, Planktothrix*) (SANT'ANNA et al., 2008), which yield extra impacts to the aquatic ecosystem (CODD, 1995; SOTERO-SANTOS et al., 2006; WIEGAND et al., 2002). Besides the porpoise of power generation, reservoirs within the studied system is used for fishing activities, water supply by cities and recreation, such as a municipal club at Nova Avanhandava reservoir. Toxins released to the medium are a threat to humans, since most of them includes: neurotoxins (CARMICHAEL, 1994; FASTNER; HEINZE; CHORUS, 1995), produced by cyanobacteria as *Anabaena* and *Aphazinizomenon* (CARMICHAEL, 1994), in lower abundance in our study; cytotoxin, produced by *Cylindrospermopsis*; and hepatotoxins, produced by as *Planktothrix* and *Microcystis* (MAGALHÃES et al., 2003). The use of water from the system to agriculture irrigation is also threatened, since toxins may affect crop growth (MCELHINEY; LAWTON; LEIFERT, 2001; PFLUGMACHER et al., 1998). Fisheries activities could also be threatened, since cyanobacterial toxins may contaminate fishes (MAGALHÃES et al., 2003).

Cyanobacteria community had its distribution better explained by at genera level and by reservoir. This fraction correlated significantly with DOC concentration, but DOC did not significantly variate along dry and rainy period, what might be attributed to frequent and dense cyanobacterial blooms of fewer species occurring along the cascade through the whole year.

The two phytoplankton communities investigated (Cyanobacteria and eukaryotic phytoplankton) were less diverse when compared with bacterial communities. The dominance of few groups along the year and the better explanation of communities'

composition by reservoir suggests that these communities are influenced by the local conditions of reservoir, rather than seasonality. In fact, our results showed that eukaryotic phytoplankton correlated with total nitrogen, phosphorous and sulphate only.

Conversely, bacterial communities were better explained by higher taxonomic level what may reflect the higher diversity and functional redundancy (TALBOT et al., 2014). Particle attached had its distribution explained strongly by reservoir. This fraction is more closely influenced by photosynthetic compounds produced by cyanobacteria and microalgae (KENT et al., 2007)**,** and distinct ecological groups of particle attached bacteria are recruited according to the stage and nature of blooms happening in each reservoir (EILER; BERTILSSON, 2007; RUSSO et al., 2016)**.** For example, members from Bacteroidetes, tends to rise in early stages of growth of phytoplankton, with Flavobacteria dominance in eutrophic systems, where phytoplankton density is high (BAGATINI et al., 2014; RUSSO et al., 2016). Some Alpha and Gammaproteobacteria members are involved in blooms demises by algicidal activity (NEMERGUT et al., 2013)**,** while others promote cells aggregation in order to improve the access the content of dying algal cell (POWELL; HILL, 2013, 2014). Moreover, particle attached correlated with variables linked to trophic state and more strongly with chlorophyll concentration, what refers to phytoplankton biomass and production, reinforcing that this community composition is tightly associated with primary producers.

Free-living bacteria had its distribution affected more strongly by rainfall regime. Since this community is nonattached to particles, patch limits are not clear due to their movement along water column, being susceptible to hydrological dynamic. Their smaller size also contributes to their higher migration along the cascade (FARJALLA et al., 2012; FINLAY, 2002), minimizing dissimilarities between reservoirs within the cascade.

In agreement with Sarmento (2012), who states that rainfall regime drives planktonic communities, seasonality promoted differentiation of free-living communities at higher taxonomic levels (Class and Phyla) from the whole cascade. The more pronounced changes in physical and chemical environmental conditions (such as temperature and pH) along the year, and hydrological dynamics, such as water flux, had stronger influence in the free-living fraction. Temperature is an important factor driving microbial communities composition in aquatic ecosystems (LINDSTROM; KAMST-VAN AGTERVELD; ZWART, 2005; WHITE et al., 1991), and we found that free-living had 30% of its distribution explained by temperature (parameter with significant variation along season) what places temperature as a major factor influencing free-living composition from dry to rainy period. Moreover, stratification is unlikely in cascade damned reservoirs, due to the permanent mixing caused by the floodgates operation (STRAŠKRABA, 1990a)**.** Thus, temperature and constant mixing of water column may have influenced free-living community composition not only at physiological, but also at migration scope from dry to rainy period.

Regarding bacterial composition, another important factor influencing the structure of local communities of reservoirs both free-living and particle-attached fractions is the input of allochthonous matter and consequently bacteria, attached and non-attached, carried together from the adjacencies, as a source of species (LINDSTRÖM; BERGSTRÖM, 2004b, 2005), with higher impact in particle-attached community. Moreover, due to the exchange of organisms along the cascade, communities 'composition is also influenced by species pool from previous reservoir, and due to the higher mobility, the impact is expected to be higher in free-living communities (LINDSTRÖM et al., 2006).

Eukaryotic Phytoplankton

Eukaryotic phytoplankton composition showed spatial variation, but persistent dominance of Cryptophyceae along the cascade. High abundance of Cryptophyceae has been reported together with diatoms and Chlorophyceae (HENRY et al., 1985) and at damned reservoirs (NOGUEIRA et al., 2010). Cryptophyceae Class is commonly found in reservoirs and with greater abundance during the rainy period (MENEZES; NOVARINO, 2003). However, in our results, Cryptophyceae Class abundance remained constant along rainfall regime. In mesotrophic environments, Crytophyceae replaced Cyanobacteria (CROSSETTI; BICUDO, 2005).

This group is a great r-strategistic, since they grow fast and are good dispersers due to their flagella (KLAVENESS, 1988). Moreover, Cryptophyceae are mixotrophic, which means that they exert phagotrophy and photossinthetic activity. (BARONE; NASELLI-FLORES, 2003; REYNOLDS, 1984b). Mixotrophy enable their ecological success in a range of environmental conditions. Their ability to scavenge alternative sources of carbon, by ingesting heterotrophic bacteria is an advantage over autotrophic phytoplankton that could be under light and/or nutrient imitation caused by the bloom. However, conditions where nitrogen was abundant have also favored *Cryptomonas*, a well-studied mixotrophic member of Class Cryptophyceae (FERRAGUT, 2004). In the studied cascade, Cryptophyceae high abundance may be due to their mixotrophic activities, where they surpass competition by resource with other Phytoplanktonic groups.

Bacillariophyceae was among the most abundant Classes during the rainy period and at Promissão and Nova Avanhandava. The presence of Bacillariophyta in high abundance has been reported in reservoir (BICUDO et al., 2006; NOGUEIRA, 2001) and lakes (HENRY; USHINOHAMA; FERREIRA, 2006) during summer in tropical cascade of reservoir (rainfall season). Diatoms are prone to sink, due to the weight of their silica

cell wall in response to nutrient stress (HARRISON et al., 1986). Thus, abudance of this group may vary at surface waters according to availability of nutrients along water. During the rainy period, with the flooding episodes related, it is possible that Bacillariophyceae, not necessarily at the bottom of reservoirs, but in sinking route have been merely resuspended and then, observed at rainy season (Fig. 1.6). Moreover, the higher mixing characteristics of reservoir in cascade formation would potentialize this during the rainy season. With respect to the diatoms (Coscinodiscophyceae, Mediophyceae and Bacillariophyceae) it is noted that they occurred in higher abundance on the surface at BB, Pr and NA, and during the rainy period. *Aulacoseira granulatea* (Coscinodiscophyceae) to is known to be common in eutrophic reservoirs around the world (Medlin, 2016) and Theriot et al. 2010). Its presence on the surface or at medium depths of the water column is mainly due to the wind turbulence that, in the main, blows towards the main axis (at least in BB) of the reservoirs, in addition to the increase in flow caused by the opening of the floodgates. This situation is valid also for the ancient Penales (Bacillariopyceae) and old polar Centrals (Mediophyceae).

Conclusions

Our results showed that the studied system has features expected for damned reservoirs in cascade, influencing environmental characteristics and microbial communities' composition through seasons, due to differences in floodgate operation, and along the cascade. The cascade was spatially structured, where environmental condition did not significantly vary between the two most close reservoirs, due to the proximity. The trophic gradient was present in both seasons, and although it was less pronounced during the rainy period, it was not significantly different between seasons. Parameters that varied along the cascade were those linked to the trophic state, such as

chlorophyll, total nitrogen, phosphorous and water transparency, while along rainfall regime, temperature, pH and residence time.

The four communities studied here had its composition explained by distinct factors at different taxonomic levels. Primary producers (cyanobacteria and eukaryotic phytoplankton) and Particle-attached bacteria communities' structure were linked to trophic state, thus the variations on their composition, better explained by reservoir rather than by rainfall regime, were induced by the cascade arrangement, since trophic state decreased downstream. Frequent cyanobacterial blooms of few species observed in all four reservoirs reflected the constant input of nutrients into the system and nutrient pulses characteristic of damned reservoir in cascade formation. In turn, free-living bacteria had greater shifts in its composition along season, mainly promoted by temperature. We were able to identify the main drivers of bacterial community composition from the cascade of reservoirs of the Medium-low Tietê River, however, for a better understanding of how these communities are structured and interacting among themselves and with the environment, futures studies are necessary addressing the dynamic of dispersion of these communities and network holding microbial communities.

This is a pioneer study of microbial communities from a tropical cascade system. Moreover, we found a great number of uncultured bacteria (~90% of sequences obtained), at some taxonomic level, that suggests that these systems harbor particular organisms and reinforces the need of studies exploring microbial communities in aquatic habitats in order to improve the knowledge of their ecological roles in biogeochemical cycles.

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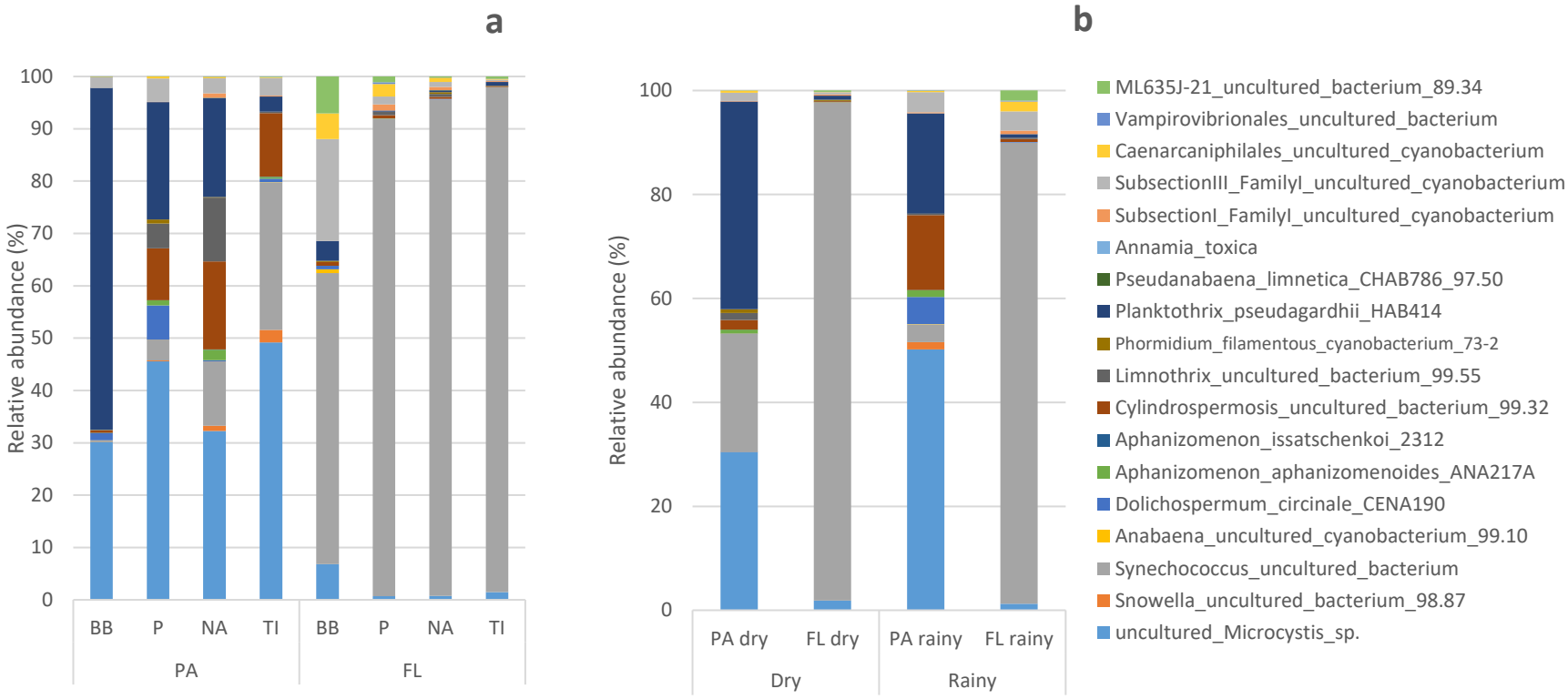
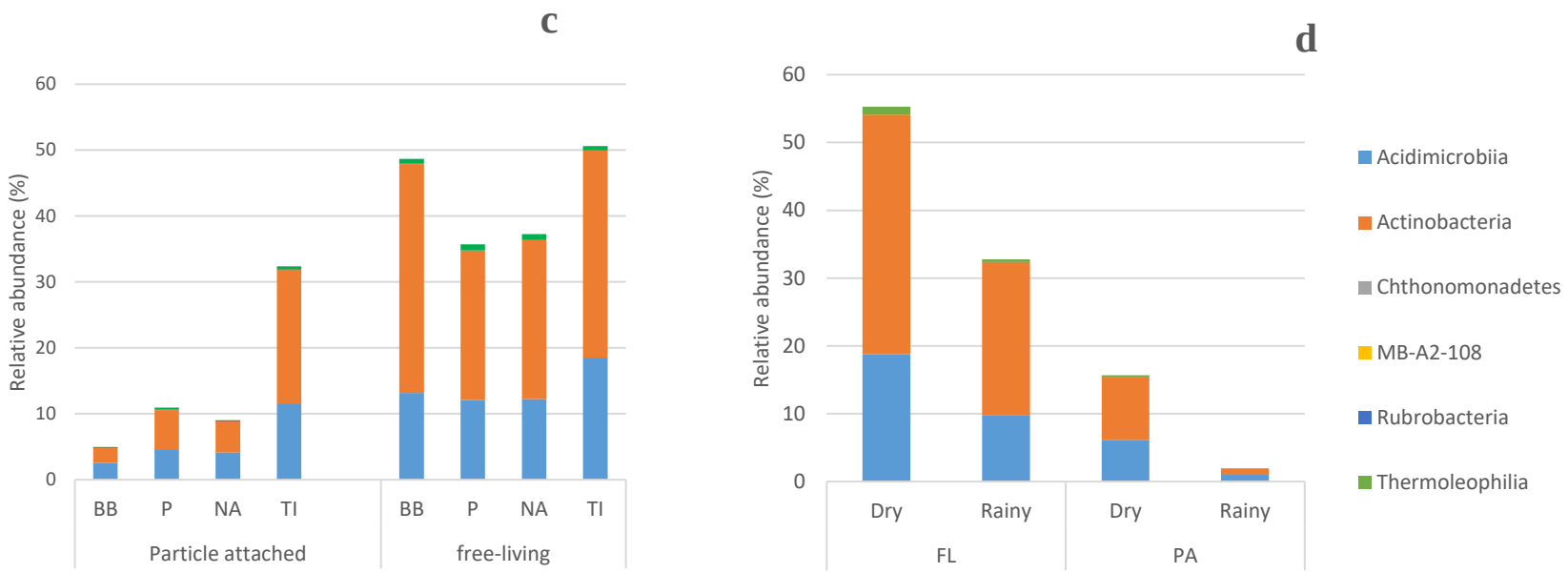
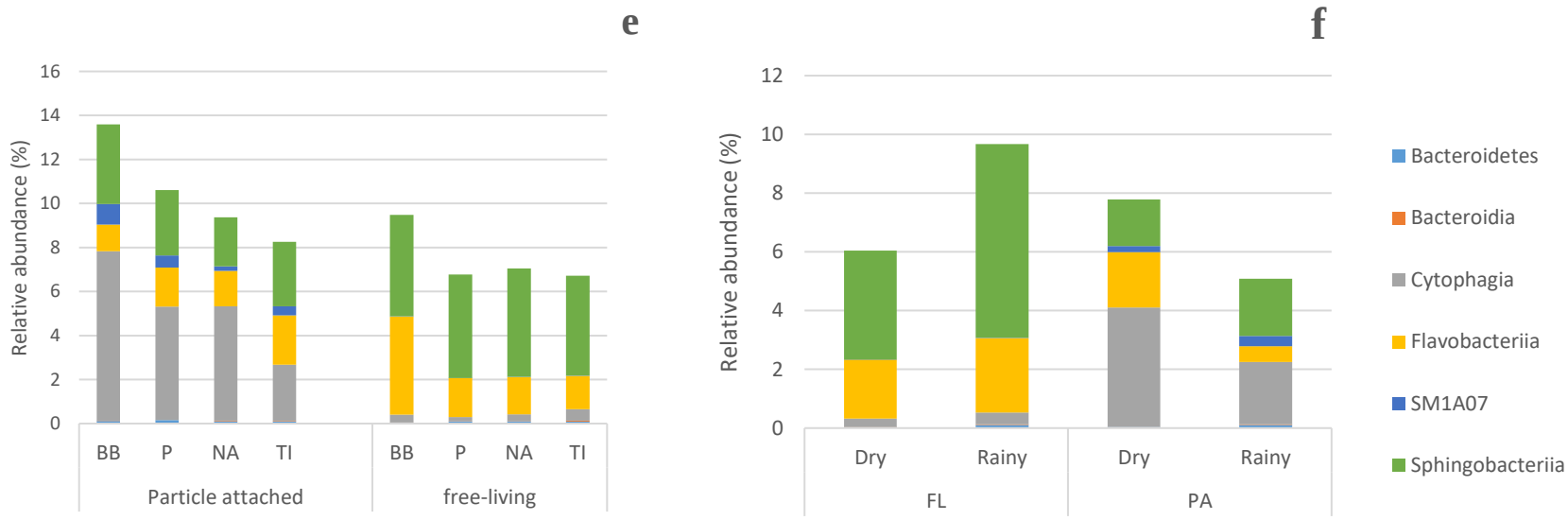
Supplementary Material



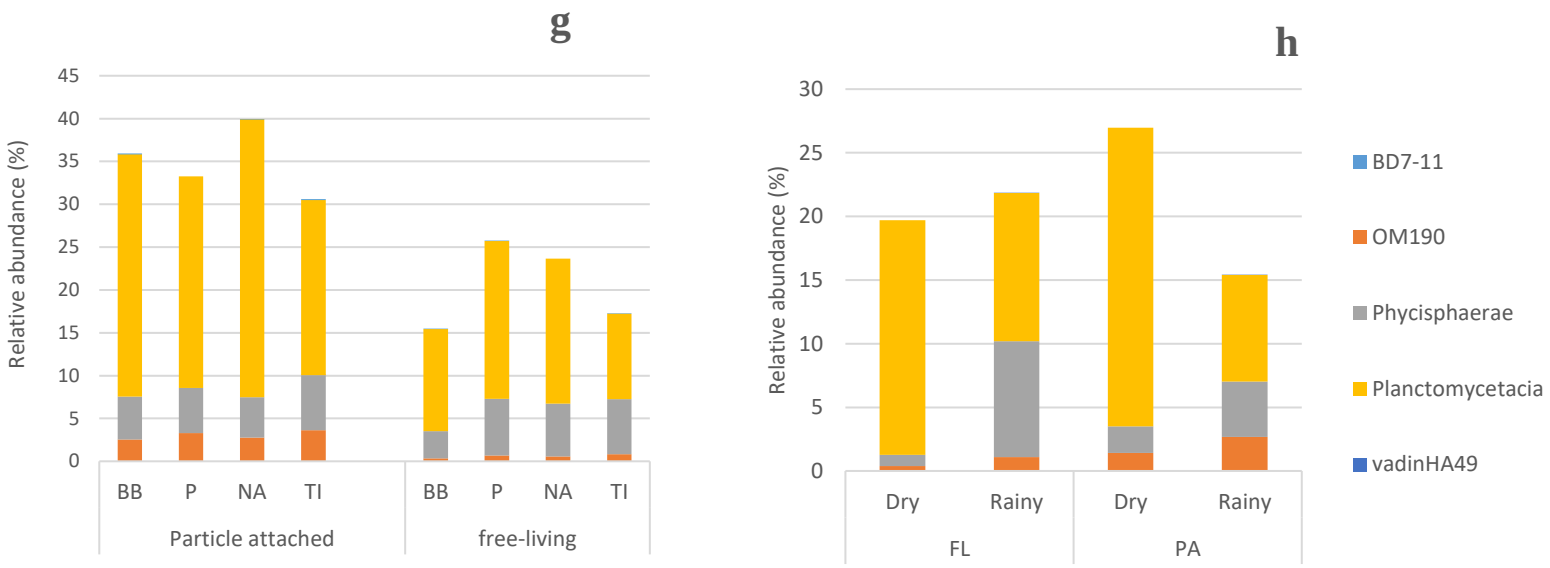
SM Figure 1.1a-b: Genera composition of *Cyanobacteria* from particel attached and free-living subcommunities by reservoir (a) and by season (b). Particle-attached (PA), Free-living (FL). Barra Bonita (BB), Promissão (P), Nova Avanhadava (NA) and Três Irmãos (TI).



