

UNIVERSIDADE FEDERAL DE SÃO CARLOS  
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE  
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA E RECURSOS NATURAIS

Mariana Câmara dos Reis

*Dinâmica espaço – temporal do bacterioplâncton em um sistema de várzea Amazônico*

São Carlos/SP

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**Folha de Aprovação**

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*“Man needs to strive for the good and the great, the rest depends on destiny”*

Alexander von Humboldt

## Lista de Abreviaturas e Siglas

ATTZ	Aquatic/terrestrial transition zone; Zona de transição/aquática terrestre
BCC	Bacterioplankton community composition; composição da comunidade do bacterioplâncton
COD	Carbono orgânico dissolvido
DNA	Deoxyribonucleic Acid
DO	Dissolved oxygen
DOC	Dissolved organic carbon
FL	Free living; vida livre
MOD	Matéria orgânica dissolvida
PA	Particle attached; aderida a partículas
PB	Produção bacteriana
PCR	Polymerase chain reaction
RB	Respiração bacteriana
rRNA	Ribosomal Ribonucleic Acid
POC	Particulate organic carbon
TDN	Total dissolved nitrogen
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphorus
TSS	Total soluble solids
OTU	Operational taxonomic unit

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## RESUMO

Planícies de inundação Amazônicas são redes complexas que desempenham papéis relevantes nos ciclos biogeoquímicos globais e a degradação da matéria orgânica é um processo chave no balanço regional de carbono desses sistemas. A Amazônia sofre variações sazonais no regime hidrológico, o que produz mudanças na paisagem e nas fontes de matéria orgânica nos sistemas de inundação. Embora essas mudanças possam afetar o bacterioplâncton, nenhum estudo abordou a questão de quais fatores direcionam os padrões espaciais e temporais dessa comunidade nesses sistemas. Neste estudo, foi usado o sequenciamento de alto rendimento (Illumina MiSeq) da região V3-V4 do gene 16S do rRNA para investigar os padrões espaço-temporais do bacterioplâncton em diferentes camadas de diversidade e frações de tamanho, e sua correlação com as variáveis ambientais em um lago de várzea Amazônico. No geral, quatro filos dominaram a comunidade ao longo do estudo: *Actinobacteria*, *Cyanobacteria*, *Proteobacteria* e *Planctomycetes*, porém suas abundâncias foram diferentes entre frações de tamanho, períodos hidrológicos e área de influência. A biosfera rara teve uma importante contribuição para os padrões de composição de toda a comunidade e mostraram ser mais influenciadas pela complexidade espacial. Nós encontramos que o pulso de inundação foi a principal força motora dos padrões de composição para a fração livre, e a distância do rio Amazonas para a fração aderida a partículas. Nós também encontramos diferenças entre a combinação de fatores locais e regionais através do espaço e do tempo modulando a comunidade. Esse é a primeira caracterização detalhada da composição da comunidade do bacterioplâncton em um lago de várzea Amazônico e nós demonstramos que a complexidade temporal e espacial deste sistema foi refletida na composição da comunidade do bacterioplâncton.

**Palavras-chave:** composição da comunidade do bacterioplâncton, sistemas de várzea, zona de transição aquática terrestre, pulso de inundação, sequenciamento de amplicons, fatores moduladores da diversidade do bacterioplâncton.

## ABSTRACT

Amazonian floodplains are complex networks that play relevant roles in global biogeochemical cycles, and the bacterioplankton degradation of organic matter is key in regional carbon budget in these systems. The Amazon undergoes seasonal variations in water level, which produces changes in landscape and in sources of organic inputs into floodplain systems. Although these changes should affect bacterioplankton, no studies have addressed the question of which factors drive spatial and temporal patterns of bacterioplankton in these systems. We used high-throughput (Illumina MiSeq) sequencing of the V3-V4 region of the 16SrRNA gene to investigate the spatiotemporal patterns of bacterioplankton community at different diversity layers and size fractions, and their correlation with environmental variables in an Amazon floodplain lake. Overall, four phyla dominated the community throughout the study: *Actinobacteria*, *Cyanobacteria*, *Proteobacteria* and *Planctomycetes*, but their abundance were different between size fractions, hydrologic periods and influence area. The rare biosphere had an important contribution for the composition patterns of all community and showed be more influenced by the spatial complexity. We found that the floodpulse was the mainly drive force of community composition patterns for the free-living fraction, and the distance from the Amazon river was the main driver for the particle-attached fraction. We also found differences between the combination of local and regional factors across space and time shaping the community. This is the first detailed characterization of the bacterioplankton community composition in an Amazon floodplain lake and we demonstrated that the spatial and temporal complexity of this system were reflected in bacterioplankton community composition.

## *Introdução geral*

### ***O papel do bacterioplâncton no ciclo do carbono***

Apesar do tamanho reduzido, os procariotos compõem uma fração significativa da biomassa planctônica em ecossistemas aquáticos e desempenham papéis importantes em todos os ciclos biogeoquímicos (Cotner e Biddanda, 2002). A remineralização da matéria orgânica e de nutrientes inicialmente era apontada como a principal função das bactérias heterotróficas em sistemas tropicais (Lindeman, 1942). Porém, parte da regeneração de nutrientes tem sido atualmente atribuída também aos protozoários que as consomem e liberam o excesso de amônia e fosfato, tanto em ecossistemas aquáticos como terrestres (Pomeroy e Wiebe, 1988), e aos fungos (Weyers e Suberkropp, 1996). Um novo enfoque que ressalta o importante papel das bactérias heterotróficas nas teias alimentares microbianas em regiões pelágicas passou a ser acrescentado a essa visão tradicional a partir de meados da década de 70, atribuindo a elas não só o papel de decompositoras, mas também como rota alternativa na transferência de carbono e nutrientes aos níveis tróficos superiores (Azam *et al.*, 1983).

A importância desses organismos nos ciclos biogeoquímicos se deve principalmente ao seu metabolismo. A produção (PB) e a respiração bacterianas (RB) são processos chave no ciclo do carbono em ecossistemas aquáticos (Del Giorgio e Cole, 1998). Através da PB as bactérias recuperam a energia liberada na cadeia trófica planctônica na forma de COD e o armazenam como carbono orgânico particulado (COP) em sua biomassa. Esse carbono pode então ser transferido para os níveis tróficos superiores através da predação por flagelados heterotróficos e microzooplâncton. Essa rota alternativa na transferência de carbono aos níveis tróficos superiores através das bactérias é chamada alça microbiana (*Microbial loop*), proposta inicialmente por Pomeroy (1974), e formalizada por Azam *et al.* (1983). O conceito de elo microbiano alterou a visão tradicional de cadeias tróficas de herbivoria e detritivoria ao evidenciar que a decomposição não é o único papel das bactérias no plâncton (Pedrós-Alió e Guerrero, 1994). Desde então, bacterioplâncton tem sido considerado um importante ator no

ciclo global carbono (Del Giorgio e Gasol, 1995; Bouvy *et al.*, 1998; Del Giorgio e Cole, 1998; Kritzberg *et al.*, 2010), sendo que o consumo bacteriano de COD é considerado o processo mais importante de remoção orgânica do carbono dissolvido em sistemas aquáticos (Williams, 2000).

Além das bactérias heterotróficas, as bactérias fotossintéticas são importantes organismos planctônicos. Pertencentes ao filo *Cyanobacteria*, esses organismos são procariotos que apresentam uma maquinaria fotossintética semelhante em função, moléculas e estrutura aos cloroplastos dos eucariotos, o que subsidiou a teoria de que os cloroplastos evoluíram de uma endossimbiose entre a célula eucariótica heterotrófica primitiva e uma cianobactéria (Stanier e Bazine, 1977; Gould *et al.*, 2008). As cianobactérias estão entre os organismos mais antigos do planeta e sua produção de oxigênio permitiu o enriquecimento da atmosfera e o surgimento dos primeiros organismos aeróbios (Falkowski *et al.*, 2008). Atualmente sabe-se que as cianobactérias dominam a produção de oxigênio nos oceanos e junto com as bactérias heterotróficas tornam os oceanos profundos gigantescos sumidouros de carbono (Jiao *et al.*, 2010).

Os membros do domínio *Archaea*, conhecidos popularmente por bactérias extremófilas, apesar de negligenciados durante muito tempo começaram a ser reconhecidos como importantes atores nos ciclos do carbono e nutrientes. A importância desses organismos passou a ser reconhecida após o advento das técnicas moleculares pois até então se acreditava que eles habitassem apenas ambientes extremos (Offre *et al.*, 2013). Atualmente sabe-se que elas podem ser encontradas em oceanos (representando mais de 20% dos procariotos), solos e em sistemas de água doce, além disso, a descoberta de funções ecológicas desses organismos vem aumentando (Berg *et al.*, 2010; Borrel *et al.*, 2012). As *Archaea* têm grande importância no ciclo global do carbono, dominando a produção e oxidação de metano e tendo papel na produção e remineralização da matéria orgânica, além de participarem do ciclo de outros

elementos como nitrogênio (fixação de nitrogênio e oxidação da amônia), enxofre (oxidação do enxofre) (Offre *et al.*, 2013).

### ***Fatores que influenciam o bacterioplâncton em sistemas de água doce***

A composição da comunidade bacteriana (BCC) em ecossistemas aquáticos pode ser regulada por fatores locais, tais como as características físicas e químicas da água, e por fatores regionais como dispersão, efeito de massa e outros processos demográficos (Figura 1). A seguir serão destacados os principais fatores locais e regionais e como eles podem afetar a comunidade bacteriana.