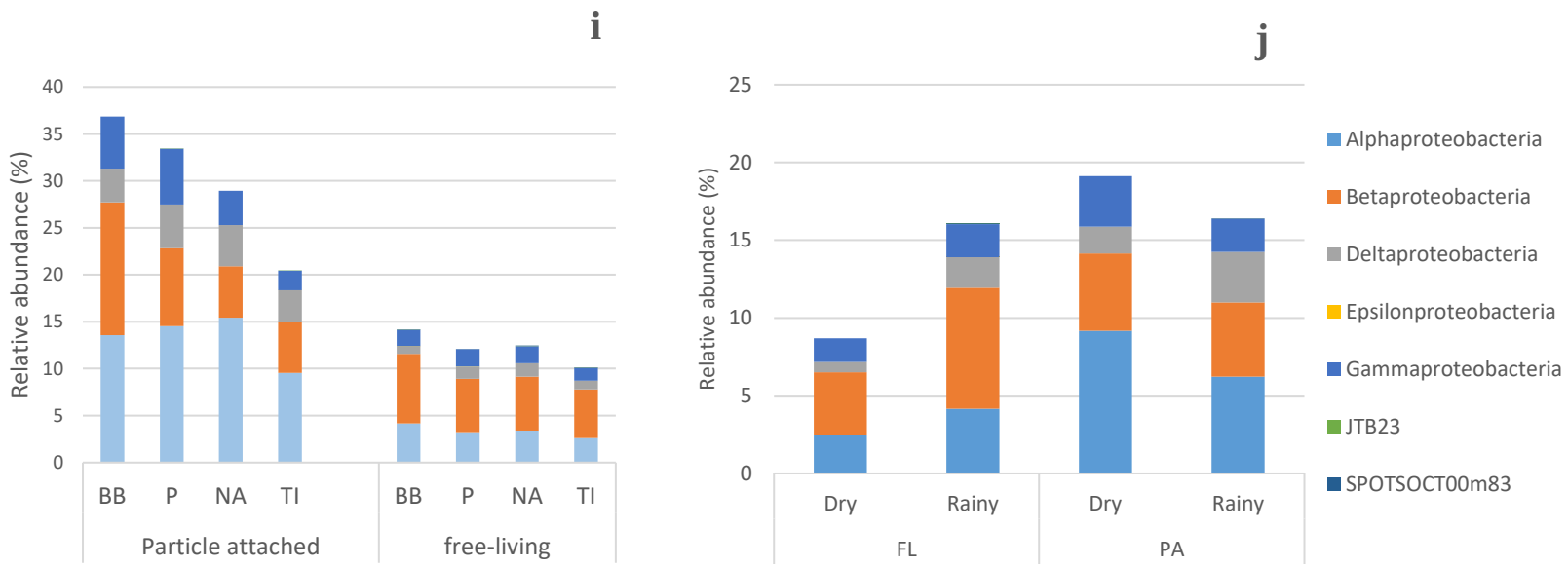
SM Figure 1.1c-d: Phylum Actinobacteria compostion from particel attached and free-living subcommunities by reservoir (c) and by season (d). Particle-attached (PA), Free-living (FL). Barra Bonita (BB), Promissão (P), Nova Avanhadava (NA) and Três Irmãos (TI).



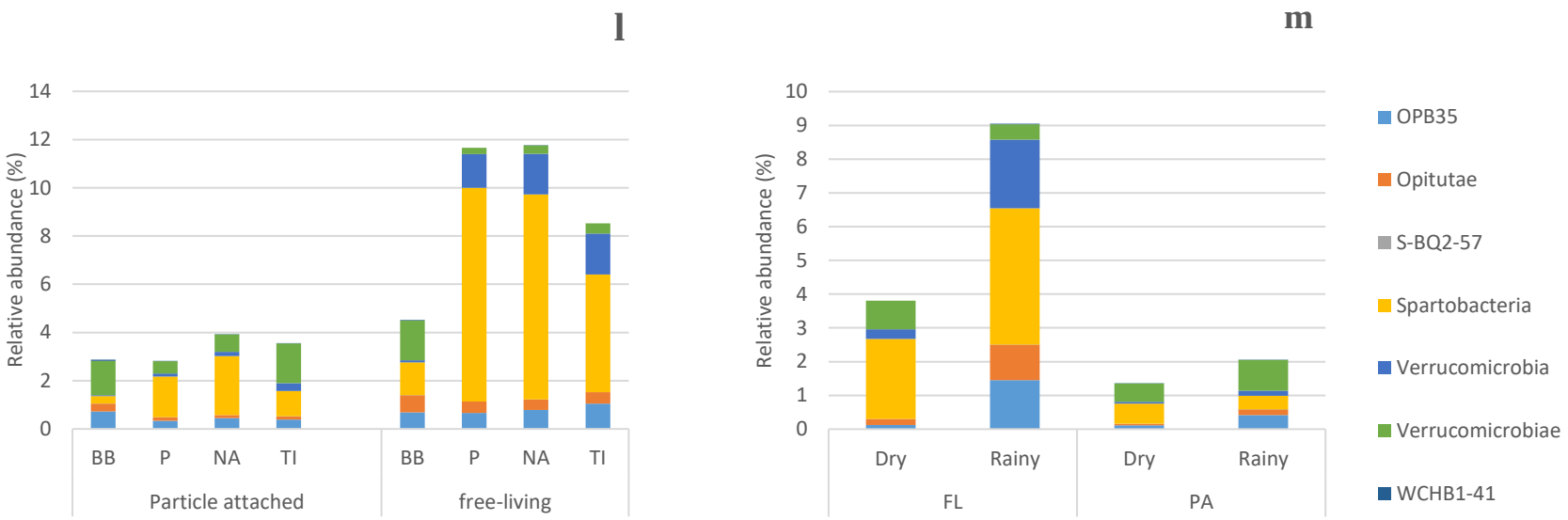
SM Figure 1.1e-f: Phylum Bacteroidetes compostion from particel attached and free-living subcommunities by reservoir (e) and by season (f). Particle-attached (PA), Free-living (FL). Barra Bonita (BB), Promissão (P), Nova Avanhadava (NA) and Três Irmãos (TI).



SM Figure 1.1g-h: Phylum Planctomycetes compostion from particel attached and free-living subcommunities by reservoir (g) and by season (h). Particle-attached (PA), Free-living (FL). Barra Bonita (BB), Promissão (P), Nova Avanhadava (NA) and Três Irmãos (TI).

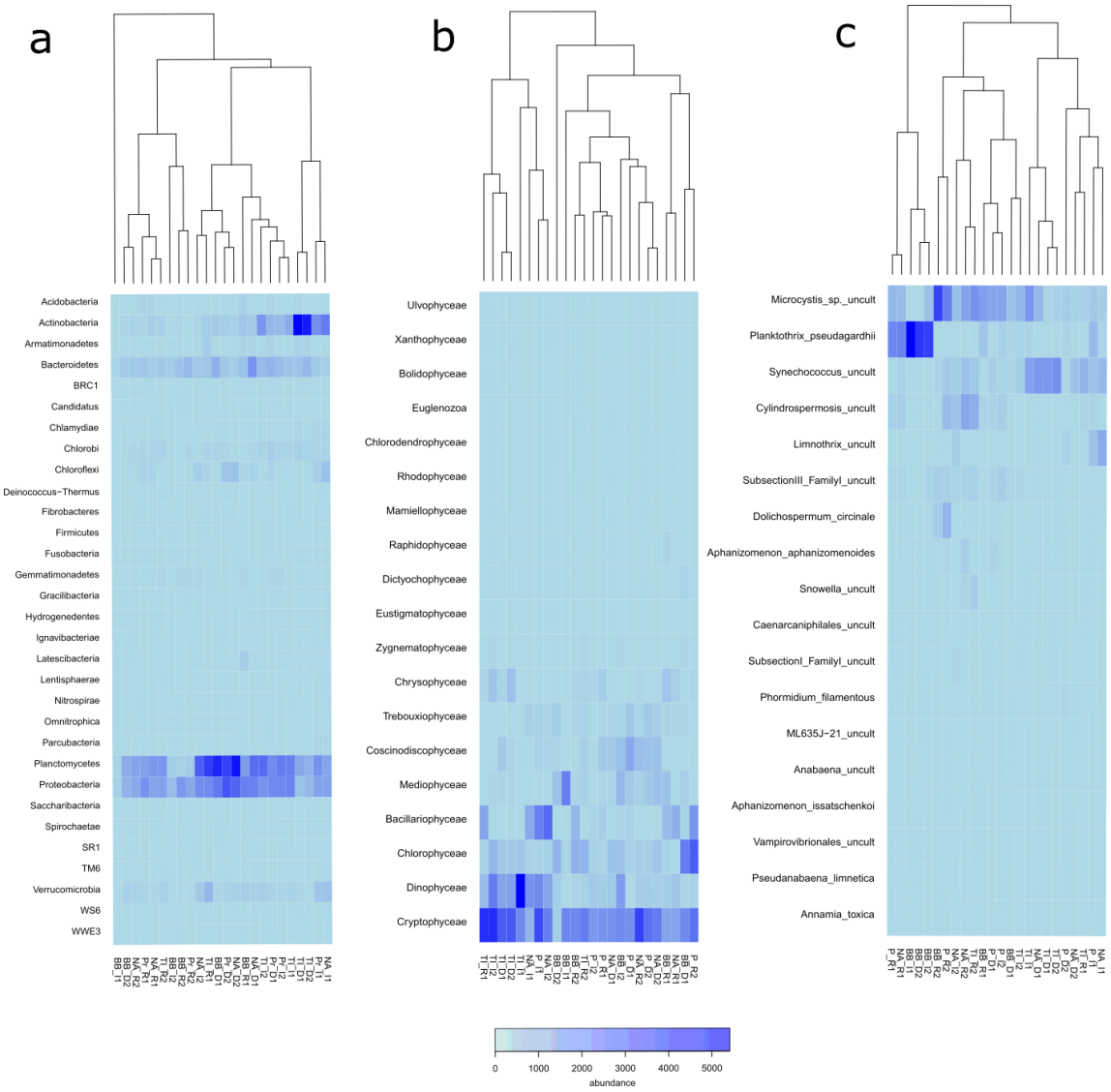


SM Figure 1.1i-j: Phylum Proteobacteria compostion from particel attached and free-living subcommunities by reservoir (i) and by season (j). Particle-attached (PA), Free-living (FL). Barra Bonita (BB), Promissão (P), Nova Avanhadava (NA) and Três Irmãos (TI).



SM Figure 1.1l-m: Phylum Verrucomicrobia compostion from particel attached and free-living subcommunities by reservoir (l) and by season (m). Particle-attached (PA), Free-living (FL). Barra Bonita (BB), Promissão (P), Nova Avanhadava (NA) and Três Irmãos (TI).

SM Fig. 1. 1– Composition of the most abundant Phyla of bacteria.



SM Fig. 1. 2 - Heatmap representation by clustering analyses of Particle-attached bacteria community by Phylum level (a), Eukaryotic Phytoplankton by Class level (b), and Cyanobacteria by Genera level (c). Sampling points are represented by Barra Bonita (BB), Promissão (Pr), Nova Avanhandava (NA) and Três Irmãos (TI). Samplings from dry period are represented by D1 (May/2015) and D2 (July/2015), from intermediary period by I1 (September/2015) and I2 (November/2015) and from rainy period by R1 (January/2016) and R2 (March/2016).

SM Table 1. 1 – Trophic state index (TSI). Reservoirs: Barra Bonita (BB), Promissão (Pr), Nova Avanhandava (NA) and Três Irmãos (TI). Samplings from dry period are represented by D1 (May/2015) and D2 (July/2015), from intermediary period by I1 (September/2015) and I2 (November/2015) and from rainy period by R1 (January/2016) and R2 (March/2016).

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site	TSI (chla)	TSI (TP)	TSI
BB_D1	57.65323	46.34293	80.8247
BB_D2	58.29129	46.64055	81.61157
BB_R1	49.72893	46.75004	73.10396
BB_R2	63.55835	49.19535	88.15602
Pr_D1	57.56744	44.84896	79.99192
Pr_D2	59.3435	43.13693	80.91197
Pr_R1	55.38027	42.15711	76.45882
Pr_R2	61.52813	42.88405	82.97016
NA_D1	53.37142	44.40463	75.57373
NA_D2	58.48124	42.21366	79.58808
NA_R1	58.04764	44.50072	80.298
NA_R2	54.7313	38.43185	73.94722
TI_D1	50.23169	43.849	72.15618
TI_D2	47.1296	36.51413	65.38667
TI_R1	52.6098	40.21417	72.71689
TI_R2	55.92658	42.56293	77.20805

	Phylum	Class	Order	Family	Specie/Strain/lineage	N° of reads	Relative abundance (%)
PARTICLE ATTACHED							
OTU_1	Cyanobacteria	Cyanobacteria	SubsectionI	FamilyI	uncultured_Microcystis_sp.	36238	15,62
OTU_8	Cyanobacteria	Cyanobacteria	SubsectionIII	FamilyI	Planktothrix_pseudagardhii_HAB414	33336	14,36
OTU_9	Cyanobacteria	Cyanobacteria	SubsectionIV	FamilyI	Cylindrospermosis_uncultured_bacterium_99.32	7893	3,40
OTU_2	**Actinobacteria**	**Actinobacteria**	**Frankiales**	**Sporichthyaceae**	**hgcI_clade_uncultured_bacterium**	**7122**	**3,07**
OTU_6	**Planctomycetes**	**Planctomycetacia**	**Planctomycetales**	**Planctomycetaceae**	**uncultured_bacterium_99.57**	**6973**	**3,00**
OTU_4	**Planctomycetes**	**Planctomycetacia**	**Planctomycetales**	**Planctomycetaceae**	**Planctomyces_uncultured_bacterium_99.35**	**6133**	**2,64**
OTU_42	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	Roseomonas_uncultured_Roseomonas_sp.	6118	2,64
OTU_14	**Cyanobacteria**	**Cyanobacteria**	**SubsectionI**	**FamilyI**	**Synechococcus_uncultured_bacterium_99.55**	**5763**	**2,48**
OTU_24	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	uncultured_bacterium_96.08	5429	2,34
OTU_63	Cyanobacteria	Cyanobacteria	SubsectionIII	FamilyI	Limnothrix_uncultured_bacterium_99.55	3652	1,57
OTU_19	Cyanobacteria	Cyanobacteria	SubsectionIII	FamilyI	uncultured_bacterium_99.32_440	2903	1,25
OTU_5	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade_uncultured_bacterium	2811	1,21
FREE-LIVING							
OTU_2	**Actinobacteria**	**Actinobacteria**	**Frankiales**	**Sporichthyaceae**	**hgcI_clade_uncultured_bacterium**	**28947**	**12,29**
OTU_5	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade_uncultured_bacterium	12960	5,50
OTU_14	**Cyanobacteria**	**Cyanobacteria**	**SubsectionI**	**FamilyI**	**Synechococcus_uncultured_bacterium_99.55**	**9912**	**4,21**
OTU_3	Verrucomicrobia	Spartobacteria	Chthoniobacterales	LD29	bacterium_enrichment_culture_clone	9450	4,01
OTU_7	Actinobacteria	Acidimicrobiia	Acidimicrobiales	Acidimicrobiaceae	CL500-29_marine_group_uncultured	6264	2,66
OTU_6	**Planctomycetes**	**Planctomycetacia**	**Planctomycetales**	**Planctomycetaceae**	**uncultured_bacterium_99.57**	**6080**	**2,58**
OTU_15	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade_uncultured_bacterium	5398	2,29
OTU_13	Planctomycetes	Phycisphaerae	Phycisphaerales	Phycisphaeraceae	CL500-3_uncultured_bacterium_98.87	5144	2,18
OTU_20	Actinobacteria	Acidimicrobiia	Acidimicrobiales	Acidimicrobiaceae	CL500-29_marine_group_uncultured	4993	2,12
OTU_103	Actinobacteria	Acidimicrobiia	Acidimicrobiales	Acidimicrobiaceae	CL500-29_marine_group_uncultured	4587	1,95
OTU_4	**Planctomycetes**	**Planctomycetacia**	**Planctomycetales**	**Planctomycetaceae**	**Planctomyces_uncultured_bacterium_99.35**	**4508**	**1,91**
OTU_10	Actinobacteria	Acidimicrobiia	Acidimicrobiales	Acidimicrobiaceae	CL500-29_marine_group_uncultured	4458	1,89
OTU_17	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	uncultured_bacterium_97.32	3856	1,64
OTU_18	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Sphaerotilus_bacterium_enrichment_culture	3004	1,28
OTU_26	Chlorobi	Chlorobia	Chlorobiales	OPB56	uncultured_bacterium_98.91_458	2837	1,20
OTU_11	Verrucomicrobia	Verrucomicrobia	Incertae	Sedis		2649	1,12
OTU_59	Actinobacteria	Acidimicrobiia	Acidimicrobiales	Acidimicrobiaceae	CL500-29_marine_group_uncultured	2444	1,04

SM Table 1. 2 - Rank and classification of the most abundant (relative abundance <1%) OTUs found in particle-attached and free-living bacterial subcommunities. OTUs shared by both subcommunities are indicated in bold

CAPITULO 2 – REGIONAL FACTORS AS MAIN DRIVER OF MICROBIAL COMMUNITIES' TURNOVER IN TROPICAL CASCADING RESERVOIRS

Running title: Stochasticity drives tropical microbial communities

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Keywords: ecological processes, microbial dispersion, metacommunities.

Abstract

Microbial community turnover across space is dictated by local and regional factors. Locally, biological interactions between organisms and environmental variables are shaping community assembly through selection, while regional factors influence microbial dispersion patterns. Typically, methods used to disentangle the effects of local and regional factors do not proceed to identify ecological processes underlying the turnover. In this paper, we identify and quantify these processes in three subcommunities (particle-attached, free-living and cyanobacteria). We sampled multiple local communities from a tropical cascade of freshwater reservoirs with decreasing trophic state over two markedly different periods of dry conditions and rainfall. We hypothesized that during the dry season, communities would be mainly under selection due to the higher environmental heterogeneity promoted by the lower water flux between reservoirs. During the rainy season, we expected a more homogenized environment and similar communities shaped by higher dispersal because of the higher water flux between reservoirs. Despite metacommunities were largely governed by regional events in both periods, free-living community was under greater influence of selection during the dry period, attributed to dissolved organic carbon concentration along the cascade. Each sub community had distinct pattern of turnover along the cascade as result of richness (Cyanobacteria), life-style and size (Free-living) and spatial dynamic (particle-attached).

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Introduction

Spatial turnover, or variation on community's species composition across space, can be driven by distinct factors. At the local scale, organisms from a given community are under pressure of the environmental conditions they share as well as biological interactions stablished among them, such as competition or facilitation (Ricklefs & Miller, 1990). These trophic interactions may promote selection of organisms inside communities and constrict species richness (Chave, 2004; Dumbrell *et al.*, 2010; Ofiteru *et al.*, 2010; Langenheder & Székely, 2011). According to the metacommunity concept, communities across space are not isolated entities and organisms are constantly being exchanged between communities (Leibold *et al.*, 2004; Logue *et al.*, 2011). The movement of organisms among communities brings the regional scale as a component influencing spatial turnover of communities.

Vellend, (2016) made an analogy of processes that undertake community's turnover across space with the four main processes governing genotypes within a population – selection, drift, mutation and gene flow. Applied for ecology communities, gene flow corresponds to the exchange of organisms (dispersion) and mutation would be speciation. For microorganisms, process of speciation does not fit, because of their high abundance and dispersion microbial communities, they are unlikely to be completely isolated. Thus, for microbial communities' ecology, the processes that can be applied are: selection, drift and dispersion.

At the regional scale, dispersion of microorganisms may be intensified or limited by regional factors that, such as distance or physical barriers between communities, flood pulse connecting communities, etc. High volume of organisms moving among communities, or homogenizing dispersion (HD), would result in communities more similar, whereas constrained movemnt results in communities less similar by dispersal

limitation (DL) (Leibold *et al.*, 2004). Moreover, drift (D), where random fluctuations in populations, such as birth/death rates and loss/gain of species, occur by chance, also contributes to spatial turnover. This process is not locally or regionally determined, and acts as a purely stochastic factor (Vellend, 2016).

Regarding microorganisms spatial turnover it can be difficult to disentangle the effects of regional and local factors (Logue et al., 2011). In general, environmental variables are correlated to spatial distance, making it challenging to partition the respective role of each component. Furthermore, the most commonly used methods to quantify the respective roles of environmental and spatial variables shaping community turnover does not discriminate between the different processes underlying (Legendre *et al.*, 2009; Tuomisto, Ruokolainen & Ruokolainen, 2012) .

An additional largely methodological challenge is that many microorganisms are not readily isolated and cultured under laboratorial conditions, hampering our ability to use traditional microbiological methods to assess their morphology, metabolism and other traits that can be used for identification (Roszak & Colwell, 1987). Accordingly, most natural microbial communities are dominated by unknown and uncultured lineages (Rappé and Giovannoni, 2003). To overcome this limitation, high throughput sequencing method of target genes, such as 16S rRNA sequence analyses, have been developed and refined, enabling precise and efficient analyses of also uncultured organisms (Stewart, 2012).

Such methods still have some challenges and limitations. For example, bacterial lineages and their taxonomic marker genes may evolve at highly variable rates (Mahé *et al.*, 2014). Using fixed identity thresholds for defining populations or operational taxonomic units (OTUs), one may aggregate or break functionally coherent groups of

organisms, hampering the possibility to assess the true ability of such groups to colonize and thrive in a given habitat (Losos, 2008; Fine & Kembel, 2011).

Hence the framework proposed by Stegen et al. (2013) seems to be more appropriate for describing the ecological distribution patterns of organisms that are as abundant, widespread and hard to cultivate as microorganisms. This method specifically addresses the ecological processes underlying observed community turnover by using the phylogenetic turnover across habitats. In brief, phylogenetically closely related species share similar traits and are assumed to share similar niches, rather than merely defining communities and make inferences based on fixed cutoff-OTU distribution data (Stegen *et al.*, 2013).

In this paper we applied Stegen *et al.* (2013) framework to quantify and compare local and regional ecological processes driving bacterial metacommunity dynamics in a cascade of four freshwater reservoirs. The cascade formation guides the dispersal of organisms from the first reservoir to the next and so on, with organisms being retained in the respective reservoir according to the hydraulic residence time, which vary with rainfall regime. Thus, we have a unidirectional dispersal where we can evaluate whether or not local factors are strong enough to shape the distribution of microorganisms along the cascade and also quantify the role of regional factors. Local factors were represented by the environmental heterogeneity (trophic state gradient) and regional factors were represented by spatial distance between reservoirs and residence time. The sampling included a dry and a rainy period that directly influenced the volume of water flowing through the reservoirs and affect the inter-system connectivity and so, the environmental homogeneity/heterogeneity. Consequently, communities seen during the dry period would mainly be influenced by local factors (selection in response to variation in environmental factors), while communities during the rainy period would mainly respond

to regional processes. We divided bacterial community in three sub-communities (Cyanobacteria, Particle-attached and Free-living bacteria) for better resolution of processes driving spatial turnover.

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Methodology

Study site

The studied site is located in the medium-low Tietê river system, Parana river basin, São Paulo state, Brazil (Fig. 1). The cascade holds six reservoirs manmade hydroelectric power reservoirs: Barra Bonita (BB), Bariri, Ibitinga, Promissão (Pr), Nova Avahandava (NA) and Três Irmãos (TI), in this order (Fig. 1). Bariri and Ibitinga were not sampled due to their lower volume. The system is located in a region surrounded by agricultural activities which injects large amounts of nutrients into the system in addition to the domestic and industrial waste inputs. In this river cascade, the water flow towards the interior of the continent, and the anthropogenic waste load is largest in the Barra Bonita reservoir because of large cities upstream, such as the São Paulo metropolitan region. Because of this, the Barra Bonita reservoir is the most eutrophic system within the cascade and the productivity and trophic state decrease along the flow of the river because of gradual nutrient removal/dilution effect (Freitas *et al.*, 2018).

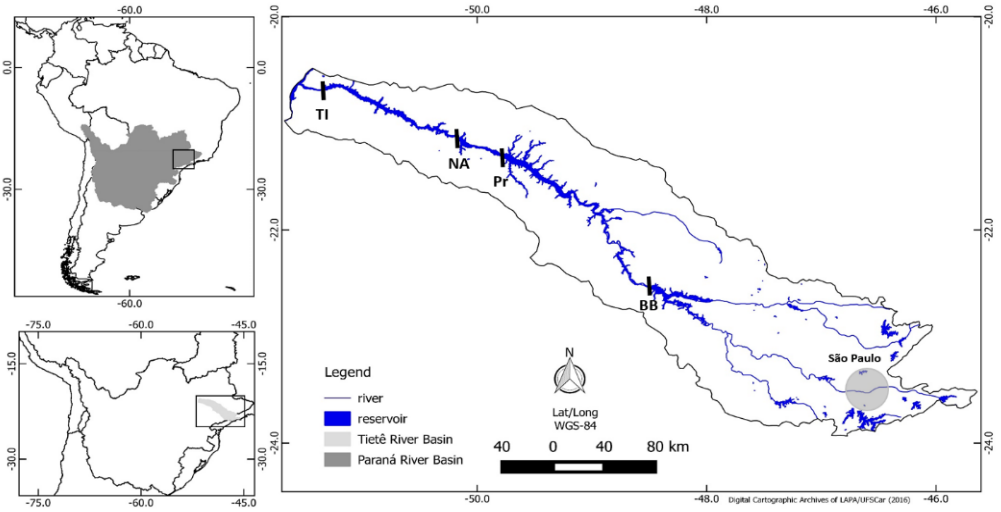

Figure 1 - Map of Paraná Basin (topleft), Tietê River (topdown) and location of the four reservoirs sampled (center). **Barra Bonita (BB), Promissão (Pr), Nova Avanhandava (NA) and Três Irmãos (TI). Modified from Freitas et al. (2018).**

The region where our system is located went through a severe drought in 2015 that compromised water supply to the basin (INMET, 2016). Since these reservoirs were originally built for hydropower generation, the hydroelectric company in charge set the floodgates to maintain the regular amount of water in the reservoirs, thus increasing the residence time. At the end of 2015, an El Niño event led to intensified rainfall in the region, thus creating abnormally wet conditions that resulted in flooding episodes (World Meteorological Organization, 2016). Frequent studies on the reservoirs within this system can be found in the literature (Rodgher, 2001; Sotero-Santos *et al.*, 2006; Smith, Espíndola & Rocha, 2014) but there is no comprehensive or sufficiently detailed study of microbial metacommunities to learn about the biogeographical processes in operation.

Table 1 Environmental variables from dry and rainy period and physical traits location of reservoirs sampled from the medium-low cascade of Tietê River. A: **Local factors, B: Regional factors. Environmental variables: Water transparency (Secchi), Temperature (Temp), pH, Chlorophyll-a (Chla-a), Total phosphorous (TP), phosphate (PO_4), Dissolved organic carbon (DOC), sulphate (SO_4), total nitrogen (TN), residence time (RT). Distance, from the first reservoir (Barra Bonita).**

		BB		Pr		NA		TI	
		Dry	**Rainy**	**Dry**	**Rainy**	**Dry**	**Rainy**	**Dry**	**Rainy**
A	**Secchi (m)**	2.7	0.35	1.2	1.3	1.4	1.5	6.9	2.4
	Temp (ºC)	21.6	26.9	23.4	28.7	24.6	28.3	24	28.9
	pH	7.9	6.9	8.7	7.6	9	7.5	7.8	7.5
	Chla-a (µg.L-1)	16.4	38.9	19.2	25.7	11.7	11.6	1.3	6.5
	TP (µM)	4.1	6.3	2.2	1.5	1.9	1.5	1.2	1.2
	PO_4 (µg.L^{-1})	43.7	15.9	3.4	8.7	2.7	4.1	4.5	18.7
	DOC (µg.C.L^{-1})	8.3	20.6	7.2	6.3	5.6	6.6	4.3	4.9
	SO_4 (µg.L^{-1})	28.3	15.9	19.1	9.8	15.6	13.2	13.3	11.8
	TN (µM)	3.1	2.9	0.7	0.9	0.7	1.0	0.5	0.4
B	**RT (days)**	174.3	35.7	243.3	47.5	83.9	17.1	755.6	79.2
	Distance (km)	0		180		235		355	
	Location (GPS)	22° 32.648’ S 048° 27.97’ W		21° 19.123’ S 049° 44.724’ W		21° 06.455’ S 050° 10.954 W		20° 40.110’ S 051° 16.855’ W	
	Volume (m^3)	3.14 10^9		7.42 10^9		2.72 10^9		13.8 10^9	

Sampling

Sampling campaigns were conducted during the dry season in May (D1) and July (D2) of 2015, and during the rainy season in January (R1) and March (R2) of 2016. Using a GPS device, we revisited specific sampling points (deepest) within in each reservoir. Around 1 liter of water from the surface of each reservoir was processed by sequential filtration that separated cyanobacteria and particle-attached bacteria (PA) on a 3µm polycarbonate filter and free-living bacteria (FL) on a 0.22 µm cellulose acetate ester membrane filter (Sterivex). Filters with immobilized bacteria were immediately frozen in liquid nitrogen and transported to the laboratory for long-term storage at -80°C.

Environmental analysis

Environmental variables such as pH, temperature and dissolved oxygen were measured with a multiparameter probe YSI 6600 V2 (YSI, Yellow Springs, OH, USA). Chlorophyll-a was extracted following Marker *et al.* (1980) and Nusch (1980) method and quantified in a spectrophotometer as described by Lorenzen (1967). Dissolved organic carbon and total nitrogen were quantified using a Shimadzu TOC-V cph analyzer, equipped with Total Nitrogen analyzer module. Free phosphate and sulphate were analyzed by ion chromatography using a Dionex ICS – 1100 system (Thermo Scientific). Water transparency was measured using a Secchi disk.

We performed a principal component analysis (PCA) with standardized environmental parameters to explore the spatial structure of environmental conditions in both rainfall period.

Communities analysis

DNA was extracted using the PowerSoil DNA Isolation kit (MoBio) and region V3-V4 of the 16S rRNA gene was amplified using primers 341F (5'CCTACGGGNGGCWGCAG-3') and 805R (5'-GACT ACHVGGGTATCTAATCC-3') (Herlemann *et al.*, 2011). High-throughput sequencing was carried out using the Illumina MiSeq platform. Sequences were processed using UPARSE (Edgar, 2013) for sequence quality control and OTU clustering at a sequence similarity ≥ 97% as previously described (Logares *et al.*, 2014; Logares, 2017). Taxonomic OTU classification was obtained with BLASTn against the SILVA 119.1 database (Zhang *et al.*, 2000).

We assessed phylogenetic turnover of communities (evolutionary distance between OTUs found in two communities being compared**)** through the analytical framework proposed by Stegen et al. (2013). This method uses a phylogenetic tree from each community for the analyses and is based on optimal OTU habitat occupation, where phylogenetically closely related OTUs are expected to share similar habitats. The method quantifies phylogenetic distance (beta-mean-nearest taxon distance, βMNTD) between each OTU in a given community and between the closest relatives in a second community to which it is being compared. By then shuffling species and abundances, βMNTD is compared to a randomly assembled community (null-model) where selection is not influencing phylogenetic turnover. Thus, in a first step, the method considers the deviation of the original community from the null-model (beta-nearest taxon index - βNTI), returning only those communities governed by selection (S), $|\beta NTI|>2$. Communities that did not fit this first group under selection ($|\beta NTI|<2$), go through a second randomization step using the Raup-Crick (Chase, 2010a) index modified by Stegen et al. (2013)**,** using Bray-Curtis for each interaction (RC_{bray}) to account for OTU relative abundance. At this second step the deviation from the null model now ranges

from -1 to 1 and corresponds to homogenizing dispersion (RC_{bray} < -0.95), limiting dispersal (RC_{bray}>0.95) or drift acting alone ($|RC_{bray}|$ >0.95). For both randomization steps, the null model analysis returned a matrix of pairwise values between all four reservoirs. We chose to interpret the output in the same sequence of the reservoir cascade, thus communities migrating from Barra Bonita to Promissão, from Promissão to Nova Avanhandava and from Nova Avanhandava to Três Irmãos. (SM Table 1).

To evaluated if community's turnover were better explained by reservoir or by rainfall regime, we performed a PERMANOVA analysis based on community composition distances matrices. We used the beta.nmtd.weighted distance, the same metric as used in null model analysis, and this index was obtained with the functions 'comdistnt' and 'cophenetic', from the Picante package (Kembel *et al.*, 2010) in R (*R* Development Core Team, 2017). We then built a non-metric multidimensional scaling analysis (NMDS) with communities that had significant values in PERMANOVA analysis.

All analyses were performed in computing environment *R* (*R* Development Core Team, 2017).

Results

Community composition

Sequencing of 16S rDNA yielded 8.141.098 reads with a minimum of 9868 reads/sample and a total of 2367 bacterial OTUs. After rarefaction, the combined dataset presented 2112 bacterial OTUs: 39 OTUs from cyanobacteria; 1728 OTUs from the particle attached subcommunity, and 1758 OTUs from the free-living subcommunity. At dry period, particle-attached community was represented by 1059 OTUs and free-living by 950 OTUs. At the rainy period, particle-attached was represented by 1433 OTUs and

free-living by 1470 OTUs. Sequences were submitted at NCBI database under reference number: ID 10996415 to 10996489.

Environmental gradient

As we observed in the PCA analysis with environmental variables, sampling points from dry period reflected the spatial structure (PC1), suggesting that the lower amount of water running through the system led to more heterogenous environmental conditions along the reservoir cascade. At dry period Samplings from BB and TI formed two groups and samplings from NA and Pr, the closest reservoirs, overlapped in the middle of PCA plot, whereas this separation was not clear during the dry period (Fig. 2). The spatial structure observed was promoted by variables related to the trophic state: Chlorophyll-a (score -0.7), DOC (score -0.92), total nitrogen (score -0.95), total phosphorous (score -1.0), phosphate (score -0.63) and Secchi disk (score 0.61) (Fig. 2). Also, samplings from dry and rainy period could be separated along the second axis (Fig. 2).

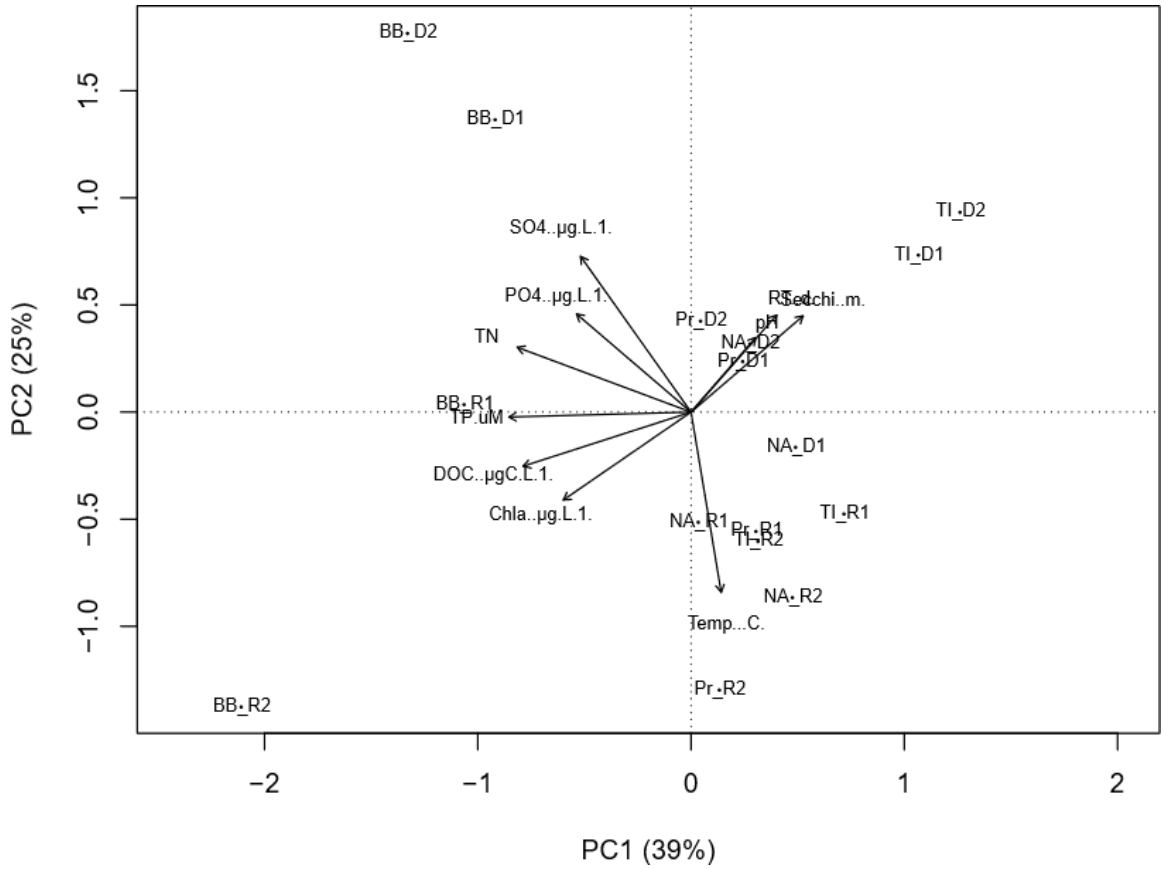

Figure 2 – Principal component analysis of environmental variables by reservoir. Environmental variables: **Residence time (RT), Water transparency (Secchi), Temperature (Temp), pH, Chlorophyll-a (Chla-a), Total phosphorous (TP), Phosphate (PO_4), Dissolved organic carbon (DOC), Sulphate (SO_4) and total nitrogen (TN). Reservoirs are represented by Barra Bonita (BB), Promissão (Pr), Nova Avanhandava (NA) and Três Irmãos (TI). Samples are represented by dry period: May-2015 (D1) and July-2015 (D2) and rainy period: January-2016 (R1) and March-2016 (R2).**

In order to investigate whether local or regional factors had higher influence on community composition, we performed PERMANOVA analysis with communities 'beta.nmtd.weighted' distance by rainfall regime and by reservoirs. We found that only free-living could be significantly explained by these two factors. Free-living communities' variation was better explained by reservoir (r^2=0.28, p=0.0009), rather than

by rainfall regime (r^2=0.25, p<0.001), with communities from BB grouped apart from remaining reservoirs.

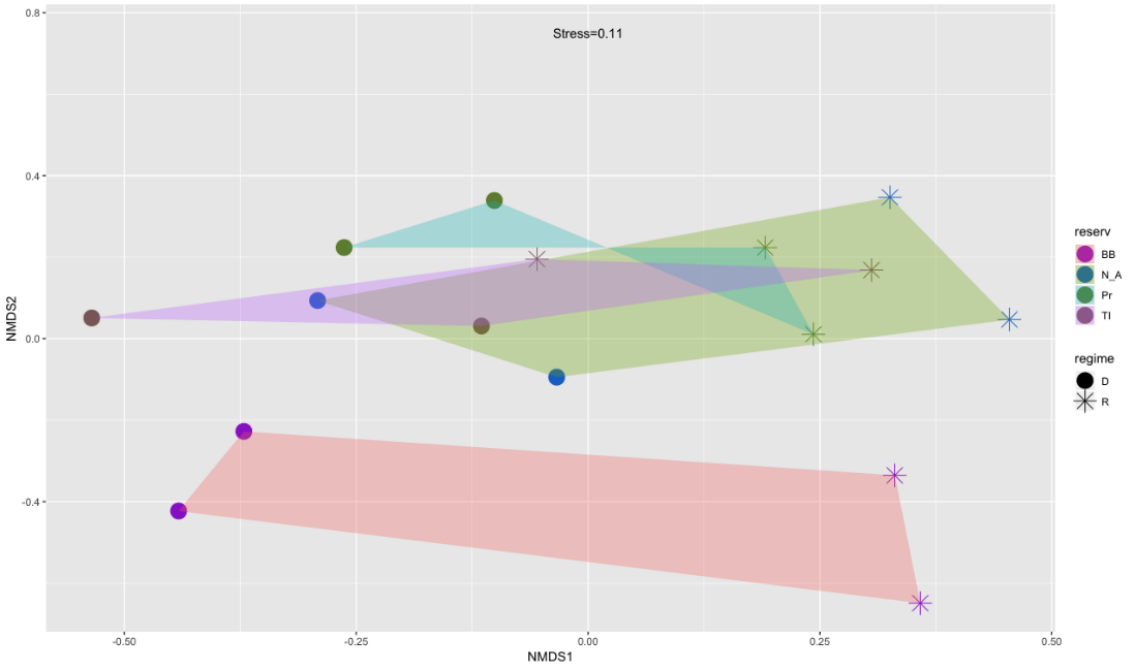

Figure 3 - Non-metric multidimensional scaling (NMDS) ordinations with 'beta.nmtd.weighted' dissimilarities of free-living communities by sampling and by reservoir. Sampling points by season (PERMANOVA r^2=0.25, p<0.001), are represented by symbols: circle=dry period samplings; asterisk=rainy period samplings; Sampling points by reservoirs are represented by polygons (PERMANOVA r^2=0.28, p<0.001), where BB=Barra Bonita; Pr=Promissão; N_A=Nova Avanhandava and TI= Três Irmãos.

Ecological processes

We choose to analyze bacterial metacommunities separately in three fractions (free living, particle attached and cyanobacteria) to get a better resolution of process governing the turnover of the whole microbial community.

Microbial communities were under major influence of regional processes during both rainfall regime. Only free-living community variation had proportional influence of local and regional factors during dry period (Table 2). Yet during the dry period, Cyanobacteria was consistently under drift (Table 2). Particle attached community

variation was under regional factors and drift during the both rainfall period (Table 2). Selection governed variation of Cyanobacterial and Free-living communities during the rainy period (Table 2).

Table 2 - Ecological processes governing microbial communities between reservoirs by sampling. BB –Barra Bonita, Pr-Promissão, NA-Nova Avanhandava and TI-Três Irmãos. Sub-communities: Cyanobaceria (Cyano), Particle-attached (PA) and Free-living (FL). Sampling names: D1-May/2015, D2-July/2015, R1- January/2016 and R2- March/2016. Ecological processes that were mainly driving community's variation are presented by sampling and between representations of reservoir in the order they are on cascade. HD-Homogenizing dispersion, DL-Dispersal limitation (Regional factor) and D-Drift (stochastic factor), and S-Selection (local factor). Percentage of ecological processes accounted from all samplings are indicated in "total" column.