Dentre os fatores locais, o pH é considerado um importante fator modulador da comunidade bacteriana, atuando como um forte filtro local selecionando espécies de um conjunto (*pool*) compartilhado em metacomunidade, ou indiretamente como um indicador de outros parâmetros que podem afetar a BCC, como a abundância do fitoplâncton e substâncias húmicas (Ruiz-González, Niño-García e Del Giorgio, 2015; Niño-García *et al.*, 2016).

Por outro lado, o fitoplâncton pode ser relacionado negativamente ou positivamente com a comunidade bacteriana, podendo alterar seu metabolismo, abundância e diversidade. Negativamente através da competição por nutrientes em determinados ambientes, visto que tanto as bactérias como o fitoplâncton assimilam nutrientes inorgânicos a partir da coluna de água (Brett *et al.*, 1999). E positivamente através da liberação de COD lábil, uma fonte de matéria orgânica de fácil assimilação para as bactérias (Morana *et al.*, 2014). Análises da composição do bacterioplâncton baseadas no gene 16S rRNA durante uma floração de fitoplâncton no mar Báltico indicaram que tanto famílias bacterianas, quanto populações bacterianas foram influenciadas diretamente pelo fitoplâncton (através das concentrações Clorofila *a*, biomassa e razão dos grupos de microalgas) e também indiretamente (através da concentração de nutrientes e matéria orgânica dissolvida - MOD) (Bunse *et al.*, 2016).



Além de serem importantes para o crescimento do fitoplâncton, nutrientes inorgânicos são importantes na modulação da BCC, podendo em alguns casos causar um aumento na riqueza de grupos bacterianos quando em altas concentrações (principalmente fósforo) se comparado com sistemas com baixas concentrações de nutrientes (Schmidt *et al.*, 2016).

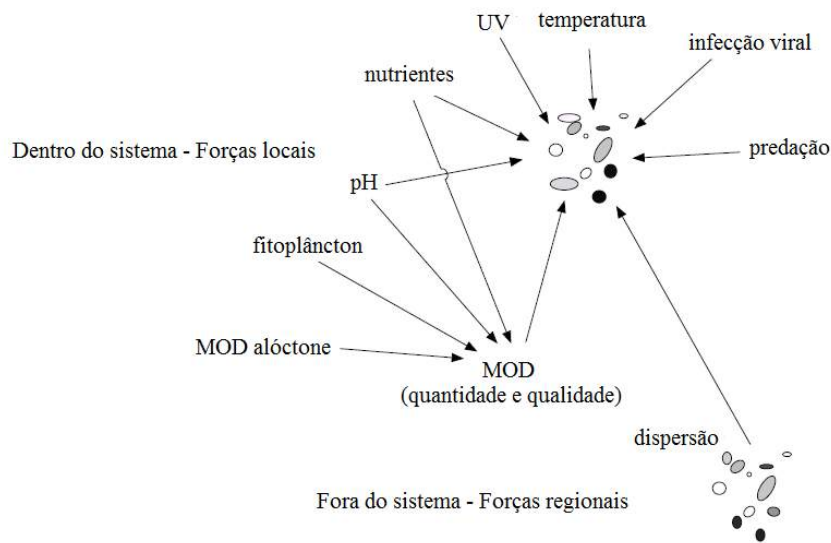
Dos fatores que influenciam a comunidade bacteriana, a matéria orgânica tem sido largamente investigada e, atualmente, sabe-se que tanto sua quantidade quanto a sua qualidade apresenta profundos efeitos na comunidade (Sarmiento e Gasol, 2012; Ruiz-González, Niño-García, Lapierre, *et al.*, 2015; Niño-García *et al.*, 2016). A matéria orgânica autóctone (proveniente do fitoplâncton e de macrófitas) tem sido considerada a principal fonte de energia para as bactérias (Kritzberg *et al.*, 2005). Evidências experimentais demonstram a existência de partição de nicho pelas bactérias na degradação da MOD do fitoplâncton, sendo que elevadas concentrações desse tipo de MOD favorecem o surgimento de hábitos generalistas entre as bactérias (Sarmiento *et al.*, 2016).

Outro importante fator controlador do metabolismo bacteriano é a temperatura que, quando elevada, estimula o crescimento da comunidade (White *et al.*, 1991). De todos os fatores ambientais, a temperatura tem um dos maiores efeitos na atividade microbiana devido à sua influência imediata nas reações enzimáticas (Kirchman, 2012), tendo um forte efeito modulador da diversidade bacteriana superior a outros fatores ambientais e geográficos (Sunagawa *et al.*, 2015).

Os principais controladores de topo da comunidade bacteriana são a predação por protistas e a morte por infecção viral. O papel da infecção viral na liberação de COD foi primeiramente descrito para sistemas marinhos como “alça viral” (Fuhrman, 1999). A lise celular bacteriana decorrente da contaminação viral libera COD e nutrientes no sistema, que podem ser utilizados por outras populações bacterianas e pelos produtores primários. Já os protistas (nanoflagelados e ciliados) têm um papel bem estabelecido na “alça microbiana” de

inserção do COP das bactérias na teia alimentar clássica (Azam *et al.*, 1983). Essas interações podem afetar a abundância, o metabolismo e as rotas de ciclagem de carbono na água, porém seus efeitos sobre a BCC em sistemas naturais ainda é um tema pouco explorado (Chow *et al.*, 2014). Evidências experimentais mostram que a comunidade bacteriana responde de forma diferente aos predadores e aos vírus (Longnecker *et al.*, 2010). Nesse estudo, a diversidade de grupos bacterianos (*Alphaproteobacteria*, *Gammaproteobacteria*, *Betaproteobacteria*, *Cytophaga*) foi estimada através da utilização de sondas específicas e quantificação em microscopia de epifluorescência, enquanto a atividade celular foi quantificada através da citometria de fluxo de bactérias que foram submetidas à incorporação de leucina radioativa. O trabalho revelou que a remoção dos predadores alterou a diversidade de células ativas, enquanto a remoção dos vírus causou impacto na atividade celular, mas não mudanças na diversidade de grupos bacterianos. Além disso, essas respostas foram mais pronunciadas em sistemas oligotróficos do que em sistemas eutróficos. Uma avaliação temporal *in situ* indicou que essas interações são complexas e que, em geral, as interações entre vírus e bactérias são mais interconectadas, com maior número de ligações, do que as interações entre protistas e bactérias, sugerindo um aumento da especificidade taxonômica entre vírus e bactérias (Chow *et al.*, 2014).

Assim, o bacterioplâncton está sujeito à influência de diferentes fatores locais. O mais importante é que esses fatores não atuam de forma isolada e sim de forma integrada. Estudos que visam elucidar o papel dos fatores locais na BCC em diferentes sistemas são escassos, porém necessários e muito importantes.



**Figura 1:** Diferentes fatores potencialmente moduladores da composição da comunidade bacteriana (BCC) em sistemas aquáticos de água doce. Modificado de Logue e Lindström (2008).

Por estarem inseridos em uma bacia hidrográfica os lagos sofrem grande influência do ambiente externo (fatores regionais). Dessa maneira, o ambiente circundante pode afetar os fatores locais como pH, quantidade e qualidade da MOD (através da entrada de matéria orgânica alóctone) e outros fatores que já foram citados como tendo um grande efeito na BCC. Essa influência é, portanto, um efeito regional indireto nos fatores locais que podem modificar a comunidade (Logue e Lindström, 2008). Estudos mostram que a MOD alóctone pode ter grandes efeitos na BCC, principalmente em sistemas oligotróficos (Roiha *et al.*, 2016).

Além dos efeitos indiretos, o ambiente circundante dos sistemas aquáticos pode influenciar a BCC através dos processos de dispersão em uma metacomunidade (Leibold *et al.*, 2004). Uma metacomunidade é definida como uma série de comunidades locais que são ligadas por processos de dispersão de múltiplas espécies (Wilson, 1992). O conceito de metacomunidade apresenta quatro paradigmas que buscam explicar a dinâmica das espécies entre as comunidades (Leibold *et al.*, 2004). O primeiro paradigma é chamado de dinâmica de fragmentos (*Patch dynamics*), que assume que os fragmentos em uma metacomunidade são

idênticos e igualmente capazes de apresentarem populações. A diversidade de espécies local, nesse caso, é limitada por dispersão e a dinâmica espacial é dominada por processos de extinção local e colonização. O segundo paradigma é o da separação de espécies (*species sorting*), no qual os fatores locais e as diferenças entre os fragmentos exercem uma forte influência sobre a dinâmica das comunidades. Nessa perspectiva ocorre um efeito conjunto entre os processos de dispersão e os filtros ambientais locais moldando as metacomunidades. Existe também o paradigma de efeito de massa (*mass effect*), que leva em consideração os efeitos dos processos de migração e emigração na dinâmica das populações. Esse paradigma enfatiza a influência da dinâmica espacial na densidade das populações locais. Nele, as comunidades são abertas e frequentemente recebem espécies de outros ambientes. Por essa razão, os filtros ambientais não têm tempo de atuar e selecionar espécies, gerando uma diversidade regional homogênea. Por fim, existe o paradigma da perspectiva neutra (*neutral perspective*) no qual todas as espécies são idênticas em sua capacidade de migração, habilidade competitiva e aptidão (*fitness*). Esse paradigma foi postulado por Hubbell (2001) e nele as interações entre espécies acontecem de forma randômica e são derivadas das probabilidades de perda (extinção e emigração) e ganho (especiação e imigração) de espécies.