		Dry		**Rainy**		
		D1	**D2**	**R1**	**R2**	**total**
Cyano	BB - Pr	D	D	D	HD	
	Pr - NA	D	D	S	D	
	NA - TI	D	D	S	D	
		Stochastic: 100%		Stochastic: 33% Regional: 16% Local: 34%		Stochastic: 75% Regional: 0.08% Local: 16%
PA	BB - Pr	DL	D	DL	DL	
	Pr - NA	HD	S	HD	DL	
	NA - TI	DL	DL	D	DL	
		Stochastic: 16% regional: 66% local:16%		Stochastic: 16 % Regional:83%		Stochastic: 16% Regional: 75% Local: 16%
FL	BB - Pr	DL	S	DL	DL	
	Pr - NA	HD	S	S	HD	
	NA - TI	HD	S	S	DL	
		regional: 50 % local: 50%		regional: 66 % local: 34 %		Regional: 58% Local: 42%

Discussion

We expected that the lower water flux between reservoirs during the dry period would result in stronger environmental gradients which in turn, would promote higher selection pressure on organisms moving along the cascade. Accordingly, during the rainy season, the higher water flux would promote environmental homogenization and increase microbial dispersion, shifting the processes controlling bacterial community composition to predominantly regional processes.

Cyanobacterial communities' variation was predominantly influenced by drift. This is not surprising, because if selection pressure is weak, drift tends to stand out in communities that are less diverse (Chase & Myers, 2011). All the four reservoir are eutrophic and sustains cyanobacterial blooms that occurs frequently throughout the entire year (Minillo, 2005). Likewise, if dispersion is limited, communities are more susceptible to effects of drift, which in turn yields higher turnover on communities (Hubbell, 2001). Blooms observed in these systems tend to be dominated by very few species (Minillo, 2005) and compared to FL and PA, cyanobacterial communities were accordingly much less diverse with only five dominant genera occurring (*Microcystis*, *Planktothrix*, *Cylindrospermopsis*, *Anabaena* and *Synechococcus*).

Variation in particle-attached communities was mainly under regional processes and likely dependent on the ability of the particles to be transported along the cascade. Particle-attached bacteria associate with several types of particles, such as cyanobacterial cells, flocculent aggregates, dissolved and particulate organic matter, clay particles, with size and transport characteristics that may vary widely (e.g. lateral, sinking). The supply of particles available for colonization may also vary, possibly dictating seasonal changes related to suspended matter in the water column (Berger *et al.*, 1996).

Compared with particle-attached fraction, free-living bacteria are usually smaller (Pedrós-Alió & Brock, 1982) and much more abundant (Kirchman & Mitchell, 1982), making the free-living subcommunity more readily dispersed. However, variation in free-living community had greater influence of selection, indicating that this community was more sensitive to environmental pressure. Since variables linked to the trophic state had major role at the environmental spatial structure observed, we believe that DOC could be a major factor promoting selection due to the following scenario: the system is highly eutrophic with a trophic state gradient, which leads to the primary production decrease downstream, (Table 1 and Fig 2). Bacterial communities profiting from labile autochthonous DOC in upstream reservoirs would leave behind a more recalcitrant organic carbon pool. These bacterial communities will then be transported to the next, more nutrient poor reservoir, where consequently, labile DOC supply from internal production would be lower.

According to the priming effect, the budget of labile DOC is important for consumption of recalcitrant DOC (Fontaine, Mariotti & Abbadie, 2003). In co-metabolism mechanism, bacteria obtain energy from labile DOC to produce extracellular enzymes necessary for decompose recalcitrant DOC (Guenet *et al.*, 2010). Because particle-attached was not often under selection as free-living community, this suggests that recalcitrant DOC decomposer from PA community could be readily profiting from the low concentration of labile DOC, released by association with phytoplankton (Kent *et al.*, 2007), and able to decomposed recalcitrant DOC. On the other hand, free-living bacteria, which feeds mainly on DOC in the medium, would be under limitation for labile DOC. In fact, Freitas et al. (2018) showed that at this system, for the same period sampled, labile DOC decreased while bacterial respiration increased towards the last reservoir, supporting a scenario where bacterial community growth was limited by DOC

availability. Likewise, selection processes observed during the rainy period could be attributed to the input of allochthonous matter carried into the system, which is considered highly recalcitrant (Thorp & Delong, 2002).

Fractioning microbial community into subcommunities revealed that distinct dispersion patterns are governing the whole community. Processes found here could be linked to features such as: *diversity*, as cyanobacteria were less rich than the other sub-communities; *life style*, as the free-living community responded more directly to environmental conditions, such as DOC availability; and *spatial dynamic*, as communities attached to particles community are more susceptible to transport limitations characteristics of particles.

It is evident that the species pools within the reservoirs are under the influence from the previous reservoir species pool along the cascade, largely because of the unidirectional water flow. The rainy period may also enhance the direct influence of species inflow on the local communities (Lindström & Bergström, 2005; Lindström *et al.*, 2006). These introduced organisms become part of sub-communities and may comprise distinct terrestrial *taxa* of those often found in aquatic environment.

However, a point that must be considered is that the method used here does not provide in which reservoir selection actually occurred, but between reservoirs. This means that communities migrating from one reservoir to the next might have been selected at any one of these two reservoirs and not necessarily at the one they are heading to. Thus, the insertion of allochthonous OTUs at one reservoir might result in selection in relation to the previous, because they were absent there. At the last sampling, R2, this introduction of alien material would be homogenized along the cascade by the intense water flux, and the community composition, as seen in the free-living subcommunity, was mainly driven by spatial distance.

Besides the implied role of DOC as a driver for selection, also other environmental variables, such as pH, luminosity and temperature can be important factors influencing microbial community composition (Dziallas and Grossart, 2011; Lindstrom et al., 2005; Lindström and Bergström, 2005; Newton et al., 2011). In our study, these variables did not seem to play major roles in shaping metacommunity dynamics, possibly due to the strong influence from regional factors guiding community turnover. Nonetheless, other environmental variables not measured in our study and also trophic interactions such as grazing and viral lysis, may also have had an influence on bacterial composition (Verreydt *et al.*, 2012; Declerck *et al.*, 2013).

Regional processes as main driver of microbial communities variation have been reported before, (Finlay, 2002; Fenchel & Finlay, 2004; Zhou *et al.*, 2013; Nemergut *et al.*, 2013), however, in some occasions, local factors may be subjected to regional factors. High rates of migration may insert organisms into environments where they are not well adapted, but persist simply due to their high abundances (Stegen *et al.*, 2013; Emily Graham & James Stegen, 2017). In other words, selection is overwhelmed by mass effects.

Microorganisms are small, abundant and rapidly reproducing and are hence unlikely to be under severe dispersal limitation (Bie *et al.*, 2012; Farjalla *et al.*, 2012). Previous studies have accordingly found that high dispersion may significantly influence microbial communities structure (Chase, 2010b; Ofiteru *et al.*, 2010; Langenheder & Székely, 2011; Bahram *et al.*, 2016). Furthermore, bacteria possess several important features that can be translated into their ability to cope with different environments and thus, overwhelming possible selection pressure. Some traits, such as metabolism and nutrition flexibility and tendency for dormancy (Jones & Lennon, 2010) dictates their resilience to adverse conditions and disturbances.

The analytical framework proposed by Stegen et al. (2013) allows to identify the main ecological process governing variation in microbial community composition, but this does not mean that distinct processes are not governing different groups within the same communities (Langenheder & Székely, 2011). More abundant bacteria may be under homogenizing dispersion, while less abundant one experiences constraints, such as physical barriers, grazing and environmental pressure, thus being under dispersal limitation. As observed by Nemergut *et al.* (2013), regional processes predominate across variable spatial scale, despite effects of local factors are more drastic for microbial communities' composition. Local (Logue & Lindström, 2010; Lindström *et al.*, 2010), regional (Lindström *et al.*, 2006) and the synergy of both factors (Langenheder & Székely, 2011) are important in controlling bacterial community structure, even if regional factors exerted a stronger influence on the three subcommunities studied here.

Dispersion rates, generation times, potential of colonization are also important traits determining turnover patterns and also varies across bacterial taxa and this deserves attention in future studies.

Conclusion

In this work we provided an overview of how local (selection), regional (dispersion: homogenizing and limiting) and stochastic (drift) factors are inducing patterns of species turnover within bacterial metacommunities along a contiguous ecosystem where these factors are acting synergistically. Each subcommunity (Particle-attached, Free-living and Cyanobacteria) was under distinct processes at the same time, as result of subcommunities features such as: *diversity*, as cyanobacterial community was mostly influenced by drift, likely because high nutrient availability allowed few species to thrive in all four reservoirs; *size* and *spatial dynamic*, as particle-attached seemed to be

submitted to particles characteristics of transport limitations, thus, mainly under regional process; and *life style*, as free-living bacteria were more susceptible to labile DOC limitation (local factor) and responded more strongly to selection. Overall, regional processes were the main driver of all three subcommunities turnover along the cascade.

Acknowledgments

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SM Table 2. 1 - Table generated with the framework used to access ecological processes guiding microbial communities. 'βNTI' gives communities that were under (S) Selection (|βNTI|>2) 'Raup-Crick' gives the regional processes and drift governing communities variation that were not under selection at βNTI step. Ecological processes: (HD) Homogenizing dispersion (RCbray < -0.95). (DL) Dispersal limitation (RCbray >-0.95) and (D) Drift (|RCbray| >0.95). Ecological processes were prospected from the table in the order reservoirs are on the cascade: (BB) Barra Bonita, (Pr) Promissão, (NA) Nova Avanhandava and (TI) Três Irmãos. Subcommunities: (PA) Particle-attached, (FL) Free-living and (Cyano) Cyanobacteria. Sampling names: D1-May/2015. D2-July/2015. R1- January/2016 and R2- March/2016.

			βNTI				**Raup-Crick**				**Ecological Process**		
	D1		**BB**	**Pr**	**NA**		**BB**	**Pr**	**NA**		**BB**	**Pr**	**NA**
		B				**BB**	0	-0.84	-0.3	**BB**			
		Pr	-1.05			**Pr**	-0.84	0	-0.57	**Pr**	**D**		
		NA	-0.69	-0.62		**NA**	-0.3	-0.57	0	**NA**		**D**	
		TI	-0.57	2.7	-0.02	**TI**	0.71	S	0.15	**TI**			**D**
	D2		**BB**	**Pr**	**NA**		**BB**	**Pr**	**NA**		**BB**	**Pr**	**NA**
		BB				**BB**	0	-0.4	-0.2	**BB**			
		Pr	0.8			**Pr**	-0.4	0	-0.34	**Pr**	**D**		
		NA	-0.51	-1.13		**NA**	-0.2	-0.34	0	**NA**		**D**	
Cyano		**TI**	-0.31	-0.66	1.46	**TI**	0.97	0.3	0.64	**TI**			**D**
	R1		**BB**	**Pr**	**NA**		**BB**	**Pr**	**NA**		**BB**	**Pr**	**NA**
		BB				**BB**	0	-0.26	-0.08	**BB**			
		Pr	-1.46			**Pr**	-0.26	0	-1	**Pr**	**D**		
		NA	0.72	-2.49		**NA**	-0.08	**S**	0	**NA**		**S**	
		TI	0.36	-1.35	2.72	**TI**	0.95	-0.39	**S**	**TI**			**S**
	R2		**BB**	**Pr**	**NA**		**BB**	**Pr**	**NA**		**BB**	**Pr**	**NA**
		BB				**BB**	0	-0.95	0.39	**BB**			
		Pr	-1.21			**Pr**	-0.95	0	-0.03	**Pr**	**HD**		
		NA	-1.22	0.37		**NA**	0.39	-0.03	0	**NA**		**D**	
		TI	-1.08	0.33	-0.5	**TI**	-0.93	-1	0.42	**TI**			**D**

		βNTI				Raup-Crick				Ecological Process		
	D1		BB	Pr	NA		BB	Pr	NA		BB	Pr	NA
		B				BB	0	1	0.04	BB			
		Pr	-0.74			Pr	1	0	-1	Pr	DL		
		NA	-0.09	0.27		NA	0.04	-1	0	NA		HD	
		TI	-1.5	0.48	-0.78	TI	1	1	1	TI			DL
	D2		BB	Pr	NA		BB	Pr	NA		BB	Pr	NA
		BB				BB	0	0.2	-0.95	BB			
		Pr	-1.29			Pr	0.2	0	-0.98	Pr	D		
		NA	-0.16	3.14		NA	-0.95	S	0	NA		S	
		TI	-0.12	0.14	0.5	TI	0.99	1	1	TI			DL
PA	R1		BB	Pr	NA		BB	Pr	NA		BB	Pr	NA
		BB				B	0	1	1	BB			
		Pr	0.92			Pr	1	0	-1	Pr	DL		
		NA	1.59	1.51		NA	1	-1	0	NA		HD	
		TI	0.87	3.09	0.44	TI	1	S	-0.68	TI			D
	R2		BB	Pr	NA		BB	Pr	NA		BB	Pr	NA
		BB				BB	0	1	1	B			
		Pr	0.92			Pr	1	0	0.58	Pr	DL		
		NA	-0.12	-0.43		NA	1	0.58	0	NA		D	
		TI	0.29	-0.45	1.974	TI	0.86	0.95	1	TI			DL

		βNTI			Raup-Crick				Ecological Process			
	D1		BB	Pr	NA		BB	Pr	NA		BB	Pr	NA
		BB				BB	0	1	-0.95	BB			
		Pr	1.38			Pr	1	0	-1	Pr	DL		
		NA	0.44	0.48		NA	-0.95	-1	0	NA		HD	
		TI	0.72	0.35	0.87	TI	0.67	1	-0.99	TI			HD
	D2		BB	Pr	NA		BB	Pr	NA		BB	Pr	NA
		BB				BB	0	1	1	BB			
		Pr	3.43			Pr	S	0	-1	Pr	S		
		NA	-0.35	2.67		NA	1	S	0	N		S	
		TI	-2.02	1.96	-5.16	TI	-0.9	0	S	TI			S
FL	R1		BB	Pr	NA		BB	Pr	NA		BB	Pr	NA
		BB				BB	0	1	1	BB			
		Pr	-0.17			Pr	1	0	-1	Pr	DL		
		NA	0.82	2.11		NA	1	S	0	NA		S	
		TI	-1.07	5.54	5.26	TI	1	-0.9	S	TI			S
	R2		BB	Pr	NA		BB	Pr	NA		BB	Pr	NA
		BB				BB	0	1	0.53	BB			
		Pr	-1			Pr	1	0	-1	Pr	DL		
		NA	-0.53	-1.2	A	NA	0.53	-1	0	NA		HD	
		TI	-1	1.71	-1.43	TI	1	-1	-0.46	TI			D

CAPÍTULO 3 – EFFECTS OF TROPHIC GRADIENT ON MICROBIAL INTERACTION NETWORKS IN A TROPICAL CASCADE OF RESERVOIRS

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Abstract

Aquatic microbial communities compose complex networks of organisms interacting with the environment and among themselves. Many intricates interactions are established between phytoplankton-bacteria and many are specie-specific and, thus, bacterioplankton covary with planktonic organisms. High proliferation of phytoplankton (blooms) is a signature of eutrophication in water bodies caused by an increase in nutrients availability, and in manmade reservoirs, prevenient of waste dumped into the system. The reduction of phytoplankton diversity caused by eutrophication of a given system would reduce the diversity of bacteria attached to Phytoplankton. In this study, we use network analyses to explore the effects of eutrophication on interactions of three components (particle-attached bacteria, cyanobacteria and eukaryotic phytoplankton) of the aquatic microbial food web from a cascade of reservoirs along a trophic state gradient. We found that the trophic state along the cascade drove phytoplanktonic diversity and influenced networks topologies, where in the most eutrophic reservoir, phytoplanktonic diversity was lower and the yielded network had less connections between organisms and higher subgroups isolated. In the less eutrophic reservoir, eukaryotic phytoplanktonic diversity and the yielded network was denser, with higher connectivity among organisms.

Keywords: Freshwater, microbial communities, network analyses, trophic gradient

Introduction

Aquatic microbial communities' make complex network of organisms interacting synergistically with their environment among themselves in intricate associations. These trophic interactions are mediated by several factors and it has been shown, that eutrophication has direct effects on microbial aquatic communities **(Cap. 1)** and imparts consequences to the ecosystem with effects on its structure and biogeochemical cycles (Azam et al. 1983).

High proliferation of phytoplankton (blooms) is a signature of eutrophication in water bodies caused by an increase in nutrients availability, and in manmade reservoirs, prevenient of waste dumped into the system (Tang et al. 2009). Phytoplanktonic blooms are often starred by few or only one phytoplankton species (Moura et al. 2013). Since many intricates interactions are established between phytoplankton-bacteria and many are specie-specific (Hold et al., 2001; Sapp et al., 2007; Sarmento et al., 2013; Schäfer et al., 2002; Schwenk et al., 2014), and that bacterioplankton covary with planktonic organisms (Kent et al. 2007; Maurice et al. 2010), the reduction of phytoplankton diversity caused by eutrophication of a given system would, consequently, reduce the diversity of bacteria attached to Phytoplankton. Thus, eutrophication effects on community's diversity could cascade through the trophic chain affecting the functionality of the system.

To explore how organisms are interacting in a given environment analysis of co-occurrence patterns provide a good overview of how these interactions are structured. Network analyses are representation of organisms (nodes) and the significant correlation (mutual exclusion or co-occurrence) linking nodes (Proulx, Promislow, e Phillips 2005). The analysis of networks relies on networks parameter such as the number of edges and nodes, the number of negative and positive interactions, if there are organisms exerting central role in the network, presence of subgroups within the network and how connected

is the network as a whole (Proulx et al. 2005). These metrics enables and simplify the evaluation of complex data of communities interacting and more, the comparison between communities interacting under distinct conditions. Bearing in mind that the greatest part of microbial communities is composed by non-identified or non-cultivable organisms, network analyses may be favor by the new sequencing technologies developed in the last years (e.g. Miseq, PacBio). Thus, efforts to evaluate which methods that better fit to these type of data and overcome some limitations of working with microbial communities, has given great contribution heading to new discoveries of microorganism's structure and function in a system (Layeghifard, Hwang, e Guttman 2017).

Here, we use network analyses to explore the effects of eutrophication on the interaction of three components (particle-attached bacteria, cyanobacteria and eukaryotic phytoplankton) of the aquatic microbial food web from a cascade of reservoirs along a trophic state gradient.

Material and Methods

Microorganisms studied were represented by particle-attached bacteria and cyanobacteria (> 3um), and by eukaryotic phytoplankton (> 3 um) (Cap. 1). We used all six sampling points to build networks of each reservoir

Networks construction and data analyses

To build the networks, we selected organisms from particle-attached bacteria, cyanobacteria and eukaryotic phytoplankton present at least in 80% of samples (four out of six) to better represent communities of each reservoir. We then normalized samples by the sum of OTUs counts in each sample. We included mean values of Chlorophyll-a Chla)

and dissolved organic carbon (DOC) from each reservoir as co-variables in the network analyses (Table 1.1). With the selected OTUs, Spearman and Pearson pairwise correlations were computed with a cut off for the correlation values higher than 0.7 ($p<0.05$). Because of this cut off value, the number of OTUs selected to compose the networks is not necessarily equal to the number of nodes integrated in the networks, as well as the mean values of Chla and DOC do not necessarily integrate the network as a node, if they had weak or not significant correlations.

In order to test for significance of the selected co-occurrences values, permutation (100x) runs were performed shuffling rows with organisms' frequencies values. This step generates a random matrix with *n* nodes (OTUs) and *e* edges (correlations weight). Using the permutation file generated at the previous step, we submitted these values to a bootstrap resampling method were edges weights from the permutation step were filtered out in order to keep only significant edges ($p<0.05$), resulting in the final network The final network representations are then built by a combination of the biotic components (nodes) and the type of interaction (edges: cooccurrence and mutual exclusion) between nodes. We also complemented our network analyses through its metrics concerning their topology. In this study, we used *network simple parameters*, obtained with Network Analyzer plugin (Cytoscape) (Table 3.1). Networks were built using the CoNet plugin developed for the platform Cytoscape (Faust e Raes 2016).

We identified the most influential OTUs in each network by selecting nodes which degrees values sum was accomplishing with 10% of total node degree sum from the network. We also explored interactions type from each network among particle-attached bacteria, cyanobacteria and eukaryotic phytoplankton. To do so, we calculated the frequency of mutual exclusion and cooccurrence occurring between and within each group.

Finally, to explore community's diversity composing networks, we calculated diversity index of Simpson (SIMPSON, 1949) and richness of particle-attached bacteria, Cyanobacteria and eukaryotic phytoplankton communities separately for each network built. Particle-attached bacteria was classified until the Class taxonomic level. When possible, Cyanobacteria was classified until Genera. Eukaryotic phytoplankton was classified until Order. Some organisms did not reach the taxonomic level previously defined, then they were classified as the previous taxonomic level available followed by "uncultured", for example: *Actinobacteria*_uncultured.

Table 3. 1 - Definition of Network metrics provided by the 'Simple Parameters' in Network Analyzer Plugin (Cytoscape).