Apesar de ainda pouco utilizada na ecologia microbiana, a abordagem de metacomunidades vem crescendo e, com ela, tem-se demonstrado que em alguns sistemas aquáticos continentais pode haver um balanço entre o efeito de massa e a separação de espécies. Ou seja, as espécies advindas do ambiente terrestre ou do sedimento entram no ambiente aquático através dos processos de imigração e sofrem seleção pelos filtros ambientais locais (separação de espécies) (Ruiz-González, Niño-García e Del Giorgio, 2015; Staley *et al.*, 2015).

### ***Composição do bacterioplâncton em sistemas de água doce***

Os procariotos são, de longe, os organismos mais diversos da biosfera, contando com 92 filos bacterianos e 26 filos de *Archaea* (Hug *et al.*, 2016) até então descritos. A diversidade bacteriana total de um ecossistema é composta por 2 elementos principais: 1) um conjunto de táxons abundantes que realizam um maior número de funções ecossistêmicas e crescem de forma ativa, visto que estão adaptados às condições do ambiente no qual se encontram. Além disso, eles sofrem intensa predação e lise viral, subsidiando o ciclo do carbono e nutrientes nos sistemas aquáticos. Esses organismos são facilmente recuperáveis por técnicas moleculares, porém, são difíceis de serem cultivados. 2) muitos táxons raros, que apresentam um crescimento lento e não são facilmente alcançados por predadores e vírus, devido à sua baixa abundância. Esses organismos desempenham funções ecossistêmicas importantes, porém em menor escala. Além disso, são de difícil recuperação com técnicas moleculares, e alguns são favorecidos em ambientes de cultura, onde a disponibilidade de recursos limitantes é alta (Pedrós-Alió, 2006).

Para entender a função das bactérias envolvidas nos processos em ecossistemas aquáticos, é essencial compreender a composição dessas duas frações da diversidade bacteriana. Esse era um ponto limitante, já que a utilização de caracteres morfológicos não permitia identificar a diversidade desses microrganismos (Pommier *et al.*, 2010). Os métodos tradicionais de cultura oferecem uma abordagem para a compreensão do potencial fisiológico, mas não fornecem informações sobre a diversidade microbiana das comunidades em ambientes naturais, visto que mais de 99 % dos microrganismos não podem ser cultivados em laboratório (Pedrós-Alió, 2006).

Nas últimas décadas, regiões do gene 16S rRNA começaram a ser analisadas usando técnicas de “fingerprinting” (impressão digital) como as RFLP (Restrição Terminal de Polimorfismos do Fragmento) (Liu *et al.*, 1997) e ARISA em combinação com a construção

de biblioteca de clones e sequenciamento (Lindström e Leskinen, 2002). No entanto, essas técnicas fornecem uma cobertura insuficiente para descrever a real diversidade bacteriana das comunidades e compará-las (Curtis *et al.*, 2006). A ecologia microbiana aquática sofreu uma revolução impulsionada pelas tecnologias de sequenciamento de DNA de alto rendimento, que permitem obter um grande número de sequências de amostras ambientais, inclusive de grupos pouco abundantes (Pedrós-Alió, 2006). As abordagens genômicas estão transformando nossa perspectiva sobre a estrutura, evolução e ecologia do mundo microbiano.

A fim de obtermos um panorama sobre a ecologia microbiana em ecossistemas de água doce, nós realizamos uma breve revisão da literatura de trabalhos que utilizaram técnicas de sequenciamento de nova geração (Roche 454, Illumina Miseq ou Hiseq e Ion torrent) para estudar a comunidade bacteriana nestes sistemas. A busca foi realizada na plataforma Web of Science (Thomson Reuters) utilizando os descritores em língua inglesa: “bacterial community composition” AND “freshwaters OR river OR lake OR floodplain” AND “amplicon OR NGS OR next generation sequencing OR Roche 454 OR Illumina OR Ion torrent”. Os trabalhos incluídos nessa revisão, bem como suas principais características, estão listados na Tabela 1. Encontramos um total de 54 trabalhos que abordaram diferentes aspectos ecológicos. Dentre eles os principais foram: influência da estratificação, inundação, variáveis ambientais e florações de cianobactérias na BCC, padrões biogeográficos e sazonais da BCC, transição ambiente terrestre-água, BCC em um gradiente de salinidade rio-estuário, entre outros.

Dos 54 trabalhos encontrados, 55,5 % realizaram uma cobertura espacial da comunidade bacteriana através de pontos distribuídos dentro do mesmo sistema, pontos distribuídos entre sistemas diferentes e diferentes profundidades. Nos trabalhos em que foi realizada cobertura temporal (11,1 %) a comunidade foi avaliada principalmente entre estações do ano, variação diária e períodos pré, durante e pós florações de cianobactérias. Os trabalhos em que foram realizados os dois tipos de cobertura (temporal e espacial) somaram

29,6 %. Encontramos também 2 trabalhos em que se avaliou a comunidade em apenas um ponto em subsuperfície.

**Tabela 1:** Revisão bibliográfica dos trabalhos que utilizaram técnicas de sequenciamento de nova geração para o estudo da composição do bacterioplâncton em sistemas de água doce. Plataformas: \* Roche 454 (Roche 454 GS-FLX, Roche 454 GS-FLX titanium system e Roche 454 GS Junior titanium); \*\* Illumina Miseq (Miseq 2000, Miseq v2, e Miseq 250); \*\*\* Illumina Hiseq (Hiseq 1500 e Hiseq 2000), \*\*\*\* Ion Torrent.

**Referências:** (Wilhelm *et al.*, 2013) (1), (Niño-García *et al.*, 2016) (2), (Bižić-Ionescu *et al.*, 2015) (3), (Edberg *et al.*, 2012) (4), (Eiler *et al.*, 2012) (5), (Grubisic *et al.*, 2017) (6), (Hauptmann *et al.*, 2016) (7), (Inceoglu *et al.*, 2015) (8), (Kurilkina *et al.*, 2016) (9), (Liu, L. *et al.*, 2015) (10), (Monard *et al.*, 2016) (11), (Newton e Mclellan, 2015) (12), (Peura *et al.*, 2012) (13), (Poretsky *et al.*, 2014) (14), (Schmidt *et al.*, 2016) (15), (Skopina *et al.*, 2015) (16), (Vila-Costa *et al.*, 2013) (17), (Woodhouse *et al.*, 2016) (18), (Zhong *et al.*, 2016) (19), (Zwirgmaier *et al.*, 2015) (20), (Logares *et al.*, 2013) (21), (Roiha *et al.*, 2016) (22), (Binh *et al.*, 2014) (23), (Severin *et al.*, 2014) (24), (Crevecoeur *et al.*, 2015) (25), (Ruiz-González, Niño-García e Del Giorgio, 2015) (26), (Chen *et al.*, 2011) (27), (Liu *et al.*, 2014) (28), (Llirós *et al.*, 2014) (29), (Yang *et al.*, 2015) (30), (Yu, Yang, Amalfitano, *et al.*, 2014) (31), (Yu, Yang e Liu, 2014) (32), (Zhang *et al.*, 2015) (33), (Brown *et al.*, 2015) (34), (Carney *et al.*, 2015) (35), (De Oliveira e Margis, 2015) (36), (Fortunato e Crp, 2015) (37), (Fortunato *et al.*, 2012) (38), (Ghai *et al.*, 2011) (39), (Gladyshev *et al.*, 2015) (40), (Ibekwe *et al.*, 2016) (41), (Jackson *et al.*, 2014) (42), (Kaevska *et al.*, 2016) (43), (Kolmakova *et al.*, 2014) (44), (Liu, J. *et al.*, 2015) (45), (Lu e Lu, 2014) (46), (Read *et al.*, 2015) (47), (Ruiz-Gonzalez *et al.*, 2015) (48), (Satinsky *et al.*, 2015) (49), (Savio *et al.*, 2015) (50), (Staley *et al.*, 2013) (51), (Staley *et al.*, 2015) (52), (Staley *et al.*, 2016) (53), (Tessler *et al.*, 2016) (54).