Cluster coeficient (C)	Degree of connections of a node with its neighbours. When computed for the network, the average cluster coefficient gives the mean of all nodes present and predicts the possibility of modular organization of the network, or subnetworks (high values) (Barabási e Oltvai 2004; Watts e Strogatz 1998)
Average number of neighbours	The mean of nodes to which a node *n* is connected *
Average Path ength (APL)	Average of shortest path between two nodes (for all possible pairs in the network) (Watts e Strogatz 1998)
Diameter	Maximum length of the shortest path between two nodes. If a network is disconnected, its diameter is the maximum of all diameters of its connected components. (Watts e Strogatz 1998)
Radius	the minimum among the non-zero eccentricities of the nodes in the network. *
Network density	How dense the network is composed by edges. *
Network heterogeneity	Tendency of network containing hub nodes (Dong e Horvath 2007)
Network centralization	Indicates how uniformly nodes are connected at the network. Ranges from 0-1, where 1 means high centralization / uniformity. *
Node degree	Number of edges connecting the node *

*(NetworkAnalyzer Help, 2019)

Results

Network analyses

Selected OTUs for the network analyses (present in at least 80% of samples) presented different proportion of microbial subcommunities in each reservoir: Barra Bonita (BB) accounted with 147 OTUs of the particle-attached bacteria (PA), 7 of

cyanobacteria and 80 OTUs of eukaryotic phytoplankton; Promissão (Pr) accounted with 293 OTUs of particle-attached bacteria, 14 of cyanobacteria and 78 of the eukaryotic phytoplankton; Nova Avanhandava (NA) had 261 OTUs of the particle-attached bacteria, 14 of cyanobacteria and 92 of the eukaryotic phytoplankton and Três Irmãos (TI) had 289 OTUs of the particle-attached bacteria, 13 of cyanobacteria and 111 of the eukaryotic phytoplankton (SM Table 3.1).

Network parameters obtained with Network Analyzer plugin showed that the community from TI reservoir was the most diverse with 281 nodes (OTUs presenting correlations weight higher than 0.7) while BB network had the lowest richness, with 133 nodes (Table 3.2). BB network nodes were composed by 71 PA bacteria, 3 cyanobacteria, 57 eukaryotic phytoplankton; Pr network by 181 PA bacteria, 12 cyanobacteria, 63 eukaryotic phytoplankton; NA by 151 PA bacteria, 13 cyanobacteria, 68 eukaryotic phytoplankton and TI by 183 PA bacteria, 11 cyanobacteria, 84 eukaryotic phytoplankton. (SM Table 3.1). TI had the shortest average path length, with 2.2 edges, whereas Pr, the longest one, with 3.8 edges (Table 3.2). Network centralization value increased along the cascade, where BB was the least centralized network and TI the most centralized (Table 3.2). Values of diameter and radius did not follow a pattern along the cascade: BB network had the widest diameter, followed by NA, Pr and TI networks (Table 3.2). BB and NA had the lowest radius, followed by TI and Pr networks (Table 3.2).

Regarding the nature of interactions, in all networks positive interactions predominated. Community from Pr had the highest proportion of positive interactions, with 91% of co-occurrences. The lowest proportion of positive interactions was found at TI (69%) (Table 3.2). Cluster coefficient showed that BB had the highest chance to have a modular structure, since it presented the lowest value for this parameter, whereas TI

was the less modular structured network (Table 3.2). The graphical representation of BB network (Fig. 3.1a) shows a network composed by more disconnected subgroups, whereas TI had a denser and cohesive network (Fig. 3.1d).

Hub nodes, or nodes that have many connections to others, may increase the average number of neighbours. In turn, average number of neighbours, may also correlates with number of edges and nodes, since it is the mean of nodes to which a node *n* is connected. Variation in values of average number of neighbours was proportional to the number of edges for all networks studied. According to network heterogeneity, parameter that predicts the tendency of containing hub nodes, NA had the highest incidence of hub nodes (Table 3.2). Exploring nodes which the sum of degrees accounted with 10% of total node degrees, we observed that BB had the lowest number of OTUs with high node degree: 5 OTUs were classified as the most influent nodes, Pr 9 OTUs, NA 8 OTUs and TI 13 OTUs, respectively **(**SM Table 3.2).

Network density values showed that BB was the less dense network in term of the number of edges (Table 3.2). TI showed the highest value for network density (Table 3.2). Values of network centralization showed that TI network density was the most uniformly distributed, whereas BB, the less uniform (Table 3.2).

As TI had the shortest APL and diameter, and thus, this network was the most connected (Table 3.2). APL, diameter and radius values predicts the connectivity of a network. A network with a high value for APL means that nodes within the network are distant and that its necessary to pass by many nodes to reach a specific one. If a network has nodes with many connections, the path to connect any two nodes tends to be shorter.

Table 3. 2 – Network metrics obtained with Network Analyzer Simple Parameters (Cytoscape) and environmental variables which were integrated at network analyses by reservoir. Percentage of negative edges (Neg %), Custer coefficient (C), Average Path length (Avg PL), Average number of neighbours (Avg No of neig.), Network diameter, Network radius, Network centralization, Network density, network heterogeneity. Environmental variable chlorophyll a (Chla) and dissolved organic carbon (DOC), when integrated at network analysis (cut off >0.7 of Spearman and Pearson correlation) are indicated by "x". Barra Bonita (BB), Promissão (Pr), Nova Avanhandava (NA) and Três Irmãos (TI).

	Nodes	Edges	Edges (+)	Edges (-)	Neg (%)	C	Avg PL	Avg Nº of neig	Net diam	Net radius	Net centr	Net density	Net heterog	DOC	Chla
BB	133	231	179	52	23	0.402	3.521	3.474	10	1	0.05	0.026	0.652	x	
Pr	260	1476	1340	136	9	0.532	3.850	9.992	8	5	0.101	0.039	0.647	x	x
NA	237	1040	893	147	14	0.504	3.628	8.059	9	1	0.115	0.034	0.762	x	x
TI	281	5230	3629	1600	31	0.553	2.281	37.217	4	3	0.244	0.133	0.662		

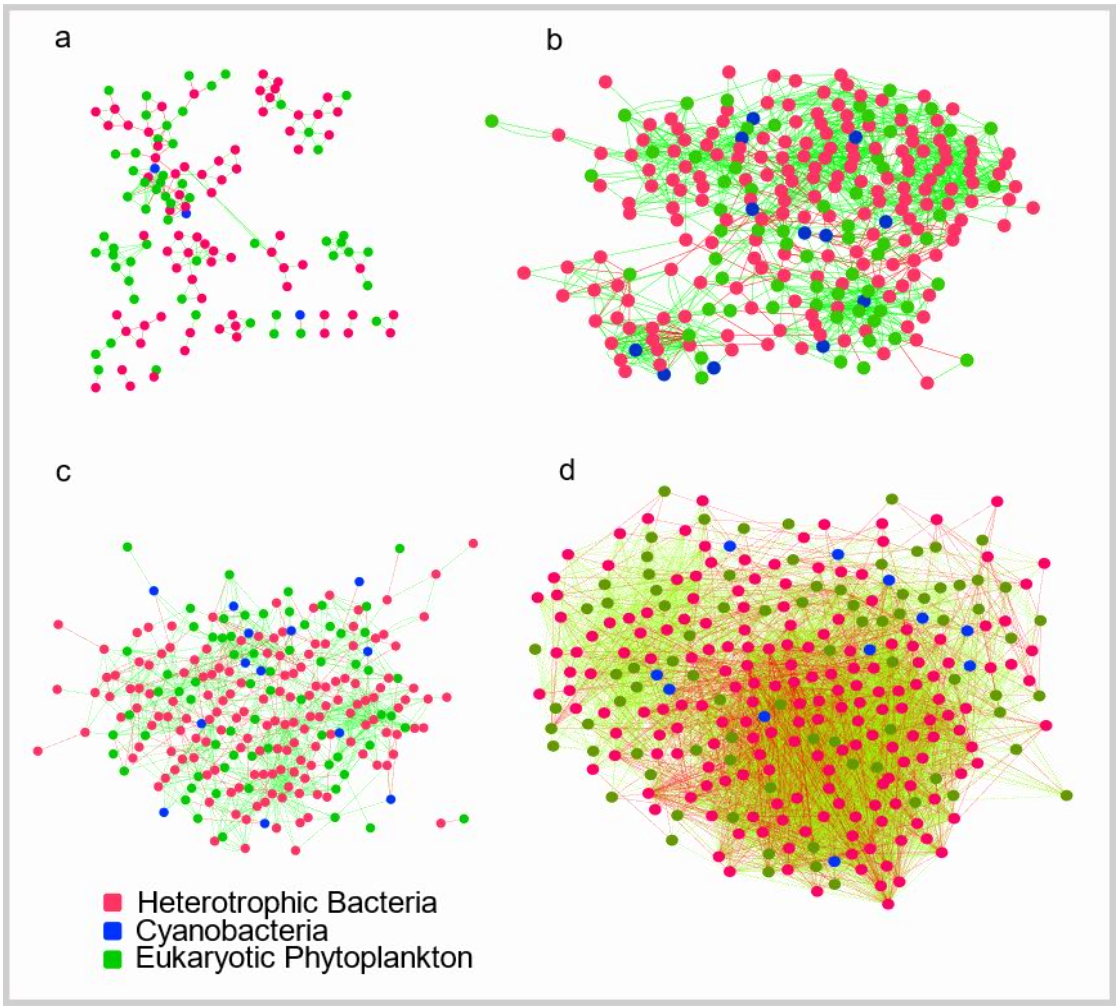

Figure 3. 1 - Networks built with OTUs present in at least four of six sampling points by reservoir. a) Network from Barra Bonita b) Network from Promissão c) Network from Nova Avanhandava and d) Network from Três Irmãos. Nodes are coloured in pink (Heterotrophic Bacteria), Blue (Cyanobacteria) and Green (Eukaryotic Phytoplankton). Green edges are cooccurrence and red edges are mutual exclusion (Spearman and Pearson correlations > 0.7)

The diameter predicts the connectivity of a network, since it is the maximum distance between connected nodes. Thus, a node which is too eccentric in a network will increase diameter. Consequently, radius values, as the minimum of eccentric nodes non-zero tend to be the lowest in network disconnected, due to the high number of eccentric nodes.

BB network had the wider value for diameter. In BB network, due to the presence of subgroups, the diameter increased and centralization was low, but APL value was not the highest, because inside sub-groups, shortest paths are small and it is accounted for the mean of this parameter. However, from diameter and radius values and considering the modular structure tendency (Table 3.2), we can say that BB network was the least connected. APL parameter may reflect in network centralization, since it depends on nodes connection. In a network where APL is long, we expect eccentricity of nodes at the network, while with a short APL we expect nodes to be easy to reach from any point at the network. Thus, TI network was the most centralized and BB the least centralized.

In all four networks, interactions between particle-associated bacteria predominated among cooccurrences and increased downstream reaching more than 50% of the interactions for the TI network (Fig. 3.2). Particle-attached bacteria also showed similar proportions of positive and negative interactions at TI (Fig. 3.2). The few interactions observed among cyanobacteria occurred at NA and TI, and they were always cooccurring (Fig. 3.2). Positive and negative interactions among eukaryotic phytoplankton showed similar rates in all four networks, however, the frequency of interactions within this group decreased downstream, along the eutrophication reduction (Fig. 3.2).

Mutual exclusion proportion between eukaryotic phytoplankton and heterotrophic bacteria was higher than co-occurrence at BB, Pr and NA. At TI, eukaryotic phytoplankton and heterotrophic bacteria showed similar proportion of mutual exclusion and cooccurrence (Fig. 3.2). Eukaryotic phytoplankton and cyanobacteria were under higher proportion of co-occurrence at BB and Pr than at NA and TI, where co-occurrence and mutual exclusion frequencies were more similar (Fig. 3.2). Finally, particle-associated bacteria and Cyanobacteria interactions had little contribution to the total

interactions observed at BB. Interactions between these two groups were observed at Pr and TI, with similar proportions of mutual exclusion and co-occurrence at NA, with mutual exclusion in higher proportion (Fig. 3.2).

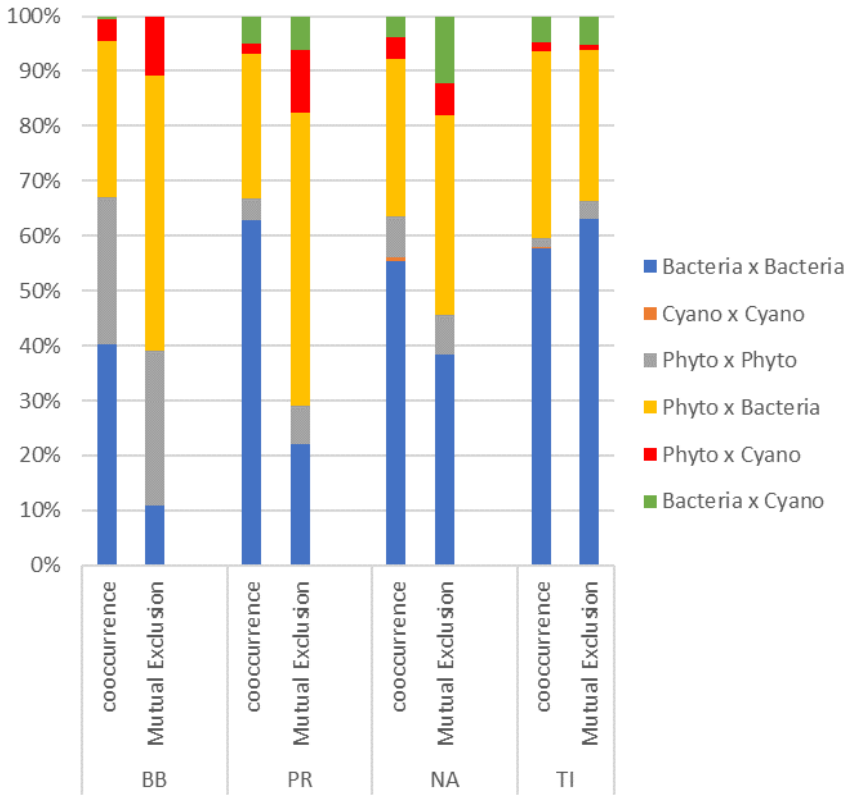

Figure 3. 2 – Contribution of cooccurrence and mutual exclusion between groups of organisms by reservoir. Cooccurrence and mutual exclusion were counted between all sub-communities: heterotrophic bacteria, Cyanobacteria and eukaryotic Phytoplankton.

Analysis of diversity values from the network's communities showed that BB had the lowest richness of particle-associated bacteria and the downstream reservoirs had higher and similar values of richness for this group (Fig. 3.3a). Cyanobacteria richness was the lowest at BB followed by TI, and higher, but similar at Pr and NA (Fig. 3.3b). Despite that the three sub-community's richness increased when trophic degree

decreased, Eukaryotic Phytoplankton richness did not variate along the trophic gradient (Fig. 3.3c).

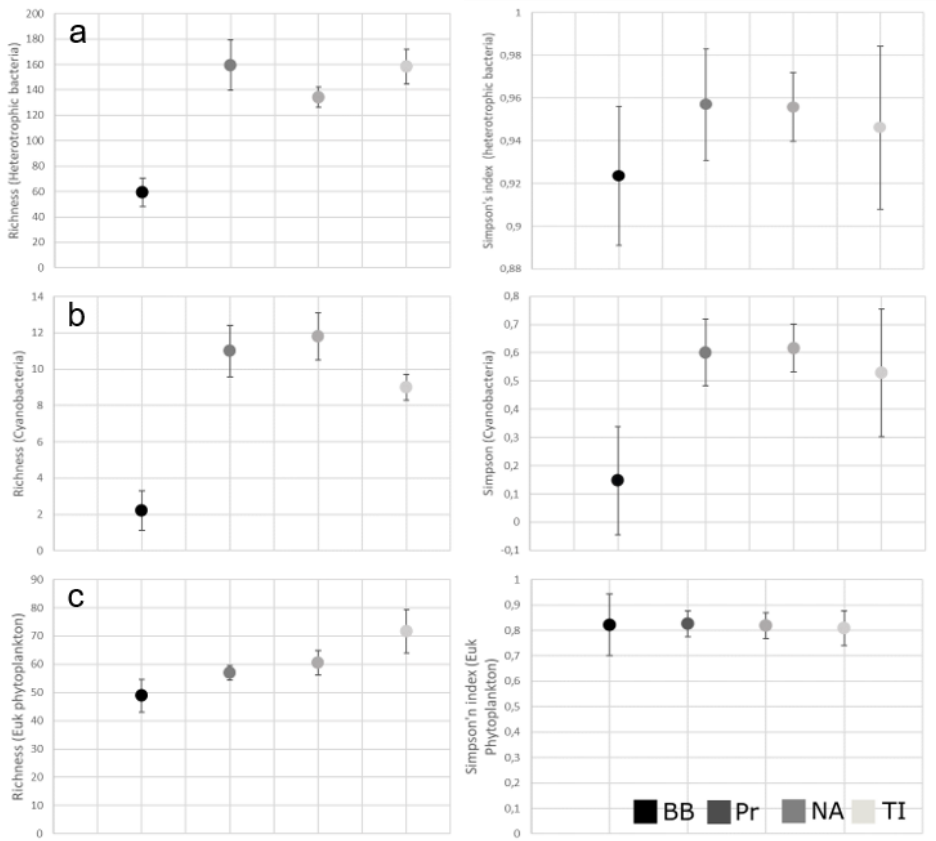

Figure 3. 3– Mean richness and index of Simpson by reservoir for (a) heterotrophic bacteria, (b) Cyanobacteria and (c) Eukaryotic Phytoplankton. Barra Bonita (BB), Promissão (PR), Nova Avanhandava (NA) and Três Irmãos (TI). Bars are standard deviation (n=6)

Discussion

Our network analyses (graphical representation and their parameters related) demonstrated changes in biotic interactions (co-occurrence and mutual exclusion) in particle-associated bacteria, cyanobacteria and eukaryotic phytoplankton along the trophic gradient for the four reservoirs in cascade. Some metrics obtained from the

networks, such as average path length and percentage of negative interactions, variated along the trophic gradient following the intermediate disturbance hypothesis (Wilkinson 1999), where extremes degree of trophy would act as strong disturbances leading changes to the microbial communities. According to the intermediate disturbance hypothesis, at these reservoirs, diversity would be reduced compared to values in the intermediate reservoirs (PR and NA). We indeed observed this trend at BB reservoir, which had the lowest diversity for all sub-communities analysed. However, the diversity pattern expected according to the hypothesis of intermediary disturbance was observed only for cyanobacteria that showed a decrease for diversity in TI. Moreover, diversity was calculated for organisms composing the networks, and not for the whole community from reservoirs.

The lowest cyanobacterial diversity at BB reservoir may be due to a cyanobacterial bloom, often with the dominance by one or few species of cyanobacteria, and frequent at this reservoir (Minillo 2005). This could also explain why Chla was not selected as a significant part of the network at BB and TI, but on Pr and NA network. Chlorophyll-a values were constantly high at BB and low at TI and it did not correlate with organism's abundance fluctuation at these networks. DOC was selected as a significant part of the network at BB, Pr and NA networks, but not at TI (Table 3.2). The decrease of DOC concentration along the cascade (Cap. 1, 2) could have led DOC in TI to be low enough to not be integrated in network from this reservoir. Moreover, DOC quality in this reservoir would be more important than quantity (Cap. 2).

As cyanobacterial bloom becomes the main source of DOC consumed by bacteria (Guillemette, McCallister, e del Giorgio 2013; Sadro, Nelson, e Melack 2011), this would favour heterotrophic bacterial lineages, which establish faster and more efficient associations with phytoplanktonic cells that grow and multiply faster, using resources

more rapidly. Bacteria that do not possess such features that enable a successful association, would have their abundance reduced by competition and as result, community goes through a bottleneck effect, reducing diversity of heterotrophic bacteria as observed in this study in BB reservoir (Fig. 3.3a).

The lower frequency of negative interactions observed at BB network among heterotrophic bacteria could be due to not only the lower diversity registered, but also to the age of reservoirs. Eutrophication is largely reported as a natural state of BB reservoir. In a stable environment, organisms are more likely to have limited physiological flexibility which means that these organisms are more sensitive to environmental changes (Balser, Kinzig, e Firestone 2002). Thus, in BB where eutrophication is known to be constant, interactions between heterotrophic bacteria would be well defined, with higher dominance of selected groups resulting in a network less diverse and more homogeneous, with lower frequency of mutual exclusion within heterotrophic bacteria. However, bacterial groups found in cyanobacterial blooms may also comprise phylogenetically close related species what could contribute to a greater frequency of positive correlations (Faust e Raes 2016). For example, Alpha, Beta and Gammaproteobacteria (Proteobacteria) show a metabolism with faster responses to nutrient pulses being frequent in association with phytoplankton. Because they can coexist, they are sharing the substrate, avoiding competition (Russo et al. 2016).

Mutual exclusion in BB was predominantly between particle-attached bacteria and eukaryotic phytoplankton and could be result of the predominance of Cryptophyceae, since many members of Cryptophyceae, such as *Cryptomonas* are able to scavenge carbon from ingestion of bacteria (Reynolds et al. 2002)**.** For the other reservoirs, as Cyanobacteria density decreases, nutrients become more available to eukaryotic phytoplankton and its diversity increased. This may have led to mixotrophic activity

loosening and the decrease of mutual exclusion frequency observed between heterotrophic bacteria and eukaryotic phytoplankton. Another possible explanation for the high frequency of mutual exclusion in BB between bacteria and eukaryotic phytoplankton is the opportunistic behaviour by some groups of bacteria associated. When the blooms events reach the senescent phase, these bacteria may produce algicidal components, inducing the lysis of phytoplanktonic cell in order to access their content (Seyedsayamdost et al. 2011).

The higher diversity of primary producers on the network observed downstream could recruit a more diverse bacteria group associated. Colonization of phytoplanktonic cell by bacteria seems to be driven be environmental factors (Grossart 1999), but also, host organisms may select bacteria attached (Hold et al. 2001; Schäfer et al. 2002). As distinct species of phytoplankton produces and releases a wide variety of organic compounds with distinct lability (Grossart e Simon 2007; Sarmento et al. 2013) this would reduce the selection for specific groups (Cyanobacteria) as source of DOC and promote higher diversity of bacteria attached (Fogg 1983; Paver et al. 2013; Sarmento e Gasol 2012).

TI network, where eukaryotic phytoplankton richness was higher, had the less modular structure, indicating that this network was highly connected with more intermediary links between different groups, provided by phytoplanktonic diversity (Fogg 1983; Paver et al. 2013; Sarmento e Gasol 2012). Bacterial taxa exert different ecological roles due to their variation in metabolism, and thus, bacterial composition will impart consequences to communities functioning (Bertilsson et al. 2007; Paver et al. 2013; Strickland et al. 2009). For example, Bacteroidetes, a group that was among the hub nodes at TI, are able to convert high into low weight molecules, which would be available for other groups (Tang et al. 2009). Comparing with BB network, where hub nodes were

forming subgroups, the subgroups at TI network, which were expected to be promoted by hub nodes, were not isolated, but highly connected across the network (Fig. 3.1). This suggests suggests that at TI reservoir, the transference of energy was mediated by different microbial groups. In fact, the average number of neighbours at TI was particularly high when compared to the other networks studied here (Table 3.2).

At TI, organisms would be competing for resources, as at this site, nutrient availability was lower. This was evident for Cyanobacteria, which relative abundance was the lowest at this reservoir (SM Fig. 3.1), suggesting that this group was under a more nutrient limited condition, since downstream, the uptake of nutrients by eukaryotic phytoplankton would increase (Straškraba 1990).

The use of ecological networks to study patterns in microbial community assembly still faces different challenges. First, working with the immense number of OTUs provided by new sequencing technologies may skew network interpretation. Second, many OTUs share similar ecological roles and may be competing at a given environment, while phylogenetically close OTUs may result in positive correlations. Edges linking nodes may not imply in exclusion or co-occurrence, as two nodes may be equally responding to an external factor or another organism (Faust e Raes 2016). However, in this work we were able to observe that trophic state of reservoirs had significant effects on microbial communities' networks topologies. The better understanding of these ecological interactions among sub-communities would contribute to map how these organisms respond to environmental change.

Conclusions

Network analyses of microbial communities showed that the trophic state gradient had influence in communities' interactions. The trophic state gradient drove phytoplanktonic community diversity in the network, where the most eutrophic reservoir had lower diversity of eukaryotic phytoplankton, cyanobacteria and particle-attached bacteria. For instance, the decrease of the trophic state promoted higher diversity of microbial communities studied. The response of phytoplankton to eutrophication influenced bacterial diversity what could be translated in changes on ecosystem functions to these communities. Moreover, hypereutrophic environments would yield networks more susceptible to disturbance due to lower number of connections among highly connected nodes and due to higher modularity isolating those nodes. On the other hand, networks from less eutrophic reservoirs show higher connectivity between nodes and less modularity. Because of the high number of edges, disturbances that could cause the exclusion of a node would be more easily bypassed due to the numerous connections exerted by neighbours of the excluded node. This would diminish the impact of species lost (nodes) on these communities compared to communities from a hypereutrophic system.

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Supplementary Material

SM Table 3. 1 - OTUs selected for network analysis (presence at 80% of the samples) by reservoir.

		Barra Bonita	
OTU Id	**Phylum**	**Class**	**Order**
BOTU525	Acidobacteria	Subgroup6	Subgroup6
BOTU109	Actinobacteria	Acidimicrobiia	Acidimicrobiales
BOTU130	Actinobacteria	Actinobacteria	Corynebacteriales
BOTU15	Actinobacteria	Actinobacteria	Frankiales
BOTU2	Actinobacteria	Actinobacteria	Frankiales
BOTU20	Actinobacteria	Acidimicrobiia	Acidimicrobiales
BOTU28	Actinobacteria	Actinobacteria	Frankiales
BOTU5	Actinobacteria	Actinobacteria	Frankiales
BOTU506	Armatimonadetes	Armatimonadia	Armatimonadales
BOTU68	Armatimonadetes	Armatimonadia	Armatimonadales
FOTU11	Bacillariophyta	Bacillariophyta	Aulacoseirales
FOTU125	Bacillariophyta	Mediophyceae	Discostella
FOTU1707	Bacillariophyta	Mediophyceae	Stephanodiscales
FOTU2376	Bacillariophyta	Bacillariophyta	Aulacoseirales
FOTU26	Bacillariophyta	Bacillariophyceae	Bacillariales
FOTU32	Bacillariophyta	Mediophyceae	Stephanodiscales
FOTU577	Bacillariophyta	Bacillariophyta	Aulacoseirales
FOTU923	Bacillariophyta	Bacillariophyta	Aulacoseirales
BOTU170	Bacteroidetes	SM1A07	SM1A07
BOTU24	Bacteroidetes	Cytophagia	Cytophagales
BOTU408	Bacteroidetes	Flavobacteriia	Flavobacteriales
BOTU436	Bacteroidetes	SM1A07	SM1A07
BOTU64	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
BOTU643	Bacteroidetes	Flavobacteriia	Flavobacteriales
BOTU124	Chlorobi	Chlorobia	Chlorobiales
BOTU26	Chlorobi	Chlorobia	Chlorobiales
FOTU1142	Chlorophyta	Trebouxiophyceae	Chlorellales
FOTU1212	Chlorophyta	Chlorophyceae	Chlorophyceae
FOTU1292	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU133	Chlorophyta	Chlorophyceae	Chlorophyceae
FOTU159	Chlorophyta	Chlorophyceae	Chlorophyceae
FOTU1637	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU17	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU293	Chlorophyta	Chlorophyceae	Chorococcales
FOTU322	Chlorophyta	Chlorophyceae	Sphaeropleales
FOTU325	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU35	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU412	Chlorophyta	Chlorophyceae	Chlamydomonadales

FOTU454	Chlorophyta	Trebouxiophyceae	Chlorellales
FOTU70	Chlorophyta	Chlorophyceae	Chlorococcales
FOTU71	Chlorophyta	Chlorophyceae	Sphaeropleales
FOTU74	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU779	Chlorophyta	Chlorophyceae	Sphaeropleales
FOTU783	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU86	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU12	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU1567	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU157	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU1672	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU178	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU181	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU22	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU29	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU3	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU31	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU4	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU44	Cryptophyta	Cryptophyceae	P131
FOTU50	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU52	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU616	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU809	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU963	Cryptophyta	Cryptophyceae	Cryptomonadales
COTU131	Cyanobacteria	Synechococcus	Synechococcus
COTU19	Cyanobacteria	Cyanobacteria	SubsectionIII
COTU8	Cyanobacteria	Planktothrix	Planktothrix
FOTU1127	Dinophyta	Dinophyceae	Gonyaulacales
FOTU164	Dinophyta	Dinophyceae	Suessiales
FOTU1823	Dinophyta	Dinophyceae	Gonyaulacales
FOTU20	Dinophyta	Dinophyceae	Gonyaulacales
FOTU2046	Dinophyta	Dinophyceae	Gonyaulacales
FOTU2284	Dinophyta	Dinophyceae	Gonyaulacales
FOTU24	Dinophyta	Dinophyceae	Suessiales
BOTU199	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales
BOTU244	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales
FOTU114	Heterokontophyta	Chrysophyceae	Ochromonadales
FOTU242	Heterokontophyta	Chrysophyceae	Ochromonadales
FOTU260	Heterokontophyta	Chrysophyceae	Synurales
FOTU316	Heterokontophyta	Eustigmatophyceae	Eustigmatales
FOTU701	Heterokontophyta	Eustigmatophyceae	Eustigmatales
FOTU829	Heterokontophyta	Chrysophyceae	Synurales
BOTU102	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU113	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU13	Planctomycetes	Phycisphaerae	Phycisphaerales
BOTU143	Planctomycetes	OM190	OM190

BOTU16	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU161	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU17	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU21	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU22	Planctomycetes	Phycisphaerae	Phycisphaerales
BOTU23	Planctomycetes	Phycisphaerae	Phycisphaerales
BOTU243	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU30	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU4	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU40	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU50	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU53	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU57	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU58	Planctomycetes	OM190	OM190
BOTU72	Planctomycetes	OM190	OM190
BOTU75	Planctomycetes	OM190	OM190
BOTU878	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU114	Proteobacteria	Betaproteobacteria	Nitrosomonadales
BOTU118	Proteobacteria	Alphaproteobacteria	Rhodobacterales
BOTU1246	Proteobacteria	Alphaproteobacteria	Rhizobiales
BOTU145	Proteobacteria	Betaproteobacteria	Burkholderiales
BOTU150	Proteobacteria	Gammaproteobacteria	Xanthomonadales
BOTU154	Proteobacteria	Alphaproteobacteria	Caulobacterales
BOTU155	Proteobacteria	Betaproteobacteria	Nitrosomonadales
BOTU157	Proteobacteria	Gammaproteobacteria	Aeromonadales
BOTU159	Proteobacteria	Alphaproteobacteria	Rhizobiales
BOTU169	Proteobacteria	Deltaproteobacteria	Desulfurellales
BOTU173	Proteobacteria	Betaproteobacteria	Burkholderiales
BOTU174	Proteobacteria	Alphaproteobacteria	Caulobacterales
BOTU214	Proteobacteria	Betaproteobacteria	Burkholderiales
BOTU224	Proteobacteria	Deltaproteobacteria	Oligoflexales
BOTU229	Proteobacteria	Gammaproteobacteria	Xanthomonadales
BOTU240	Proteobacteria	Alphaproteobacteria	Rhodobacterales
BOTU245	Proteobacteria	Alphaproteobacteria	Rhizobiales
BOTU343	Proteobacteria	Betaproteobacteria	Burkholderiales
BOTU42	Proteobacteria	Alphaproteobacteria	Rhodospirillales
BOTU424	Proteobacteria	Deltaproteobacteria	Oligoflexales
BOTU65	Proteobacteria	Alphaproteobacteria	Sphingomonadales
BOTU76	Proteobacteria	Betaproteobacteria	Burkholderiales
BOTU85	Proteobacteria	Betaproteobacteria	Burkholderiales
BOTU88	Proteobacteria	Alphaproteobacteria	Rhizobiales
BOTU90	Proteobacteria	Gammaproteobacteria	Xanthomonadales
BOTU93	Proteobacteria	Betaproteobacteria	Nitrosomonadales
BOTU98	Proteobacteria	Alphaproteobacteria	Caulobacterales
BOTU1257	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales
BOTU179	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales

BOTU92	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales

Promissão

OTU Id	Phylum	Class	Order
BOTU184	Acidobacteria	Blastocatellia	Blastocatellales
BOTU10	Actinobacteria	Acidimicrobiia	Acidimicrobiales
BOTU103	Actinobacteria	Acidimicrobiia	Acidimicrobiales
BOTU109	Actinobacteria	Acidimicrobiia	Acidimicrobiales
BOTU129	Actinobacteria	Actinobacteria	ActinobacteriaPeM15
BOTU15	Actinobacteria	Actinobacteria	Frankiales
BOTU163	Actinobacteria	Thermoleophilia	Solirubrobacterales
BOTU2	Actinobacteria	Actinobacteria	Frankiales
BOTU20	Actinobacteria	Acidimicrobiia	Acidimicrobiales
BOTU212	Actinobacteria	Acidimicrobiia	Acidimicrobiales
BOTU222	Actinobacteria	Thermoleophilia	Solirubrobacterales
BOTU223	Actinobacteria	Acidimicrobiia	Acidimicrobiales
BOTU28	Actinobacteria	Actinobacteria	Frankiales
BOTU293	Actinobacteria	Acidimicrobiia	Acidimicrobiales
BOTU31	Actinobacteria	Acidimicrobiia	Acidimicrobiales
BOTU33	Actinobacteria	Actinobacteria	Frankiales
BOTU35	Actinobacteria	Acidimicrobiia	Acidimicrobiales
BOTU38	Actinobacteria	Acidimicrobiia	Acidimicrobiales
BOTU5	Actinobacteria	Actinobacteria	Frankiales
BOTU59	Actinobacteria	Acidimicrobiia	Acidimicrobiales
BOTU7	Actinobacteria	Acidimicrobiia	Acidimicrobiales
BOTU84	Actinobacteria	Acidimicrobiia	Acidimicrobiales
BOTU68	Armatimonadetes	Armatimonadia	Armatimonadales
FOTU11	Bacillariophyta	Coscinodiscophyceae	Aulacoiseirales
FOTU125	Bacillariophyta	Mediophyceae	Discostella
FOTU2376	Bacillariophyta	Coscinodiscophyceae	Aulacoiseirales
FOTU26	Bacillariophyta	Bacillariophyceae	Bacillariales
FOTU32	Bacillariophyta	Mediophyceae	Stephanodiscales
FOTU6	Bacillariophyta	Bacillariophyceae	Fragilariales
FOTU923	Bacillariophyta	Coscinodiscophyceae	Aulacoseirales
BOTU135	Bacteroidetes	Flavobacteriia	Flavobacteriales
BOTU170	Bacteroidetes	SM1A07	uncultured
BOTU219	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
BOTU24	Bacteroidetes	Cytophagia	Cytophagales
BOTU262	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
BOTU271	Bacteroidetes	Cytophagia	Cytophagales
BOTU421	Bacteroidetes	Cytophagia	Cytophagales
BOTU436	Bacteroidetes	SM1A07	uncultured
BOTU438	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
BOTU469	Bacteroidetes	Flavobacteriia	Flavobacteriales
BOTU618	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
BOTU64	Bacteroidetes	Sphingobacteriia	Sphingobacteriales

BOTU643	Bacteroidetes	Flavobacteriia	Flavobacteriales
BOTU91	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
BOTU94	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
BOTU206	BRC1	uncultured	bacterium
FOTU163	Charophyta	Zygnematophyceae	Desmidiales
FOTU287	Charophyta	Zygnematophyceae	Desmidiales
FOTU327	Charophyta	Zygnematophyceae	Desmidiales
FOTU65	Charophyta	Zygnematophyceae	Desmidiales
BOTU177	Chlamydiae	Chlamydiae	Chlamydiales
BOTU464	Chlamydiae	Chlamydiae	Chlamydiales
BOTU80	Chlamydiae	Chlamydiae	Chlamydiales
BOTU124	Chlorobi	Chlorobia	Chlorobiales
BOTU246	Chlorobi	Chlorobia	Chlorobiales
BOTU26	Chlorobi	Chlorobia	Chlorobiales
BOTU283	Chlorobi	Chlorobia	Chlorobiales
BOTU350	Chlorobi	Chlorobia	Chlorobiales
BOTU36	Chlorobi	Chlorobia	Chlorobiales
BOTU167	Chloroflexi	Caldilineae	Caldilineales
BOTU318	Chloroflexi	Anaerolineae	Anaerolineales
BOTU564	Chloroflexi	Caldilineae	Caldilineales
BOTU74	Chloroflexi	Caldilineae	Caldilineales
BOTU89	Chloroflexi	Caldilineae	Caldilineales
FOTU1142	Chlorophyta	Trebouxiophyceae	Chlorellales
FOTU1292	Chlorophyta	Chlorophyceae	Chlamydomonadaceae
FOTU1485	Chlorophyta	Trebouxiophyceae	Trebouxiales
FOTU1588	Chlorophyta	Trebouxiophyceae	Chlorellales
FOTU159	Chlorophyta	Chlorophyceae	Chlorophyceae
FOTU1637	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU17	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU173	Chlorophyta	Chlorophyceae	Chlorophyceae
FOTU174	Chlorophyta	Trebouxiophyceae	Trebouxiales
FOTU310	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU322	Chlorophyta	Chlorophyceae	Sphaeropleales
FOTU35	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU371	Chlorophyta	Chlorophyceae	Chlorophyceae
FOTU412	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU46	Chlorophyta	Trebouxiophyceae	Chlorellales
FOTU70	Chlorophyta	Chlorophyceae	Chlorococcales
FOTU71	Chlorophyta	Chlorophyceae	Sphaeropleales
FOTU74	Chlorophyta	Chlorophyceae	Chlamydomonadaceae
FOTU85	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU86	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU406	Chrysophyceae	NA	NA
FOTU112	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU12	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU1567	Cryptophyta	Cryptophyceae	Cryptomonadales

FOTU1672	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU178	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU181	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU22	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU29	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU295	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU3	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU31	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU4	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU44	Cryptophyta	Cryptophyceae	CryptophyceaeP131
FOTU52	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU647	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU809	Cryptophyta	Cryptophyceae	Cryptomonadales
COTU1	Cyanobacteria	Microcystis	Microcystis
COTU121	Cyanobacteria	Snowella	Snowella
COTU131	Cyanobacteria	Synechococcus	Synechococcus
COTU14	Cyanobacteria	Synechococcus	Synechococcus
COTU19	Cyanobacteria	Cyanobacteria	Cyanobacteria
COTU237	Cyanobacteria	Melainabacteria	Caenarcaniphilales
COTU25	Cyanobacteria	Anabaena	Anabaena
COTU362	Cyanobacteria	Phormidium	Phormidium
COTU41	Cyanobacteria	Synechococcus	Synechococcus
COTU63	Cyanobacteria	Limnothrix	Limnothrix
COTU8	Cyanobacteria	Planktothrix	Planktothrix
COTU9	Cyanobacteria	Cylindrospermosis	Cylindrospermosis
FOTU164	Dinophyta	Dinophyceae	Gymnodiniphycidae
FOTU1758	Dinophyta	Dinophyceae	Peridiniphycidae
FOTU20	Dinophyta	Dinophyceae	Gonyaulacales
FOTU2284	Dinophyta	Dinophyceae	Gonyaulacales
FOTU24	Dinophyta	Dinophyceae	Gymnodiniphycidae
FOTU338	Dinophyta	Dinophyceae	Gymnodiniphycidae
FOTU98	Dinophyta	Dinophyceae	Peridiniphycidae
BOTU291	Firmicutes	Clostridia	Halanaerobiales
BOTU199	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales
BOTU244	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales
FOTU114	Heterokontophyta	Chrysophyceae	Ochromonadales
FOTU1501	Heterokontophyta	Chrysophyceae	Ochromonadales
FOTU233	Heterokontophyta	Chrysophyceae	Ochromonadales
FOTU242	Heterokontophyta	Chrysophyceae	Ochromonadales
FOTU328	Heterokontophyta	Chrysophyceae	Ochromonadales
FOTU376	Heterokontophyta	Chrysophyceae	Chrysophyceae
FOTU425	Heterokontophyta	Chrysophyceae	ChrysophyceaeP3445
FOTU603	Heterokontophyta	Chrysophyceae	Ochromonadales
BOTU253	Hydrogenedentes	uncultured	bacterium
BOTU208	Nitrospirae	Nitrospira	Nitrospirales
BOTU811	Nitrospirae	Nitrospira	Nitrospirales

BOTU102	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU104	Planctomycetes	Phycisphaerae	Phycisphaerales
BOTU111	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU113	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU115	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU116	Planctomycetes	Phycisphaerae	Phycisphaerales
BOTU12	Planctomycetes	Phycisphaerae	Phycisphaerales
BOTU13	Planctomycetes	Phycisphaerae	Phycisphaerales
BOTU132	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU136	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU143	Planctomycetes	OM190	uncultured
BOTU144	Planctomycetes	OM190	uncultured
BOTU16	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU161	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU164	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU165	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU17	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU175	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU180	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU194	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU198	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU202	Planctomycetes	OM190	uncultured
BOTU21	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU22	Planctomycetes	Phycisphaerae	Phycisphaerales
BOTU23	Planctomycetes	Phycisphaerae	Phycisphaerales
BOTU239	Planctomycetes	Phycisphaerae	Phycisphaerales
BOTU243	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU259	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU264	Planctomycetes	Phycisphaerae	Phycisphaerales
BOTU273	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU279	Planctomycetes	Phycisphaerae	Phycisphaerales
BOTU29	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU30	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU32	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU360	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU4	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU40	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU403	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU409	Planctomycetes	Phycisphaerae	Phycisphaerales
BOTU434	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU49	Planctomycetes	Phycisphaerae	Phycisphaerales
BOTU50	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU52	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU53	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU56	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU57	Planctomycetes	Planctomycetacia	Planctomycetales