Sistema	Primers	Plataforma	Frações (µm)	Filos dominantes	Cobertura	Zona climática	Ref.
Córrego	515F e 926R	*	0.2	<i>Proteobacteria (Betaproteobacteria, Alphaproteobacteria), Bacteroidetes, Actinobacteria, Acidobacteria e Firmicutes</i>	Espacial	Temperada	1
Córrego, lago e rio	515F e 806R	**	0.2	<i>Actinobacteria, Proteobacteria (Betaproteobacteria, Alphaproteobacteria e Gammaproteobacteria), Bacteroidetes e Verrucomicrobia</i>	Espacial	Boreal	2
Lago	28F e 519R	*	0.2	<i>Actinobacteria, Bacteroidetes, Proteobacteria (Betaproteobacteria e Alphaproteobacteria), Verrucomicrobia</i>	Temporal e espacial	Temperada	3
			5	<i>Proteobacteria (Betaproteobacteria e Alphaproteobacteria), Bacteroidetes, Planctomycetes e Actinobacteria</i>			
Lago	341F e 805R	*	0.2	<i>Actinobacteria, Proteobacteria (Betaproteobacteria, Alphaproteobacteria e Deltaproteobacteria), Bacteroidetes e Cyanobacteria</i>	Espacial	Temperada	4
Lago	341F e 805R	*	0.2	<i>Actinobacteria, Proteobacteria (Alphaproteobacteria, Betaproteobacteria e Gammaproteobacteria), Bacteroidetes e Verrucomicrobia</i>	Temporal e espacial	Temperada	5
Lago	341F e 805R	*	0.2	<i>Actinobacteria, Proteobacteria (Alphaproteobacteria), Bacteroidetes e Verrucomicrobia</i>	Temporal	Temperada	6
Lago	515F e 806R	**	0.2	<i>Proteobacteria (Gammaproteobacteria, Betaproteobacteria), Bacteroidetes, Actinobacteria e Acidobacteria</i>	Espacial	Zona polar	7
Lago	27F e 519R / ARCH 349F e ARCH 806R	*	0.2	<i>Actinobacteria, Cyanobacteria, Proteobacteria (Alphaproteobacteria, Betaproteobacteria e Gammaproteobacteria) e Cyanobacteria</i>	Temporal e espacial	Tropical	8
Lago	U341F e U515R	*	0.2	<i>Proteobacteria (Betaproteobacteria, Alphaproteobacteria e Deltaproteobacteria), Actinobacteria, Chloroflexi, Bacteroidetes, Firmicutes</i>	Temporal e Espacial	Temperada	9
Lago	520F e 802R	**	0.2	<i>Actinobacteria, Verrucomicrobia, Proteobacteria (Alphaproteobacteria, Betaproteobacteria e Gammaproteobacteria), Bacteroidetes e Cyanobacteria</i>	Espacial	Temperada	10
Lago	341F e 805R	*	0.2	<i>Actinobacteria, Proteobacteria (Alphaproteobacteria e Betaproteobacteria) e Bacteroidetes</i>	Espacial	Temperada	11
Lago	518F e 1064R	*	0.2	<i>Actinobacteria, Proteobacteria (Betaproteobacteria e Alphaproteobacteria), Bacteroidetes e Verrucomicrobia.</i>	Espacial	Temperada	12
Lago	341F e 805R	*	0.2	<i>Actinobacteria, Proteobacteria (Betaproteobacteria e Alphaproteobacteria), Verrucomicrobia, Bacteroidetes e Cyanobacteria</i>	Temporal e espacial	Temperada	13
Lago	27F e 534R	*	0.2	<i>Proteobacteria (Betaproteobacteria e Alphaproteobacteria), Actinobacteria, Cyanobacteria, Bacteroidetes, Verrucomicrobia</i>	Temporal	Temperada	14
Lago	515F e 806R	**	0.2	<i>Bacteroidetes, Actinobacteria e Proteobacteria (Betaproteobacteria)</i>	Espacial	Temperada	15
			3	<i>Bacteroidetes, Cyanobacteria e Planctomycetes</i>			
Lago	515F e 806R	*	0.45	<i>Proteobacteria (Betaproteobacteria, Alphaproteobacteria, Gammaproteobacteria e Deltaproteobacteria), Actinobacteria, Bacteroidetes, Verrucomicrobia e Cyanobacteria.</i>	Espacial	Temperada	16
Lago	967F e 1046R	*	0.2	<i>Bacteroidetes, Proteobacteria (Betaproteobacteria, Alphaproteobacteria e Gammaproteobacteria) e Actinobacteria</i>	Temporal	Temperada	17
Lago	28F e 519R	**	0.2	<i>Actinobacteria, Proteobacteria (Alphaproteobacteria, Betaproteobacteria e Gammaproteobacteria), Cyanobacteria e Bacteroidetes</i>	Temporal	Tropical	18
Lago	515F e 806R	**	0.2	<i>Proteobacteria (Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria), Bacteroidetes, Actinobacteria e Planctomycetes</i>	Espacial	Temperada	19
Lago	S-D-Bact-0785-a-S-18 e S*-Univ-1392-a-A- 15 1392R	*	0.2	<i>Verrucomicrobia, Proteobacteria (Gammaproteobacteria, Betaproteobacteria, Alphaproteobacteria e Deltaproteobacteria), Planctomycetes e Cyanobacteria</i>	Temporal e espacial	Temperada	20
Lago costeiro	341F e 805R	*	0.2	<i>Actinobacteria, Proteobacteria (Betaproteobacteria e Gammaproteobacteria) e Bacteroidetes</i>	Espacial	Zona polar	21
Lago e lagoa	341F e 805R	*	não filtrada	<i>Actinobacteria, Verrucomicrobia, Proteobacteria (Betaproteobacteria) e Bacteroidetes</i>	Temporal e espacial	Subártica	22
Lago e Rio	28F e 519R	*	0.2	<i>Proteobacteria (Betaproteobacteria, Alphaproteobacteria e Gammaproteobacteria), Actinobacteria, Bacteroidetes e Armatimonadetes</i>	Espacial	Temperada	23
Lago e rio	341F e 805R	*	0.2	<i>Proteobacteria, Bacteroidetes, Actinobacteria, Verrucomicrobia e Planctomycetes</i>	Temporal e espacial	Temperada	24
Lagoa	sem nome	*	0.2	<i>Proteobacteria (Betaproteobacteria e Gammaproteobacteria), Bacteroidetes, Verrucomicrobia e Actinobacteria</i>	Espacial	Temperada	25
			3	<i>Proteobacteria (Betaproteobacteria e Gammaproteobacteria), Bacteroidetes, Verrucomicrobia e Cyanobacteria</i>			
Lagoa, lago e rio	515F e 806R	**	0.2	<i>Proteobacteria (Betaproteobacteria e Alphaproteobacteria), Actinobacteria, Acidobacteria, Bacteroidetes e Verrucomicrobia</i>	Espacial	Boreal	26
Reservatório	27F e 543R	*	-	<i>Cyanobacteria, Actinobacteria, Proteobacteria (Betaproteobacteria e Alphaproteobacteria), Bacteroidetes e Planctomycetes</i>	Temporal	Temperada	27
Reservatório	357F e 926R	*	0.2	<i>Actinobacteria, Proteobacteria (Betaproteobacteria, Alphaproteobacteria e Gammaproteobacteria), Bacteroidetes e Firmicutes</i>	Temporal	Subtropical	28
Reservatório	28F e 519R	*	0.2	<i>Proteobacteria (Betaproteobacteria e Alphaproteobacteria), CFB (Cytophaga, Flavobacteria e Bacteroidetes), Actinobacteria e Cyanobacteria</i>	Temporal e espacial	Temperada	29

Reservatório	8F e 533R	*	0.2	<i>Proteobacteria (Betaproteobacteria e Alphaproteobacteria), Actinobacteria, Cyanobacteria e Bacteroidetes</i>	Temporal e espacial	Temperada	30
Reservatório	357F e 926R	*	0.2	<i>Actinobacteria, Proteobacteria (Alphaproteobacteria, Betaproteobacteria e Deltaproteobacteria), Cyanobacteria, Bacteroidetes e Firmicutes</i>	Temporal e espacial	Subtropical	31
Reservatório	357F e 926R	*	0.2	<i>Actinobacteria, Proteobacteria (Betaproteobacteria, Alphaproteobacteria, Deltaproteobacteria e Gammaproteobacteria), Bacteroidetes, Cyanobacteria e Firmicutes</i>	Espacial	Subtropical	32
Reservatório	515F e 806R	**	0.2	<i>Verrucomicrobia, Actinobacteria, Proteobacteria (Betaproteobacteria e Gammaproteobacteria)</i>	Espacial	Subtropical	33
Rio	341F e 518R	**	0.2	<i>Proteobacteria (Betaproteobacteria, Gammaproteobacteria e Alphaproteobacteria), Bacteroidetes, Actinobacteria, Cyanobacteria, e Verrucomicrobia</i>	Ponto único	Temperada	34
Rio	27F e 519R	*	0.2	<i>Proteobacteria (Betaproteobacteria, Alphaproteobacteria e Epsilonproteobacteria), Actinobacteria e Cyanobacteria</i>	Temporal e espacial	Temperada	35
			5	<i>Cyanobacteria, Proteobacteria (Betaproteobacteria, Gammaproteobacteria e Alphaproteobacteria) e Actinobacteria</i>			
Rio	515F e 806R	**	0.45	<i>Proteobacteria (Betaproteobacteria, Alphaproteobacteria), Bacteroidetes, Actinobacteria e Cyanobacteria</i>	Temporal e espacial	Subtropical	36
Rio	27F e 338R	*	0.2	<i>Actinobacteria, Proteobacteria (Gammaproteobacteria, Alphaproteobacteria e Betaproteobacteria), Bacteroidetes e Verrucomicrobia</i>	Espacial	Temperada	37
Rio	27F e 338R	*	0.2	<i>Proteobacteria (Betaproteobacteria), Bacteroidetes, Actinobacteria e Verrucomicrobia</i>	Temporal e espacial	Temperada	38
Rio	-	*	0.2	<i>Actinobacteria, Proteobacteria (Betaproteobacteria, Gammaproteobacteria, Alphaproteobacteria) e Acidobacteria</i>	Ponto único	Tropical	39
Rio	343F e 806R	**	0.2	<i>Actinobacteria, Proteobacteria (Betaproteobacteria e Alphaproteobacteria), Cyanobacteria e Planctomycetes</i>	Espacial	Temperada	40
Rio	8FM e 357R	*	-	<i>Proteobacteria (Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria e Alphaproteobacteria), Bacteroidetes, Verrucomicrobia, Actinobacteria e Acidobacteria</i>	Espacial	Temperada	41
Rio	515F e 806R	****	0.2	<i>Cyanobacteria, Bacteroidetes, Proteobacteria (Alphaproteobacteria, Betaproteobacteria e Gammaproteobacteria), Planctomycetes e Verrucomicrobia</i>	Espacial	Temperada	42
			3.0	<i>Cyanobacteria, Bacteroidetes, Planctomycetes e Proteobacteria (Alphaproteobacteria, Betaproteobacteria e Gammaproteobacteria)</i>			
Rio	347F e 803R	*	0.2	<i>Proteobacteria, Bacteroidetes, Actinobacteria, Firmicutes e Cyanobacteria</i>	Temporal e espacial	Temperada	43
Rio	343F e 806R	**	0.2	<i>Actinobacteria, Proteobacteria (Alphaproteobacteria e Betaproteobacteria), Bacteroidetes e Verrucomicrobia</i>	Espacial	Temperada	44
Rio	28F e 519R	*	0.2	<i>Proteobacteria (Betaproteobacteria, Alphaproteobacteria e Gammaproteobacteria), Actinobacteria, Bacteroidetes</i>	Espacial	Temperada	45
Rio	27F e 533R	*	0.45	<i>Proteobacteria (Betaproteobacteria e Gammaproteobacteria), Bacteroidetes e Cyanobacteria</i>	Espacial	Temperada	46
Rio	Gray28F e Gray519R	*	0.2	<i>Actinobacteria, Proteobacteria (Betaproteobacteria, Alphaproteobacteria e Gammaproteobacteria), Bacteroidetes e Verrucomicrobia</i>	Espacial	Temperada	47
Rio	341F e 907R	*	0.2	<i>Actinobacteria, Proteobacteria (Betaproteobacteria, Alphaproteobacteria e Gammaproteobacteria), e Bacteroidetes</i>	Espacial	Temperada	48
Rio	-	***	0.2	<i>Actinobacteria, Planctomycetes, Proteobacteria (Betaproteobacteria) e Verrucomicrobia</i>	Espacial	Tropical	49
			2.0	<i>Actinobacteria, Planctomycetes, Proteobacteria (Betaproteobacteria) e Verrucomicrobia</i>			
Rio	8F e 338R	**	0.2	<i>Actinobacteria, Proteobacteria (Betaproteobacteria e Alphaproteobacteria), Bacteroidetes, Verrucomicrobia e Candidate division ODI</i>	Espacial	Temperada	50
			3.0	<i>Proteobacteria (Betaproteobacteria e Alphaproteobacteria), Actinobacteria, Verrucomicrobia e Candidate division ODI</i>			
Rio	967F e 1046R	**	0.45	<i>Proteobacteria (Betaproteobacteria e Alphaproteobacteria), Actinobacteria, Bacteroidetes, Cyanobacteria e Verrucomicrobia</i>	Espacial	Temperada	51
Rio	967F e 1046R	***	0.45	<i>Proteobacteria (Betaproteobacteria, Gammaproteobacteria e Alphaproteobacteria), Bacteroidetes, Actinobacteria e Verrucomicrobia</i>	Temporal e espacial	Temperada	52
Rio	B5F784 e 1046R	**	0.45+0.2	<i>Actinobacteria, Proteobacteria (Betaproteobacteria), Cyanobacteria, Bacteroidetes</i>	Espacial	Temperada	53
Sistemas de inundação	341F e 806R	*	0.2	<i>Proteobacteria (Betaproteobacteria e Alphaproteobacteria), Bacteroidetes, Actinobacteria, Cyanobacteria</i>	Espacial	Tropical	54