BOTU58	Planctomycetes	OM190	uncultured
BOTU6	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU628	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU66	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU69	Planctomycetes	Phycisphaerae	CPla3
BOTU70	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU71	Planctomycetes	OM190	uncultured
BOTU72	Planctomycetes	OM190	uncultured
BOTU75	Planctomycetes	OM190	uncultured
BOTU878	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU110	Proteobacteria	Alphaproteobacteria	Caulobacterales
BOTU114	Proteobacteria	Betaproteobacteria	Nitrosomonadales
BOTU118	Proteobacteria	Alphaproteobacteria	Rhodobacterales
BOTU1233	Proteobacteria	Deltaproteobacteria	Myxococcales
BOTU142	Proteobacteria	Alphaproteobacteria	Rickettsiales
BOTU145	Proteobacteria	Betaproteobacteria	Burkholderiales
BOTU150	Proteobacteria	Gammaproteobacteria	Xanthomonadales
BOTU154	Proteobacteria	Alphaproteobacteria	Caulobacterales
BOTU155	Proteobacteria	Betaproteobacteria	Nitrosomonadales
BOTU157	Proteobacteria	Gammaproteobacteria	Aeromonadales
BOTU159	Proteobacteria	Alphaproteobacteria	Rhizobiales
BOTU160	Proteobacteria	Alphaproteobacteria	Rhodospirillales
BOTU174	Proteobacteria	Alphaproteobacteria	Caulobacterales
BOTU1753	Proteobacteria	Betaproteobacteria	Burkholderiales
BOTU18	Proteobacteria	Betaproteobacteria	Burkholderiales
BOTU195	Proteobacteria	Betaproteobacteria	Burkholderiales
BOTU197	Proteobacteria	Betaproteobacteria	Burkholderiales
BOTU203	Proteobacteria	Alphaproteobacteria	Rhodospirillales
BOTU210	Proteobacteria	Gammaproteobacteria	Xanthomonadales
BOTU211	Proteobacteria	Alphaproteobacteria	Rhodospirillales
BOTU214	Proteobacteria	Betaproteobacteria	Burkholderiales
BOTU215	Proteobacteria	Deltaproteobacteria	Myxococcales
BOTU240	Proteobacteria	Alphaproteobacteria	Rhodobacterales
BOTU241	Proteobacteria	Gammaproteobacteria	Cellvibrionales
BOTU245	Proteobacteria	Alphaproteobacteria	Rhizobiales
BOTU285	Proteobacteria	Alphaproteobacteria	Sphingomonadales
BOTU321	Proteobacteria	Betaproteobacteria	Nitrosomonadales
BOTU323	Proteobacteria	Alphaproteobacteria	Sphingomonadales
BOTU326	Proteobacteria	Betaproteobacteria	Burkholderiales
BOTU332	Proteobacteria	Deltaproteobacteria	Myxococcales
BOTU343	Proteobacteria	Betaproteobacteria	Burkholderiales
BOTU351	Proteobacteria	Gammaproteobacteria	Xanthomonadales
BOTU365	Proteobacteria	Alphaproteobacteria	Sphingomonadales
BOTU373	Proteobacteria	Deltaproteobacteria	Bdellovibrionales
BOTU393	Proteobacteria	Deltaproteobacteria	Myxococcales
BOTU413	Proteobacteria	Gammaproteobacteria	Xanthomonadales

BOTU419	Proteobacteria	Betaproteobacteria	TRA320
BOTU42	Proteobacteria	Alphaproteobacteria	Rhodospirillales
BOTU424	Proteobacteria	Deltaproteobacteria	Oligoflexales
BOTU441	Proteobacteria	Gammaproteobacteria	Methylococcales
BOTU456	Proteobacteria	Alphaproteobacteria	Rhodospirillales
BOTU46	Proteobacteria	Betaproteobacteria	Burkholderiales
BOTU521	Proteobacteria	Alphaproteobacteria	Rhodospirillales
BOTU523	Proteobacteria	Alphaproteobacteria	Rickettsiales
BOTU524	Proteobacteria	Deltaproteobacteria	Myxococcales
BOTU568	Proteobacteria	Deltaproteobacteria	Myxococcales
BOTU581	Proteobacteria	Gammaproteobacteria	Methylococcales
BOTU60	Proteobacteria	Gammaproteobacteria	Xanthomonadales
BOTU601	Proteobacteria	Betaproteobacteria	Burkholderiales
BOTU65	Proteobacteria	Alphaproteobacteria	Sphingomonadales
BOTU689	Proteobacteria	Deltaproteobacteria	Bdellovibrionales
BOTU76	Proteobacteria	Betaproteobacteria	Burkholderiales
BOTU77	Proteobacteria	Alphaproteobacteria	SAR11
BOTU83	Proteobacteria	Betaproteobacteria	Burkholderiales
BOTU85	Proteobacteria	Betaproteobacteria	Burkholderiales
BOTU88	Proteobacteria	Alphaproteobacteria	Rhizobiales
BOTU90	Proteobacteria	Gammaproteobacteria	Xanthomonadales
BOTU93	Proteobacteria	Betaproteobacteria	Nitrosomonadales
BOTU98	Proteobacteria	Alphaproteobacteria	Caulobacterales
BOTU148	Saccharibacteria	uncultured	bacterium
BOTU11	Verrucomicrobia	Verrucomicrobia	Incertae
BOTU1257	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales
BOTU126	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales
BOTU27	Verrucomicrobia	Spartobacteria	Chthoniobacterales
BOTU3	Verrucomicrobia	Spartobacteria	Chthoniobacterales
BOTU417	Verrucomicrobia	OPB35	soil

Nova Avanhandava

OTU Id	**Phylum**	**Class**	**Order**
FOTU497	Chlorophyta	Trebouxiophyceae	Chlorellales
BOTU33	Actinobacteria	Actinobacteria	Frankiales
BOTU247	Proteobacteria	Alphaproteobacteria	Rhizobiales
BOTU130	Actinobacteria	Actinobacteria	Corynebacteriales
BOTU132	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU434	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU31	Actinobacteria	Acidimicrobiia	Acidimicrobiales
BOTU10	Actinobacteria	Acidimicrobiia	Acidimicrobiales
BOTU292	Proteobacteria	Gammaproteobacteria	Pseudomonadales
BOTU403	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU391	Proteobacteria	Alphaproteobacteria	Rhizobiales
BOTU264	Planctomycetes	Phycisphaerae	Phycisphaerales
BOTU165	Planctomycetes	Planctomycetacia	Planctomycetales

BOTU350	Chlorobi	Chlorobia	Chlorobiales
BOTU1246	Proteobacteria	Alphaproteobacteria	Rhizobiales
COTU81	Cyanobacteria	Cyanobacteria	Cyanobacteria
FOTU369	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU372	Chlorophyta	Chlorophyceae	Chlorophyceae
BOTU38	Actinobacteria	Acidimicrobiia	Acidimicrobiales
BOTU273	Planctomycetes	Planctomycetacia	Planctomycetales
FOTU233	Heterokontophyta	Chrysophyceae	Ochromonadales
BOTU240	Proteobacteria	Alphaproteobacteria	Rhodobacterales
BOTU135	Bacteroidetes	Flavobacteriia	Flavobacteriales
FOTU325	Chlorophyta	Chlorophyceae	Chlamydomonadales
COTU404	Cyanobacteria	Cyanobacteria	Cyanobacteria
BOTU76	Proteobacteria	Betaproteobacteria	Burkholderiales
BOTU274	Verrucomicrobia	OPB35	OPB35
BOTU239	Planctomycetes	Phycisphaerae	Phycisphaerales
BOTU23	Planctomycetes	Phycisphaerae	Phycisphaerales
BOTU12	Planctomycetes	Phycisphaerae	Phycisphaerales
FOTU412	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU1637	Chlorophyta	Chlorophyceae	Chlamydomonadales
BOTU605	Proteobacteria	Deltaproteobacteria	Myxococcales
BOTU235	Proteobacteria	Deltaproteobacteria	SAR324
BOTU18	Proteobacteria	Betaproteobacteria	Burkholderiales
FOTU652	Dinophyta	Dinophyceae	Peridiniphycidae
BOTU521	Proteobacteria	Alphaproteobacteria	Rhodospirillales
BOTU339	Planctomycetes	OM190	OM190
BOTU279	Planctomycetes	Phycisphaerae	Phycisphaerales
BOTU157	Proteobacteria	Gammaproteobacteria	Aeromonadales
BOTU150	Proteobacteria	Gammaproteobacteria	Xanthomonadales
FOTU29	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU178	Cryptophyta	Cryptophyceae	Cryptomonadales
COTU9	Cyanobacteria	Cylindrospermosis	Cylindrospermosis
BOTU74	Chloroflexi	Caldilineae	Caldilineales
BOTU628	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU506	Armatimonadetes	Armatimonadia	Armatimonadales
BOTU413	Proteobacteria	Gammaproteobacteria	Xanthomonadales
BOTU323	Proteobacteria	Alphaproteobacteria	Sphingomonadales
BOTU161	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU151	Proteobacteria	Alphaproteobacteria	Rhizobiales
BOTU118	Proteobacteria	Alphaproteobacteria	Rhodobacterales
BOTU116	Planctomycetes	Phycisphaerae	Phycisphaerales
FOTU52	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU24	Dinophyta	Dinophyceae	Suessiales
FOTU173	Chlorophyta	Chlorophyceae	Chlorophyceae
BOTU6	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU57	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU50	Planctomycetes	Planctomycetacia	Planctomycetales

BOTU4	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU393	Proteobacteria	Deltaproteobacteria	Myxococcales
BOTU259	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU245	Proteobacteria	Alphaproteobacteria	Rhizobiales
BOTU194	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU175	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU15	Actinobacteria	Actinobacteria	Frankiales
BOTU126	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales
BOTU111	Planctomycetes	Planctomycetacia	Planctomycetales
FOTU1212	Chlorophyta	Chlorophyceae	Chlorophyceae
COTU334	Cyanobacteria	Synechococcus	Synechococcus
BOTU66	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU409	Planctomycetes	Phycisphaerae	Phycisphaerales
BOTU30	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU3	Verrucomicrobia	Spartobacteria	Chthoniobacterales
BOTU293	Actinobacteria	Acidimicrobiia	Acidimicrobiales
BOTU271	Bacteroidetes	Cytophagia	Cytophagales
BOTU241	Proteobacteria	Gammaproteobacteria	Cellvibrionales
BOTU207	Actinobacteria	Acidimicrobiia	Acidimicrobiales
BOTU167	Chloroflexi	Caldilineae	Caldilineales
BOTU159	Proteobacteria	Alphaproteobacteria	Rhizobiales
BOTU145	Proteobacteria	Betaproteobacteria	Burkholderiales
BOTU114	Proteobacteria	Betaproteobacteria	Nitrosomonadales
FOTU647	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU637	Trebouxiophyceae	Oocystacea	uncult
FOTU485	Chlorophyta	Chlorophyceae	Sphaeropleales
FOTU295	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU286	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU1485	Chlorophyta	Trebouxiophyceae	Trebouxiales
BOTU90	Proteobacteria	Gammaproteobacteria	Xanthomonadales
BOTU59	Actinobacteria	Acidimicrobiia	Acidimicrobiales
BOTU564	Chloroflexi	Caldilineae	Caldilineales
BOTU42	Proteobacteria	Alphaproteobacteria	Rhodospirillales
BOTU24	Bacteroidetes	Cytophagia	Cytophagales
BOTU184	Acidobacteria	Blastocatellia	Blastocatellales
BOTU160	Proteobacteria	Alphaproteobacteria	Rhodospirillales
BOTU110	Proteobacteria	Alphaproteobacteria	Caulobacterales
BOTU103	Actinobacteria	Acidimicrobiia	Acidimicrobiales
FOTU86	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU85	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU164	Dinophyta	Dinophyceae	Gymnodiniphycidae
FOTU159	Chlorophyta	Chlorophyceae	Chlorophyceae
FOTU133	Chlorophyta	Chlorophyceae	Chlorophyceae
FOTU1142	Chlorophyta	Trebouxiophyceae	Chlorellales
COTU63	Cyanobacteria	Limnothrix	Limnothrix
BOTU93	Proteobacteria	Betaproteobacteria	Nitrosomonadales

BOTU878	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU54	Planctomycetes	OM190	OM190
BOTU253	Hydrogenedentes	Hydrogenedentes	Hydrogenedentes
BOTU215	Proteobacteria	Deltaproteobacteria	Myxococcales
BOTU212	Actinobacteria	Acidimicrobiia	Acidimicrobiales
BOTU211	Proteobacteria	Alphaproteobacteria	Rhodospirillales
BOTU20	Actinobacteria	Acidimicrobiia	Acidimicrobiales
BOTU196	Planctomycetes	Phycisphaerae	Phycisphaerales
FOTU70	Chlorophyta	Chlorophyceae	Chlorococcales
FOTU4	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU396	Bacillariophyta	Bacillariophyceae	Bacillariales
FOTU371	Chlorophyta	Chlorophyceae	Chlorophyceae
FOTU32	Bacillariophyta	Mediophyceae	Stephanodiscales
FOTU3	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU125	Bacillariophyta	Mediophyceae	Discostella
COTU41	Cyanobacteria	Synechococcus	Synechococcus
COTU237	Cyanobacteria	Melainabacteria	Caenarcaniphilales
COTU1	Cyanobacteria	Microcystis	Microcystis
BOTU75	Planctomycetes	OM190	OM190
BOTU32	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU203	Proteobacteria	Alphaproteobacteria	Rhodospirillales
BOTU199	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales
BOTU198	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU142	Proteobacteria	Alphaproteobacteria	Rickettsiales
BOTU13	Planctomycetes	Phycisphaerae	Phycisphaerales
BOTU129	Actinobacteria	Actinobacteria	ActinobacteriaPeM15
BOTU102	Planctomycetes	Planctomycetacia	Planctomycetales
FOTU71	Chlorophyta	Chlorophyceae	Sphaeropleales
FOTU65	Charophyta	Zygnematophyceae	Desmidiales
FOTU620	Dinophyta	Dinophyceae	Gymnodiniphycidae
FOTU455	Heterokontophyta	Eustigmatophyceae	Eustigmatales
FOTU338	Dinophyta	Dinophyceae	Gymnodiniphycidae
FOTU327	Charophyta	Zygnematophyceae	Desmidiales
FOTU269	Bacillariophyta	Bacillariophyceae	Fragilariales
FOTU2284	Dinophyta	Dinophyceae	Gonyaulacales
FOTU129	Chlorophyta	Chlorophyceae	Chlorophyceae
BOTU94	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
BOTU88	Proteobacteria	Alphaproteobacteria	Rhizobiales
BOTU70	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU643	Bacteroidetes	Flavobacteriia	Flavobacteriales
BOTU58	Planctomycetes	OM190	OM190
BOTU56	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU52	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU36	Chlorobi	Chlorobia	Chlorobiales
BOTU29	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU26	Chlorobi	Chlorobia	Chlorobiales

BOTU22	Planctomycetes	Phycisphaerae	Phycisphaerales
BOTU214	Proteobacteria	Betaproteobacteria	Burkholderiales
BOTU202	Planctomycetes	OM190	OM190
BOTU2	Actinobacteria	Actinobacteria	Frankiales
BOTU143	Planctomycetes	OM190	OM190
FOTU923	Bacillariophyta	Coscinodiscophyceae	Aulacoseirales
FOTU74	Chlorophyta	Chlorophyceae	Chlamydomonadaceae
FOTU35	Heterokontophyta	Chrysophyceae	Chrysophyceae
FOTU26	Bacillariophyta	Bacillariophyceae	Bacillariales
FOTU1567	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU114	Heterokontophyta	Chrysophyceae	Ochromonadales
COTU19	Cyanobacteria	Cyanobacteria	Cyanobacteria
BOTU87	Armatimonadetes	Armatimonadia	Armatimonadales
BOTU7	Actinobacteria	Acidimicrobiia	Acidimicrobiales
BOTU69	Planctomycetes	Phycisphaerae	CPla3
BOTU60	Proteobacteria	Gammaproteobacteria	Xanthomonadales
BOTU523	Proteobacteria	Alphaproteobacteria	Rickettsiales
BOTU5	Actinobacteria	Actinobacteria	Frankiales
BOTU17	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU16	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU136	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU1257	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales
BOTU124	Chlorobi	Chlorobia	Chlorobiales
BOTU123	Proteobacteria	Alphaproteobacteria	Rickettsiales
BOTU113	Planctomycetes	Planctomycetacia	Planctomycetales
FOTU6	Bacillariophyta	Bacillariophyceae	Fragilariales
FOTU44	Cryptophyta	Cryptophyceae	CryptophyceaeP131
FOTU310	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU31	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU264	Dinophyta	Dinophyceae	Dinophyceae
FOTU22	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU1672	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU12	Cryptophyta	Cryptophyceae	Cryptomonadales
COTU592	Cyanobacteria	Cyanobacteria	Cyanobacteria
COTU14	Cyanobacteria	Synechococcus	Synechococcus
COTU131	Cyanobacteria	Synechococcus	Synechococcus
BOTU89	Chloroflexi	Caldilineae	Caldilineales
BOTU83	Proteobacteria	Betaproteobacteria	Burkholderiales
BOTU72	Planctomycetes	OM190	OM190
BOTU64	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
BOTU524	Proteobacteria	Deltaproteobacteria	Myxococcales
BOTU421	Bacteroidetes	Cytophagia	Cytophagales
BOTU332	Proteobacteria	Deltaproteobacteria	Myxococcales
BOTU321	Proteobacteria	Betaproteobacteria	Nitrosomonadales
BOTU170	Bacteroidetes	SM1A07	SM1A07
BOTU154	Proteobacteria	Alphaproteobacteria	Caulobacterales

BOTU140	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
BOTU115	Planctomycetes	Planctomycetacia	Planctomycetales
FOTU98	Dinophyta	Dinophyceae	Peridiniphycidae
FOTU2376	Bacillariophyta	Coscinodiscophyceae	Aulacoseirales
FOTU181	Cryptophyta	Cryptophyceae	Cryptomonadales
BOTU85	Proteobacteria	Betaproteobacteria	Burkholderiales
BOTU84	Actinobacteria	Acidimicrobiia	Acidimicrobiales
BOTU77	Proteobacteria	Alphaproteobacteria	SAR11
BOTU285	Proteobacteria	Alphaproteobacteria	Sphingomonadales
BOTU27	Verrucomicrobia	Spartobacteria	Chthoniobacterales
BOTU21	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU206	BRC1	BRC2	BRC3
BOTU155	Proteobacteria	Betaproteobacteria	Nitrosomonadales
BOTU11	Verrucomicrobia	Verrucomicrobia	Verrucomicrobia
FOTU809	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU46	Chlorophyta	Trebouxiophyceae	Chlorellales
FOTU20	Dinophyta	Dinophyceae	Gonyaulacales
FOTU11	Bacillariophyta	Coscinodiscophyceae	Aulacoseirales
BOTU53	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU243	Planctomycetes	Planctomycetacia	Planctomycetales
BOTU173	Proteobacteria	Betaproteobacteria	Burkholderiales
BOTU169	Proteobacteria	Deltaproteobacteria	Desulfurellales
FOTU376	Dinophyta	Dinophyceae	Gymnodiniumclade
FOTU322	Chlorophyta	Chlorophyceae	Sphaeropleales
FOTU163	Charophyta	Zygnematophyceae	Desmidiales
FOTU1588	Chlorophyta	Trebouxiophyceae	Chlorellales
COTU8	Cyanobacteria	Planktothrix	Planktothrix
BOTU98	Proteobacteria	Alphaproteobacteria	Caulobacterales
BOTU71	Planctomycetes	OM190	OM190
BOTU424	Proteobacteria	Deltaproteobacteria	Oligoflexales
BOTU244	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales
BOTU210	Proteobacteria	Gammaproteobacteria	Xanthomonadales
BOTU148	Saccharibacteria	Saccharibacteria	Saccharibacteria
BOTU104	Planctomycetes	Phycisphaerae	Phycisphaerales

		Três Irmãos	
OTU Id	**Phylum**	**Class**	**Order**
BOTU10	Actinobacteria	Acidimicrobiia	Acidimicrobiia
BOTU101	Actinobacteria	Actinobacteria	Actinobacteria
BOTU102	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU103	Actinobacteria	Acidimicrobiia	Acidimicrobiia
BOTU104	Planctomycetes	Phycisphaerae	Phycisphaerae
BOTU108	Bacteroidetes	Flavobacteriia	Flavobacteriia
BOTU109	Actinobacteria	Acidimicrobiia	Acidimicrobiia
BOTU11	Verrucomicrobia	Verrucomicrobia	Verrucomicrobia
BOTU110	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria

BOTU111	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU112	Actinobacteria	Actinobacteria	Actinobacteria
BOTU113	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU114	Proteobacteria	Betaproteobacteria	Betaproteobacteria
BOTU116	Planctomycetes	Phycisphaerae	Phycisphaerae
BOTU118	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria
BOTU119	Bacteroidetes	Sphingobacteriia	Sphingobacteriia
BOTU12	Planctomycetes	Phycisphaerae	Phycisphaerae
BOTU123	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria
BOTU124	Chlorobi	Chlorobia	Chlorobia
BOTU128	Actinobacteria	Thermoleophilia	Thermoleophilia
BOTU13	Planctomycetes	Phycisphaerae	Phycisphaerae
BOTU130	Actinobacteria	Actinobacteria	Actinobacteria
BOTU135	Bacteroidetes	Flavobacteriia	Flavobacteriia
BOTU136	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU139	Bacteroidetes	Cytophagia	Cytophagia
BOTU142	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria
BOTU143	Planctomycetes	OM190	OM190
BOTU144	Planctomycetes	OM190	OM190
BOTU147	Actinobacteria	Actinobacteria	Actinobacteria
BOTU15	Actinobacteria	Actinobacteria	Actinobacteria
BOTU151	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria
BOTU154	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria
BOTU155	Proteobacteria	Betaproteobacteria	Betaproteobacteria
BOTU1568	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria
BOTU157	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria
BOTU158	Actinobacteria	Actinobacteria	Actinobacteria
BOTU159	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria
BOTU1595	Proteobacteria	Betaproteobacteria	Betaproteobacteria
BOTU16	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU160	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria
BOTU161	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU163	Actinobacteria	Thermoleophilia	Thermoleophilia
BOTU164	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU165	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU168	Bacteroidetes	Sphingobacteriia	Sphingobacteriia
BOTU169	Proteobacteria	Deltaproteobacteria	Deltaproteobacteria
BOTU17	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU170	Bacteroidetes	SM1A07	SM1A07
BOTU172	Bacteroidetes	Flavobacteriia	Flavobacteriia
BOTU173	Proteobacteria	Betaproteobacteria	Betaproteobacteria
BOTU174	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria
BOTU175	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU176	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadetes
BOTU177	Chlamydiae	Chlamydiae	Chlamydiae
BOTU18	Proteobacteria	Betaproteobacteria	Betaproteobacteria

BOTU180	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU1819	Actinobacteria	Acidimicrobiia	Acidimicrobiia
BOTU1893	Actinobacteria	Actinobacteria	Actinobacteria
BOTU192	Proteobacteria	Betaproteobacteria	Betaproteobacteria
BOTU194	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU195	Proteobacteria	Betaproteobacteria	Betaproteobacteria
BOTU196	Planctomycetes	Phycisphaerae	Phycisphaerae
BOTU197	Proteobacteria	Betaproteobacteria	Betaproteobacteria
BOTU198	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU199	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadetes
BOTU2	Actinobacteria	Actinobacteria	Actinobacteria
BOTU20	Actinobacteria	Acidimicrobiia	Acidimicrobiia
BOTU203	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria
BOTU205	Verrucomicrobia	OPB35	OPB35
BOTU207	Actinobacteria	Acidimicrobiia	Acidimicrobiia
BOTU2088	Acidobacteria	Subgroup6	Subgroup6
BOTU21	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU210	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria
BOTU212	Actinobacteria	Acidimicrobiia	Acidimicrobiia
BOTU214	Proteobacteria	Betaproteobacteria	Betaproteobacteria
BOTU215	Proteobacteria	Deltaproteobacteria	Deltaproteobacteria
BOTU218	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria
BOTU22	Planctomycetes	Phycisphaerae	Phycisphaerae
BOTU221	Bacteroidetes	Sphingobacteriia	Sphingobacteriia
BOTU225	Bacteroidetes	Flavobacteriia	Flavobacteriia
BOTU227	Bacteroidetes	Sphingobacteriia	Sphingobacteriia
BOTU23	Planctomycetes	Phycisphaerae	Phycisphaerae
BOTU233	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU239	Planctomycetes	Phycisphaerae	Phycisphaerae
BOTU24	Bacteroidetes	Cytophagia	Cytophagia
BOTU242	Bacteroidetes	Sphingobacteriia	Sphingobacteriia
BOTU244	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadetes
BOTU245	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria
BOTU247	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria
BOTU252	Planctomycetes	Phycisphaerae	Phycisphaerae
BOTU253	Hydrogenedentes	Hydrogenedentes	Hydrogenedentes
BOTU259	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU26	Chlorobi	Chlorobia	Chlorobia
BOTU264	Planctomycetes	Phycisphaerae	Phycisphaerae
BOTU267	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU271	Bacteroidetes	Cytophagia	Cytophagia
BOTU279	Planctomycetes	Phycisphaerae	Phycisphaerae
BOTU28	Actinobacteria	Actinobacteria	Actinobacteria
BOTU282	Bacteroidetes	Flavobacteriia	Flavobacteriia
BOTU288	Bacteroidetes	Flavobacteriia	Flavobacteriia
BOTU29	Planctomycetes	Planctomycetacia	Planctomycetacia

BOTU290	Actinobacteria	Acidimicrobiia	Acidimicrobiia
BOTU3	Verrucomicrobia	Spartobacteria	Spartobacteria
BOTU30	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU31	Actinobacteria	Acidimicrobiia	Acidimicrobiia
BOTU314	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU317	Actinobacteria	Acidimicrobiia	Acidimicrobiia
BOTU318	Chloroflexi	Anaerolineae	Anaerolineae
BOTU33	Actinobacteria	Actinobacteria	Actinobacteria
BOTU332	Proteobacteria	Deltaproteobacteria	Deltaproteobacteria
BOTU335	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU34	Bacteroidetes	Sphingobacteriia	Sphingobacteriia
BOTU35	Actinobacteria	Acidimicrobiia	Acidimicrobiia
BOTU354	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria
BOTU36	Chlorobi	Chlorobia	Chlorobia
BOTU360	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU363	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria
BOTU37	Bacteroidetes	Sphingobacteriia	Sphingobacteriia
BOTU38	Actinobacteria	Acidimicrobiia	Acidimicrobiia
BOTU391	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria
BOTU392	Proteobacteria	Deltaproteobacteria	Deltaproteobacteria
BOTU4	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU40	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU408	Bacteroidetes	Flavobacteriia	Flavobacteriia
BOTU412	Bacteroidetes	SM1A07	SM1A07
BOTU413	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria
BOTU42	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria
BOTU421	Bacteroidetes	Cytophagia	Cytophagia
BOTU44	Planctomycetes	Phycisphaerae	Phycisphaerae
BOTU45	Actinobacteria	Actinobacteria	Actinobacteria
BOTU455	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU46	Proteobacteria	Betaproteobacteria	Betaproteobacteria
BOTU461	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU47	Actinobacteria	Acidimicrobiia	Acidimicrobiia
BOTU48	Proteobacteria	Betaproteobacteria	Betaproteobacteria
BOTU49	Planctomycetes	Phycisphaerae	Phycisphaerae
BOTU5	Actinobacteria	Actinobacteria	Actinobacteria
BOTU50	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU506	Armatimonadetes	Armatimonadia	Armatimonadia
BOTU52	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU53	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU54	Planctomycetes	OM190	OM190
BOTU56	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU57	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU58	Planctomycetes	OM190	OM190
BOTU59	Actinobacteria	Acidimicrobiia	Acidimicrobiia
BOTU597	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria

BOTU6	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU60	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria
BOTU601	Proteobacteria	Betaproteobacteria	Betaproteobacteria
BOTU62	Verrucomicrobia	OPB35	OPB35
BOTU628	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU64	Bacteroidetes	Sphingobacteriia	Sphingobacteriia
BOTU65	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria
BOTU66	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU67	Armatimonadetes	Fimbriimonadia	Fimbriimonadia
BOTU68	Armatimonadetes	Armatimonadia	Armatimonadia
BOTU7	Actinobacteria	Acidimicrobiia	Acidimicrobiia
BOTU70	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU71	Planctomycetes	OM190	OM190
BOTU72	Planctomycetes	OM190	OM190
BOTU73	Bacteroidetes	Flavobacteriia	Flavobacteriia
BOTU74	Chloroflexi	Caldilineae	Caldilineae
BOTU75	Planctomycetes	OM190	OM190
BOTU76	Proteobacteria	Betaproteobacteria	Betaproteobacteria
BOTU77	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria
BOTU78	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU80	Chlamydiae	Chlamydiae	Chlamydiae
BOTU83	Proteobacteria	Betaproteobacteria	Betaproteobacteria
BOTU84	Actinobacteria	Acidimicrobiia	Acidimicrobiia
BOTU841	Proteobacteria	Betaproteobacteria	Betaproteobacteria
BOTU85	Proteobacteria	Betaproteobacteria	Betaproteobacteria
BOTU87	Armatimonadetes	Armatimonadia	Armatimonadia
BOTU878	Planctomycetes	Planctomycetacia	Planctomycetacia
BOTU88	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria
BOTU90	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria
BOTU91	Bacteroidetes	Sphingobacteriia	Sphingobacteriia
BOTU93	Proteobacteria	Betaproteobacteria	Betaproteobacteria
BOTU94	Bacteroidetes	Sphingobacteriia	Sphingobacteriia
BOTU96	Bacteroidetes	Sphingobacteriia	Sphingobacteriia
BOTU97	Chloroflexi	ChloroflexiSL56	ChloroflexiSL56
BOTU98	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria
BOTU99	Proteobacteria	Betaproteobacteria	Betaproteobacteria
COTU1	Cyanobacteria	Microcystis	Microcystis
COTU121	Cyanobacteria	Snowella	Snowella
COTU131	Cyanobacteria	Synechococcus	Synechococcus
COTU14	Cyanobacteria	Synechococcus	Synechococcus
COTU19	Cyanobacteria	Cyanobacteria	Cyanobacteria
COTU25	Cyanobacteria	Anabaena	Anabaena
COTU334	Cyanobacteria	Synechococcus	Synechococcus
COTU41	Cyanobacteria	Synechococcus	Synechococcus
COTU8	Cyanobacteria	Planktothrix	Planktothrix
COTU81	Cyanobacteria	Anabaena	Anabaena

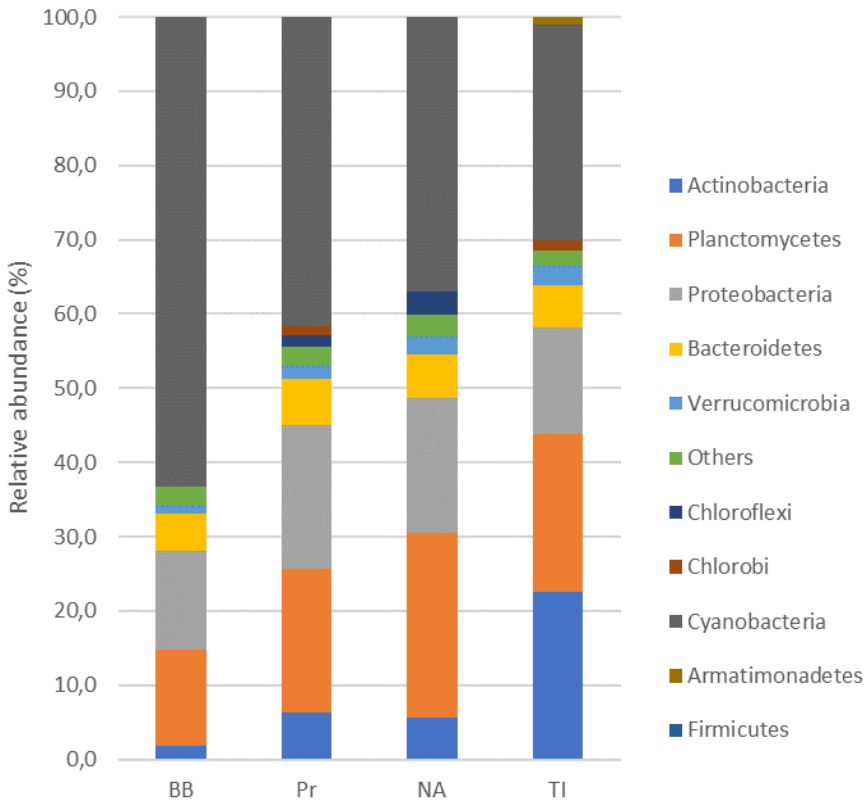
COTU9	Cyanobacteria	Cylindrospermosis	Cylindrospermosis
FOTU11	Bacillariophyta	Coscinodiscophyceae	Aulacoseirales
FOTU1127	Dinophyta	Dinophyceae	Gonyaulacales
FOTU114	Heterokontophyta	Chrysophyceae	Ochromonadales
FOTU12	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU1219	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU125	Bacillariophyta	Mediophyceae	Discostella
FOTU129	Chlorophyta	Chlorophyceae	Chlorophyceae
FOTU133	Chlorophyta	Chlorophyceae	Chlorophyceae
FOTU1485	Chlorophyta	Trebouxiophyceae	Trebouxiales
FOTU1567	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU163	Charophyta	Zygnematophyceae	Desmidiales
FOTU1637	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU164	Dinophyta	Dinophyceae	Gymnodiniphycidae
FOTU1672	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU172	Heterokontophyta	Chrysophyceae	Ochromonadales
FOTU173	Chlorophyta	Chlorophyceae	Chlorophyceae
FOTU174	Chlorophyta	Trebouxiophyceae	Trebouxiales
FOTU1758	Dinophyta	Dinophyceae	Peridiniphycidae
FOTU181	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU1823	Dinophyta	Dinophyceae	Gonyaulacales
FOTU20	Dinophyta	Dinophyceae	Gonyaulacales
FOTU2046	Dinophyta	Dinophyceae	Gonyaulacales
FOTU2047	Dinophyta	Dinophyceae	Gymnodiniphycidae
FOTU2065	Dinophyta	Dinophyceae	Gonyaulacales
FOTU22	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU2284	Dinophyta	Dinophyceae	Gonyaulacales
FOTU233	Heterokontophyta	Chrysophyceae	Ochromonadales
FOTU237	Heterokontophyta	Chrysophyceae	ChrysophyceaeTKR07M93
FOTU2376	Bacillariophyta	Coscinodiscophyceae	Aulacoseirales
FOTU24	Dinophyta	Dinophyceae	Gymnodiniphycidae
FOTU242	Heterokontophyta	Chrysophyceae	Ochromonadales
FOTU26	Bacillariophyta	Bacillariophyceae	Bacillariales
FOTU264	Dinophyta	Dinophyceae	Dinophyceae
FOTU267	Heterokontophyta	Chrysophyceae	Chromulinales
FOTU269	Bacillariophyta	Bacillariophyceae	Fragilariales
FOTU273	Dinophyta	Dinophyceae	Gymnodiniphycidae
FOTU286	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU29	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU290	Heterokontophyta	Chrysophyceae	Chromulinales
FOTU295	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU3	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU31	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU310	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU311	Heterokontophyta	Chrysophyceae	Chrysophyceae
FOTU313	Heterokontophyta	Chrysophyceae	Ochromonadales

FOTU32	Bacillariophyta	Mediophyceae	Stephanodiscales
FOTU322	Chlorophyta	Chlorophyceae	Sphaeropleales
FOTU325	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU327	Charophyta	Zygnematophyceae	Desmidiales
FOTU338	Dinophyta	Dinophyceae	Gymnodiniphycidae
FOTU35	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU350	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU368	Heterokontophyta	Chrysophyceae	Chromulinales
FOTU372	Chlorophyta	Chlorophyceae	Chlorophyceae
FOTU4	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU406	Heterokontophyta	Chrysophyceae	ChrysophyceaeA1
FOTU44	Cryptophyta	Cryptophyceae	Cryptophyceae
FOTU454	Chlorophyta	Trebouxiophyceae	Chlorellales
FOTU46	Chlorophyta	Trebouxiophyceae	Chlorellales
FOTU461	Heterokontophyta	Chrysophyceae	Chrysophyceae
FOTU477	Chlorophyta	Chlorophyceae	Tetrasporales
FOTU479	Heterokontophyta	Chrysophyceae	ChrysophyceaeTKR07M93
FOTU498	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU50	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU513	Heterokontophyta	Chrysophyceae	ChrysophyceaeTKR07M93
FOTU52	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU59	Dinophyta	Dinophyceae	Glenodinium
FOTU6	Bacillariophyta	Bacillariophyceae	Fragilariales
FOTU620	Dinophyta	Dinophyceae	Gymnodiniphycidae
FOTU621	Heterokontophyta	Chrysophyceae	ChrysophyceaeTKR07M92
FOTU647	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU65	Charophyta	Zygnematophyceae	Desmidiales
FOTU652	Dinophyta	Dinophyceae	Peridiniphycidae
FOTU655	Bacillariophyta	Bacillariophyceae	Cymbellales
FOTU70	Chlorophyta	Chlorophyceae	Chlorococcales
FOTU71	Chlorophyta	Chlorophyceae	Sphaeropleales
FOTU726	Chlorophyta	Chlorophyceae	Sphaeropleales
FOTU737	Chlorophyta	Chlorophyceae	Tetrasporales
FOTU74	Chlorophyta	Chlorophyceae	Chlamydomonadaceae
FOTU809	Cryptophyta	Cryptophyceae	Cryptomonadales
FOTU85	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU86	Chlorophyta	Chlorophyceae	Chlamydomonadales
FOTU909	Dinophyta	Dinophyceae	Dinophyceae
FOTU98	Dinophyta	Dinophyceae	Peridiniphycidae

SM Table 3. 2 - OTUs which node degree sum accounted with 10% of total node degree from each network

	Phyla	Class	Order	degree
		Barra Bonita		
BOTU506	Armatimonadetes	Armatimonadia	Armatimonadales	10
COTU131	Cyanobacteria	Synechococcus	Synechococcus	10
FOTU1707	Bacillariophyta	Mediophyceae	Stephanodiscales	10
FOTU242	Heterokontophyta	Chrysophyceae	Ochromonadales	10
BOTU179	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	9
			Sum total degree	454
		Promissão		
BOTU469	Bacteroidetes	Flavobacteriia	Flavobacteriales	38
BOTU438	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	31
BOTU464	Chlamydiae	Chlamydiae	Chlamydiales	34
BOTU273	Planctomycetes	Planctomycetacia	Planctomycetales	36
BOTU434	Planctomycetes	Planctomycetacia	Planctomycetales	33
BOTU132	Planctomycetes	Planctomycetacia	Planctomycetales	32
BOTU285	Proteobacteria	Alphaproteobacteria	Sphingomonadales	37
BOTU321	Proteobacteria	Betaproteobacteria	Nitrosomonadales	35
BOTU195	Proteobacteria	Betaproteobacteria	Burkholderiales	33
			Sum total degree	2952
		Nova Avanhandava		
			Sum total degree	2080
BOTU33	Actinobacteria	Actinobacteria	Frankiales	33
BOTU130	Actinobacteria	Actinobacteria	Corynebacteriales	32
BOTU10	Actinobacteria	Acidimicrobiia	Acidimicrobiales	28
FOTU497	Chlorophyta	Trebouxiophyceae	Chlorellales	35
BOTU132	Planctomycetes	Planctomycetacia	Planctomycetales	30
BOTU434	Planctomycetes	Planctomycetacia	Planctomycetales	29
BOTU247	Proteobacteria	Alphaproteobacteria	Rhizobiales	32
		Três Irmãos		
BOTU163	Actinobacteria	Thermoleophilia	Thermoleophilia	95
BOTU147	Actinobacteria	Actinobacteria	Actinobacteria	93
BOTU47	Actinobacteria	Acidimicrobiia	Acidimicrobiia	92
BOTU1893	Actinobacteria	Actinobacteria	Actinobacteria	91
BOTU67	Armatimonadetes	Fimbriimonadia	Fimbriimonadia	94
BOTU221	Bacteroidetes	Sphingobacteriia	Sphingobacteriia	89
BOTU73	Bacteroidetes	Flavobacteriia	Flavobacteriia	89
BOTU80	Chlamydiae	Chlamydiae	Chlamydiae	90
BOTU97	Chloroflexi	ChloroflexiSL56	ChloroflexiSL56	89
FOTU2065	Dinophyta	Dinophyceae	Gonyaulacales	90

BOTU176	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadetes	103
BOTU1568	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria	105
BOTU205	Verrucomicrobia	OPB35	OPB35	93
			Sum total degree	10458



SM Fig. 3. 1 - Relative abundance of most abundant (relative abundance>1%) Phyla of particle attached (PA) bacterial community by reservoir. Barra Bonita (BB), Promissão (Pr), Nova Avanhadava (NA) and Três Irmãos (TI).

CONCLUSÕES GERAIS

1) O sistema estudado possui características esperadas para reservatórios em cascata, influenciando as características ambientais. A cascata foi espacialmente estruturada, onde a condição ambiental não variou significativamente entre os dois reservatórios intermediários, devido à proximidade. O gradiente trófico esteve presente em ambas as estações e, embora tenha sido menos pronunciado durante o período chuvoso, não foi significativamente diferente entre as estações. Parâmetros que variaram ao longo da cascata foram aqueles ligados ao estado trófico, como clorofila, nitrogênio total, fósforo e transparência da água, enquanto ao longo do regime de chuvas, temperatura, pH e tempo de residência.

2) A disposição em cascata também teve influência nas estruturas das comunidades microbianas. As comunidades de Produtores primários (cianobactérias e fitoplâncton eucariótico) e bacteriana aderida à partículas tiveram sua estrutura respondendo ao estado trófico, e assim, as variações em suascomposições, foram melhor explicadas por reservatórios e não pelo regime de chuvas. Florações frequentes de cianobactérias foram observadas em todos os quatro reservatórios e refletiram a entrada de nutrientes alóctones nos sistemas, assim como pulsos de nutrientes característicos do reservatório dispostos em cascata. Por sua vez, as bactérias de vida livre apresentaram maiores mudanças em sua composição entre os dois períodos de seca e chuva, promovidas principalmente pela temperatura.

3) Encontramos um grande número de bactérias não cultivadas (~ 90% das seqüências obtidas), em algum nível taxonômico, que sugere que esses sistemas possam abrigar organismos específicos.

4) Fatores estocásticos foram dominantes governando a variação na estrutura das comunidades. Entretanto, cada sub comunidade foi governada por processos ecológicos distintos: comparado com bactérias aderidas à partículas e cianobactérias, a bactérias de vida livre estavam mais

frequentemente sob efeito seletivo de fatores locais; Bactérias aderidas a partículas tiveram variação na sua estrutura ao longo da cascata dependente da dispersão de partículas e raramente sob seleção. Cianobactérias estavam sob maior influência da deriva, que foi atribuído à a alta disponibilidade de nutrientes, favorecendo que poucas espécies prosperassem em todos os quatro reservatórios.

5)A presença de reservatórios intermediários e a distância espacial entre os reservatórios influenciaram a dispersão de bactérias aderidas e de vida livre, enquanto que distâncias maiores impuseram limitação apenas para as comunidades de partículas aderidas.

6) O regime de chuvas influenciou a variação das comunidades bacterianas por inserir material e organismos alóctones no sistema.

7) O estado trófico teve grande influência nas interações das comunidades, onde o reservatório mais eutrofizado apresentou menor diversidade de produtores primários e bactérias aderidas partículas. A rede gerada apresentou menor conexão entre os organismos e estrutura modular, com sub-grupos externos a redes. A diminuição da trofia promoveu maior diversidade fitoplanctônica e portanto, maior diversidade de bactérias aderidas. A rede gerada apresentou maior conexão entre os organismos, baixa tendência a modularidade