Os sistemas mais estudados foram rios (38,7%) e lagos (38,7%), seguidos de reservatórios (12,9%), lagoas (4,8%), córregos (3,2%), sendo que em 7 trabalhos foram avaliados diferentes tipos de sistemas em conjunto, córregos lagos e rios (Niño-García *et al.*, 2016); lagos e rios (Binh *et al.*, 2014; Severin *et al.*, 2014); lagoas, lagos e rios (Ruiz-González, Niño-García e Del Giorgio, 2015); lagos e lagoas (Roiha *et al.*, 2016); lagos e reservatórios (Liu, L. *et al.*, 2015). Os sistemas de inundação foram avaliados em apenas um trabalho (Tessler *et al.*, 2016).

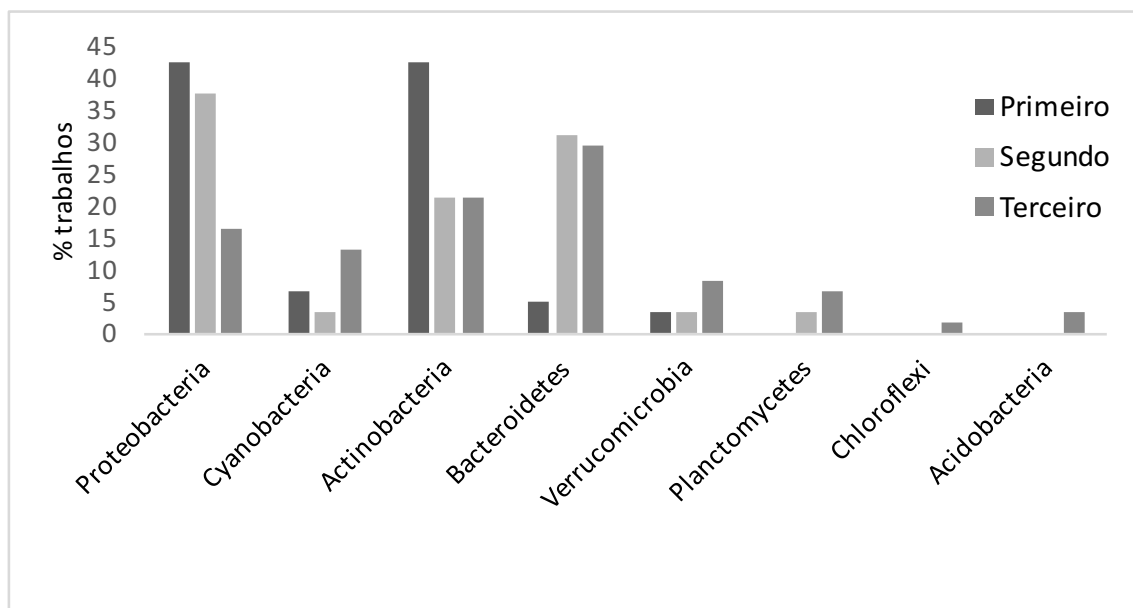
Entre as frações de tamanho, nós observamos que 89 % dos trabalhos se concentraram em apenas uma fração de tamanho (principalmente 0.2  $\mu\text{m}$  e 0.45  $\mu\text{m}$ ), e que em 11,1% dos trabalhos foram avaliadas duas frações de tamanho, grande/aderida a partículas (3  $\mu\text{m}$  ou 5  $\mu\text{m}$ ) e pequena/vida livre (0.2  $\mu\text{m}$ ).

Além disso, do total de trabalhos encontrados, 5 (9,2 %) foram realizados em sistemas tropicais, sendo que 3 destes trabalhos foram em sistemas brasileiros (Rio Solimões, Rio Amazonas, Rio Paraná, Rio Araguaia e Pantanal), 1 em um lago africano (Lago Kivu) e 1 em um lago Australiano (Lago Yanga). Os sistemas temperados foram os mais representados (72,2 %), enquanto as zonas boreais, subárticas, subtropicais e polares corresponderam a 18,5 % dos trabalhos.

Esses resultados mostram que, apesar dos avanços proporcionados pelas técnicas de sequenciamento de nova geração nos últimos 10 anos, a comunidade bacteriana em sistemas de água doce tropicais ainda é pouco explorada, principalmente nos sistemas de inundação. Além disso, os trabalhos que avaliam a comunidade em diferentes frações de tamanho e realizam uma cobertura espacial e temporal ainda são poucos.

Entre os filos dominantes encontrados, nós podemos observar que *Proteobacteria* e *Actinobacteria* ocuparam o primeiro lugar entre os trabalhos (Figura 2). Sendo que o filo *Proteobacteria* também foi o segundo mais abundante. Em terceiro lugar, *Bacteroidetes* foi o

que mais apareceu entre os trabalhos. Os filos *Chloroflexi* e *Acidobacteria* apareceram apenas como terceiro grupo mais abundante. Estes resultados estão de acordo com o trabalho de Newton *et al.*, (2011), uma meta-análise que reuniu todos os dados de sequência do gene 16S rRNA do epilimnio de lagos de água doce publicados. Essa meta-análise identificou um total de 21 filos sendo que os mais comuns foram: *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, *Verrucomicrobia* e *Cyanobacteria*. A pesquisa ainda revelou que estes filos compõem cerca de 97% (> 11.400 sequências) das sequências analisadas.



**Figura 2:** Filos dominantes encontrados nos 54 trabalhos que utilizaram técnicas de sequenciamento de nova geração para o estudo da composição do bacterioplâncton em sistemas de água doce e a frequência (% de trabalhos) em que apareceram em cada posição de abundância (primeiro, segundo e terceiro).

Dentro de *Proteobacteria*, as classes mais abundantes foram *Betaproteobacteria*, *Alphaproteobacteria*, *Gammaproteobacteria* e *Deltaproteobacteria*. Este resultado é semelhante ao encontrado em Newton *et al.*, (2011). A classe *Betaproteobacteria* é a mais bem estudada, visto que é o grupo mais abundante em lagos e sistemas de água doce, apesar de serem suscetíveis à predação (Newton *et al.*, 2011). Podem ser encontradas em associação com algas e são mais abundantes em condições neutras e alcalinas, além de possuírem uma alta capacidade de responder às mudanças ambientais. Já a classe

*Alphaproteobacteria*, apresenta organismos que resistem à predação através da formação de filamentos e são bons competidores em situações de escassez de nutrientes, pois são eficientes em degradar a matéria orgânica (Newton *et al.*, 2011).

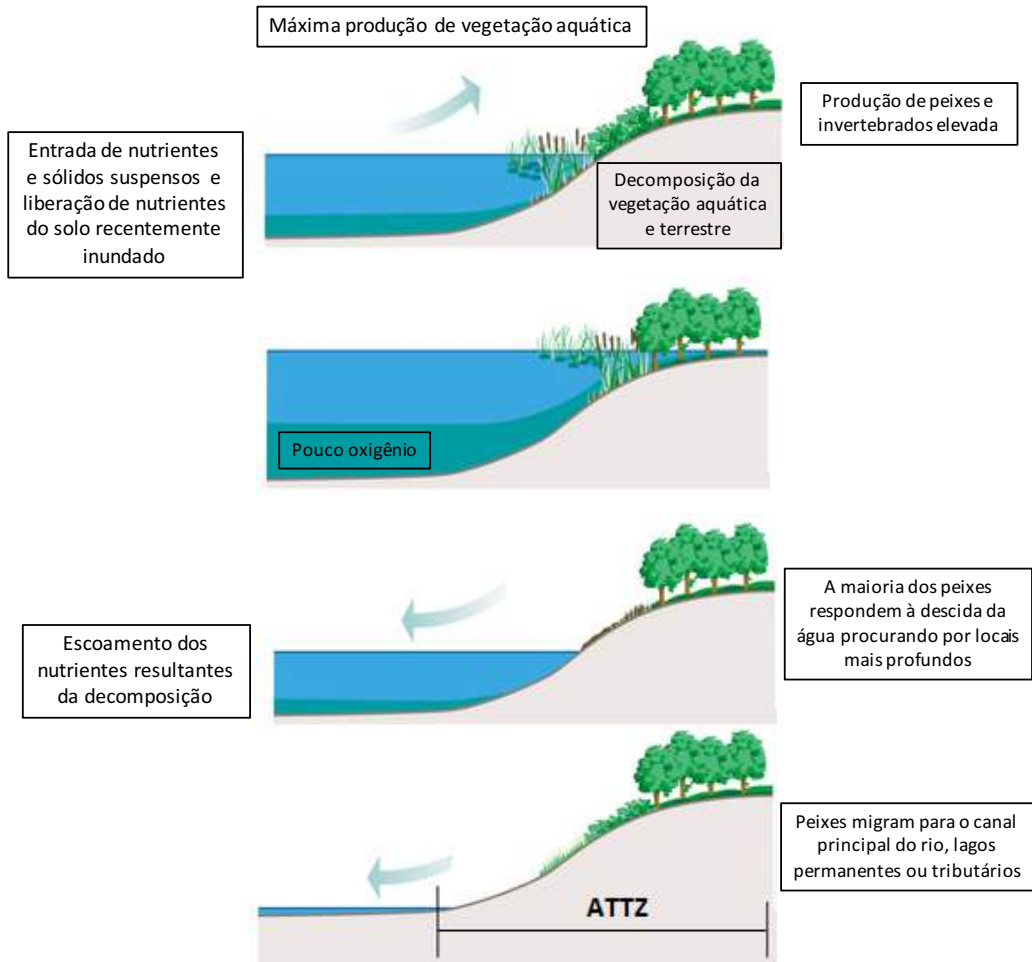
### ***Importância dos sistemas de várzeas tropicais***

Os sistemas de várzeas são áreas periodicamente inundadas pelo transbordamento lateral de rios ou lagos e/ou por precipitação direta ou água subterrânea. Esses sistemas compreendem uma complexa rede formada pelos habitats lóticos permanentes (canal principal), habitats lênticos permanentes conectados pelas extensas zonas húmidas (Junk *et al.*, 1989). As zonas úmidas são áreas de solo saturados de água e incluem as grandes planícies de inundação e pantanais (Kayranli *et al.*, 2010). Estas áreas de solo saturadas por água compreendem cerca de 7 a 10 milhões de km<sup>2</sup>, o que corresponde à aproximadamente 5 - 8% da superfície do globo (Mitsch e Gosselink, 2007). Todos os continentes são cobertos por uma rede de zonas úmidas, ao longo de cursos d'água e em depressões que assumem importantes funções na paisagem, principalmente na retenção da água, como filtros, sumidouros e fontes de substâncias, e como habitats para plantas específicas, animais e atividade humana (Junk, 2002). A interação dinâmica entre água e terra é o principal processo que cria e mantém a rede rio – várzea e afeta a biota desses ambientes (Bayley, 1995).

A maioria dos ambientes de inundação estão sujeitos a flutuações de acordo com as estações secas e chuvosas. O conceito do pulso de inundação (*flood pulse concept*) foi postulado para resumir os efeitos das oscilações cíclicas no nível da água na biota (comportamento e fisiologia), na disponibilidade de nutrientes orgânicos e inorgânicos e na produtividade desses sistemas, usando informações disponíveis para ambientes tropicais e temperados (Junk *et al.*, 1989). De acordo com este conceito, a principal força controladora nos ambientes de inundação é a hidrologia (Junk *et al.*, 1989). As planícies de inundação são, então, uma parte do sistema rio – várzea que é regularmente inundada e drenada e

representam um tipo de zona úmida (Bayley, 1995). Por apresentarem uma natureza efêmera, o estudo desses sistemas é muitas vezes enviesado, concentrado apenas no canal principal do rio, ou concentrando-se apenas nos lagos de inundação, tratando-os como sistemas clássicos. Por essa razão, os sistemas de várzea foram por muito tempo negligenciados.

Esses sistemas apresentam uma região de elevada atividade que é a compreendida na zona de transição aquática/terrestre (Figura 3 – ATTZ, do inglês *aquatic/terrestrial transition zone*) (Junk *et al.*, 1989). A ATTZ alterna entre estados aquáticos e terrestres e liga o rio aos sistemas lênticos permanentes e à terra firme. Por essa razão, a ciclagem de nutrientes e matéria orgânica na ATTZ é muito elevada. Durante a fase de inundação, os nutrientes fixados na fase seca pelas plantas são dissolvidos na água e/ou são transportados para o canal principal do rio, o que eleva as taxas de produção primária e também de decomposição (Bayley, 1995). Quando a elevação da água cessa, as taxas de decomposição excedem a produção primária, o que pode levar à depleção dos níveis de oxigênio na água (Figura 3). Assim como a dinâmica dos nutrientes e da vegetação, a dinâmica da comunidade de peixes também se altera com as variações da hidrologia. Com a enchente, a produção de peixes e invertebrados cresce na ATTZ (Figura 3). Por outro lado, com a descida da água, os peixes buscam lugares mais profundos e migram para o canal principal, lago permanente ou tributário.



**Figura 3:** Esquema do conceito de pulso de inundação mostrando as diferentes fases e as alterações nas características do sistema (Extraído e modificado de Bayley (1995)).

A quantidade e qualidade do material suspenso e dissolvido determinam a produção primária e secundária nesses sistemas (Junk, 2002). A região central do rio Amazonas e suas planícies de inundação, chegam a emitir cerca de  $0,21 \text{ Pg C}^{-1} \text{ ano}^{-1}$  na forma de  $\text{CO}_2$ , sendo que o metabolismo bacteriano é o que confere altas taxas de emissão nesses sistemas (Abril *et al.*, 2014). A qualidade da MOD nos sistemas amazônicos tem grande influência sobre o metabolismo bacteriano, sendo que em a RB excede a PB (Vidal *et al.*, 2015). O mesmo acontece na bacia do Orinoco na Venezuela, onde as emissões totais de metano são estimadas em  $0,17 \text{ Tg ano}^{-1}$ , sendo que as regiões de maior emissão são as de floresta alagada, águas abertas e regiões com alta cobertura de macrófitas, onde a disponibilidade de matéria orgânica é maior (Smith *et al.*, 2000). Por serem ambientes altamente produtivos, esses sistemas têm

recebido atenção crescente e estudos destacam a importância de se inventariar, conhecer e preservar esses sistemas (Downing, 2009).

Por serem regiões de transição e sofrerem influência do pulso de inundação, os sistemas de várzeas são interessantes para se estudar os efeitos dos processos de filtragem ambiental e de dispersão sobre a BCC e produtividade bacteriana. Porém, como evidenciado pela revisão que realizamos, esses sistemas ainda são pouco estudados, especialmente em um ecossistema com fauna e flora altamente diversas como a Amazônia, pouco se sabe sobre a diversidade microbiana

Dada a importância do bacterioplâncton no ciclo do carbono e de nutrientes, dos sistemas de várzea como ecossistemas altamente produtivos, da influência dos fatores locais e regionais na BCC em sistemas aquáticos e tendo como norteadora a escassez de estudos que abordam a composição do bacterioplâncton de forma aprofundada (diferentes frações de tamanho e com cobertura espacial e temporal) nesses sistemas, objetivamos realizar, nesta dissertação, a primeira caracterização detalhada da composição do bacterioplâncton em um sistema de várzea Amazônico, buscando contribuir para a expansão do conhecimento de ecossistemas aquáticos continentais.



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## Capítulo I

*Bacterioplankton community composition reflects the temporal and spatial complexity of  
the Amazon hydrologic network*

## Introduction

Drained by a complex hydrological network, the Amazonian rainforest is one of the largest and most biodiverse biomes in the world (Sioli, 1984). The Amazon river-floodplain system comprises a complex network of permanent lotic habitats and permanent lentic habitats interconnected in extensive floodplains (Junk *et al.*, 1989). These floodplains contain a complex mosaic of wetland habitats, including open water environments, alluvial forests and grasslands, which are collectively referred to as floodplain lakes. Altogether, these systems play key biogeochemical processes of global relevance. In the Amazon, the annual CO<sub>2</sub> outgassing is estimated at 470 TgCyr<sup>-1</sup> (Richey *et al.*, 2002), which is one order of magnitude higher than fluvial export of organic carbon to the ocean (Richey *et al.*, 1990). A more updated budget (Melack, 2016) indicated that the emissions from Amazon wetlands is comparable to that emitted by rivers and streams globally (Raymond *et al.*, 2013). A large percentage of these emissions come from floodplain lakes and flooded areas, especially those with high macrophyte coverage (Abril *et al.*, 2014). Moreover, the Amazon comprises a huge number of endemic species of fauna and flora, but little is known about prokaryotic diversity in Amazon floodplains, which account for most of the CO<sub>2</sub> emissions through organic matter degradation.

In addition to spatial complexity, the Amazon basin has three contrasting types of water, based on optical and physicochemical characteristics (Sioli, 1984): “White water”, with a high content of suspended materials and nutrients, and pH near to neutral; “Black water”, with low concentration of nutrients, high content of dissolved humic substances and acid pH ( $4 > \text{pH} < 7$ ); “Clear water”, which is an intermediate type and presents pH near to neutral (Sioli, 1984). Another factor of great importance is the seasonality of hydrologic conditions: the “flood pulse” (Junk *et al.*, 1989). The flood pulse is the major driving force in tropical floodplain systems, affecting the behavior and physiology of animals and plants (Junk *et al.*,

1989). Hydrologic fluctuations create a region that alternates between aquatic and terrestrial states called aquatic/terrestrial transition zone (ATTZ). The ATTZ links permanent water bodies to permanent terrestrial system, consequently they receive large amounts of terrestrial organic matter (Junk *et al.*, 1989), which has been considered a strong driver of BCC in freshwater systems (Ruiz-González, Niño-García, Lapierre, *et al.*, 2015). In addition, terrestrial influence also enhances the entrance of surrounding communities from the soil into the aquatic system (Ruiz-González, Niño-García e Del Giorgio, 2015).

Another factor that contributes to spatiotemporal complexity are the changes of organic matter sources into the floodplains lakes resulting from the flood pulse (Moreira-Turcq *et al.*, 2013). During rising waters the main source of organic matter is the Amazon river (Moreira-Turcq *et al.*, 2013). Otherwise, during the high and falling waters the organic matter origin is mainly from *in situ* production, which in some floodplain lakes is dominated by macrophytes and others by phytoplankton, including cyanobacteria, as the Curuai floodplain (Moreira-Turcq *et al.*, 2013). The aquatic vegetation is composed of different species, mostly emergent macrophytes, that cover vast areas especially in the ATTZ (Silva *et al.*, 2009). These macrophytes are sources of dissolved organic matter for prokaryotic activity (Mortillaro *et al.*, 2016). Recent studies highlighted trends of BCC changes along a riverine continuum (Read *et al.*, 2015; Savio *et al.*, 2015; Staley *et al.*, 2015). However, the influence of the flood pulse typical in large tropical rivers on spatiotemporal variability of bacterioplankton is still poorly understood.

A first metagenome of bacterioplankton in Amazonian waters carried out in a single sample from the Solimões River, indicated that the community was more similar to lake samples than marine or soil samples (Ghai *et al.*, 2011). The phylum *Actinobacteria* was dominant, followed by *Proteobacteria* (*Betaproteobacteria*, *Alphaproteobacteria* and *Gammaproteobacteria*, respectively) (Ghai *et al.*, 2011). A microbiome announcement in the

lower Amazon river (main channel) and river plume (ocean) reported that the most abundant genes belonged to *Actinobacteria*, *Planctomycetes*, *Betaproteobacteria*, *Verrucomicrobia*, *Nitrospirae*, and *Acidobacteria* (Satinsky *et al.*, 2015). A study in Brazilian floodplain systems (Amazon, Pantanal, Araguaia, and Paraná) demonstrated that the BCC were similar to global sites at phyla level, with a dominance of *Actinobacteria*, *Cyanobacteria*, *Proteobacteria*, *Bacteroidetes* and *Verrucomicrobia* (Tessler *et al.*, 2016). A recent clone libraries study from Solimões river, adjacent rivers and lakes of the Amazon basin showed that the *Proteobacteria* phylum was the most abundant in all systems, accounting for up to 20% of the total microbial population, being followed by *Actinobacteria* (Toyama *et al.*, 2017). With regards to bacterial genus, the most abundant in the river system were related to *Proteobacteria* phyla (*Aquabacterium* and *Acinetobacter*), otherwise, in lake systems the most abundant genus belonged to *Cyanobacteria* phyla (*Synechococcus* and *Cyanobium*) (Toyama *et al.*, 2017).

To fully understand bacterioplankton function and how diversity is structured in aquatic ecosystems it is essential to know the composition of all layers of diversity and their relationship with environmental factors. Firstly, most bacterioplankton taxa are present in low abundance, and few abundant taxa (Pedrós-Alió, 2006). Second, some taxa may be found associated with particles. In general, operational taxonomic unit (OTU) richness in the particle attached (PA) fraction is higher than in free living (FL) (Mohit *et al.*, 2014; Bižić-Ionescu *et al.*, 2015; Savio *et al.*, 2015). Among the factors that influence the colonization and richness of prokaryotes on particles is their size (Mestre *et al.*, 2017), quality and composition of suspended material (Luef *et al.*, 2007).

## **Objective**

Given the global relevance of biogeochemical processes carried out in the Amazon basin, and the crucial role of prokaryotes on these processes, this study addressed the question of how BCC varies in space and time across a complex floodplain lake. The main objective is to understand which environmental factors drive BCC in a complex Amazonian floodplain lake, and how diversity layers reflects spatial and temporal variations in this system. Therefore, we analyzed FL and PA prokaryotic communities through Illumina MiSeq 16S rRNA amplicon sequencing from 6 locations of Lago Grande do Curuai a typical Amazonian floodplain system. Our sampling strategy covered the environmental conditions from the ATTZ to the lake open waters, in two contrasted hydrological periods. This is the first study that addresses BCC from a floodpulse perspective in an Amazonian floodplain lake system, covering variations in space and time.

## **Hypothesis**

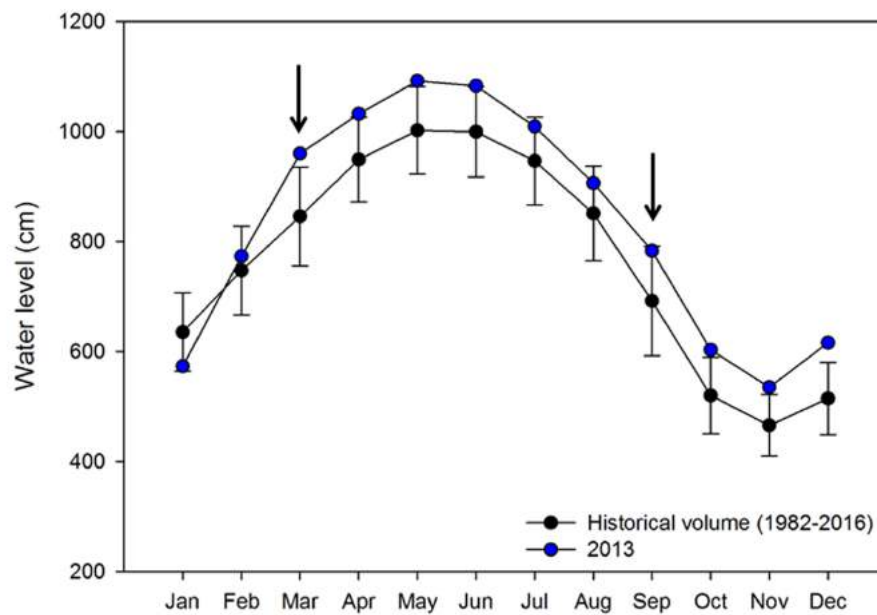
We hypothesize that the flood pulse is the great driver of bacterioplankton community composition. With regards to spatial patterns, we expect that the lake points will have similar community composition, different from points in the ATTZ which will be more influenced by terrestrial inputs. Also, the flood pulse will cause a change in the combination between local factors and regional factors across the time. In rising period, the processes of mass effect will have a great influence, modifying the community composition, on the other hand, in the falling period the community will be more influenced by local factors (e.g. water chemistry).

## Materials and Methods

### *Study site and sampling procedures*

This study was carried out in Lago Grande do Curuai, a floodplain lake located in the lower Amazon portion (from 56.10°W to 55.00°W, and 2.3°S to 1.9°S), in Pará State (Brazil). The Curuai floodplain is a typical Amazon complex formed by more than 30 interconnected lakes, linked to the Amazon River by 9 channels. The watershed is approximately 3660 km<sup>2</sup> including open water areas. The flooded area ranges between 575 km<sup>2</sup> and 2300 km<sup>2</sup> with the water level ranging between 3 m and 11 m (Bonnet *et al.*, 2008). Lago Grande do Curuai is representative of lakes on the Amazon floodplain and contains a wide range of distinct habitats, such as lakes, ATTZ and *igarapés* (low order Amazonian streams or channels) with different geochemical characteristics, comprising black and white waters (Perez *et al.*, 2011).

To evaluate the spatiotemporal complexity of this system, sampling sites were selected according to their main influence: three sites located in the ATTZ (points 2,10 and 30), three sites in the open waters lake (points 15, 24 and 43) Samples were also taken in two different phases of the hydrological cycle, rising waters (March 2013), and falling waters (September 2013) (Figure I – 1) during expeditions of CLIM-FABIAM Project.



**Figure I - 1:** Mean water level and standard deviation in the Lago Grande do Curuai system. Black points indicate historical water level. Blue points indicate water level from the sampling year. Arrows indicate sampling dates. Data source: Agência Nacional de Águas, Hidroweb (data from 1982 to 2016)

Water samples were collected at 1 m below the surface with Van Dorn bottle and kept in carboys pre-cleaned with 10% HCl and rinsed with Milli-Q water. In all cases, DNA filtration was performed *in situ* less than 6 hours after collection.

### ***Physical and chemical parameters***

Water temperature, pH, electrical conductivity, turbidity and concentration of dissolved oxygen (DO) on the epilimnion layer were measured *in situ* using a multiparameter probe YSI EXO2 (YSI, Yellow Springs, OH, USA). Water transparency was determined with a Secchi disc. The alkalinity was determined by titrimetric methods. The concentration of total phosphorus (TP), organic phosphorus (OP) and Silica (Si) was measured with colorimetric methods (Mackereth *et al.*, 1978). For chlorophyll *a*, 250 ml of water was filtered in 0.7 pore size (Whatman GF/F glass microfibre filters) in triplicates, with low-pressure vacuum pump. Chlorophyll *a* was extracted with buffered acetone (90% acetone + 10%



saturated magnesium carbonate), and the extracts were kept for 24h in the refrigerator before colorimetric determination (Rice *et al.*, 2012). To evaluate TSS concentration, 350 ml of lake water was filtered under moderate pressure onto acetate cellulose membranes (0.45  $\mu\text{m}$  pore size) pre-dried and pre-weighted. Filters were dried for 24 h at 50°C and TSS concentration were determined gravimetrically using the dry weight of the filtered material. Concentration of total organic carbon (TOC) and dissolved organic carbon (DOC) was measured with a non-dispersive infrared method (TOC V - Shimadzu 5000). The concentration of POC was calculated by the difference of TOC and DOC. The total nitrogen (TN) and the total dissolved nitrogen (TDN) were obtained a non-dispersive infrared method (TOC V - Shimadzu 5000). The DOC/Chlorophyll *a* ratio was calculated by the product between DOC and Chlorophyll *a* concentrations. The C:N, C:P and N:P ratios, were calculated by the product of the molar concentration of total fraction for each component. The distance between the sites and the Amazon river was calculated by the distance between the exact coordinate of the sites in the map and the nearest point of the river in a straight line.

### ***DNA extraction and sequencing***

Working with particle-rich Amazonian waters require some adjustments of standard methodological procedures. It worth mentioning (might be useful for future studies), that we experienced difficulties in all steps from filtration to DNA amplification. First, in filtering the water (due to the high amount of particles), in DNA extraction (regular tools such as MoBio Power Soil DNA extraction kit were not efficient), in performing PCR (humic substances inhibited PCR in most samples). The problems faced in each of those steps conditioned our choices in terms of the methodologies applied.

Water samples were filtered through 3  $\mu\text{m}$  polycarbonate pore size (47mm diameter) for PA fraction, and then filtered again through 1.2  $\mu\text{m}$  pore size, to remove large organisms

and particles. Lastly, FL prokaryotes were collected by filtration in 0.2 µm pore size (47mm diameter). Filters were kept in an ultrafreezer at -80°C until DNA extraction.

Before extraction, filters containing total DNA were submitted to enzymatic digestion with lysozyme (final concentration 1 mg ml<sup>-1</sup>) and Proteinase K (final concentration 0.2 mg ml<sup>-1</sup>). Then, total DNA was extracted using phenol-chloroform protocol followed by purification in Amicon columns (Millipore® 100KDa/100.000MWCO). An additional purification step with cetyl trimethyl ammonium bromide (CTAB) 10% was realized to remove PCR inhibitory humic substances (Schneegurt *et al.*, 2003). After PCR amplification, high-throughput sequencing of the V3-V4 regions of the 16S rRNA gene was performed in an Illumina MiSeq sequencing platform.

Each DNA sample was PCR-amplified in duplicated 25 µl reactions including an initial step of 95°C for 3 min, followed by 25 cycles of 98°C 20 s, 62°C for 15 s, 72°C for 15 s, and finally 72°C for 1 min, using primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3') (Herlemann *et al.*, 2011). Each reaction contained 12,5 µl of Kapa High-fidelity HOTSTART ready MIX, 0.3 µM of each primer, 10 µl of PCR-grade water, and 10 ng of DNA.

PCR products (50 µl) were purified with magnetic beads AMPURE XP kit (Bechman Coulter) and Indexed with Nextera XT kit V2 (Illumina) to separate samples. Another step of purification with magnetic beads was realized, and then the metagenomic pool was assembled with 5 µl of each library.

### ***Data processing and community analyses***

Sequencing data were processed using UPARSE (Edgar, 2013) in a pipeline internally implemented (Logares *et al.*, 2014; Logares, 2017). Paired-end reads were merged with PEAR (Zhang *et al.*, 2014). Sequences were quality controlled with the following steps: all

sequences shorter than 100 pb were discarded, quality dereplication checking, OTU clustering (UPARSE algorithm, similarity  $\geq 97\%$ ), and filtering of chimeras (with SILVA v.119 as reference database (Quast *et al.*, 2013)) with USEARCH (Edgar, 2010). Taxonomic classification was done through the BLASTn 119.1 SILVA (Zhang *et al.*, 2000) (at least 75% of similarity). All chloroplasts sequences were excluded.

For further analyses, the OTU table was rarefied and converted in to relative abundances. Abundant OTUs were that with a relative abundance over 1% within a sample, and rare OTUs were defined as having an abundance under 0.1 % as previously described (Pedrós-Alió, 2012; Lynch e Neufeld, 2015).

To evaluate the beta diversity among hydrological periods and influence area, we used Bray-Curtis (Bray e Curtis, 1957) indices. A nonmetric multidimensional scaling (NMDS) ordination and cluster analyses were used to visualize this metric. We tested the beta dispersion of the data using the function *betadisper* of vegan package. The differences among fractions, hydrologic periods and influence area were tested using permutational multivariate analysis of variance (PERMANOVA) with Bray-Curtis indice performing 10000 permutations (Anderson, 2005). We also calculated the individual value (IndVal) for each OTUs to find indicative species of fraction, hydrologic periods and influence area using 1000 iterations (Dufrene e Legendre, 1997). We considered as indicative species those that presented a p value  $< 0.05$ . An IndVal near 1 indicates that the specie is strongly indicative of that environment.

We performed a simple Mantel test for each taxonomic resolution, with matrices of FL and PA fractions separately, to determine the relative contribution of environmental variables to the bacterioplankton composition patterns. The distance matrices of all OTUs, abundant and rare OTUs, and community composition were obtained using Bray-Curtis distance. Otherwise, for environmental matrices, Euclidean distance was used. Co-variance among

environmental variables was tested using linear regression models. The comparison between environmental variables in the two sampling periods was done with a paired t-test. All statistical analyses were carried out in R software 3.3.1 (R Core Team, 2016) using the packages *vegan* (Oksanen *et al.*, 2016), *labdsv* (Roberts, 2007), and *GUnifrac* (Chen, 2012).

## Results

### *Environmental parameters*

A paired t-test comparison between environmental variables in the two sampling periods revealed significant differences between rising and falling waters for alkalinity, conductivity, total suspended solids (TSS), Silica (Si), pH, particulate organic carbon (POC), DOC/Chlorophyll *a* ratio and N:P ratio (Table I - 1). Alkalinity, conductivity, TSS, POC and DOC/Chlorophyll *a* were higher during rising water and Si, pH and N:P ratio were higher during falling waters. Other parameters such as nitrogen and phosphorus concentrations had higher means in the rising waters period but no statistically significant differences ( $p > 0.05$ ).

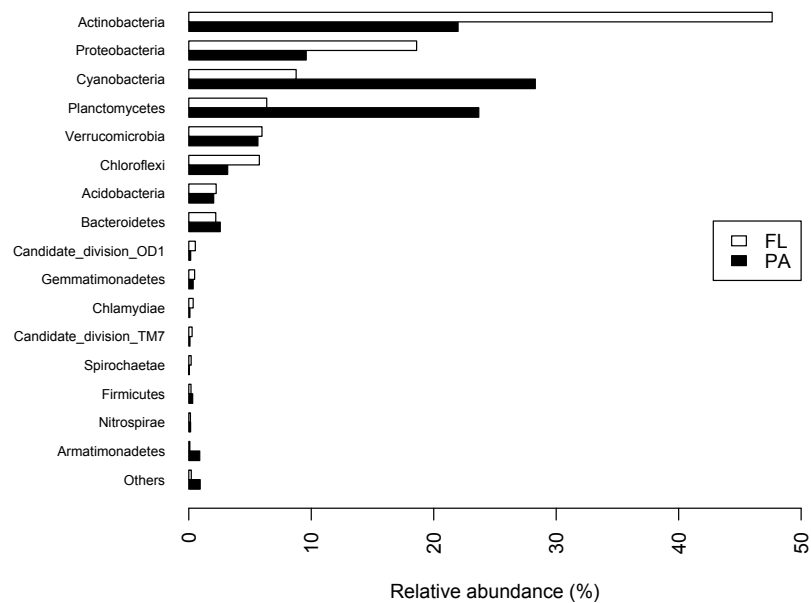
**Table I - 1:** Minimum, mean and maximum values of environmental parameters sampled in both hydrological periods rising and falling waters. Asterisks indicate statistical significant differences tested by a Paired t-test (\* $p < 0.05$ , \*\* $p < 0.01$ ).

	Rising			Falling			
	Min.	Avg.	Max.	Min.	Avg.	Max.	
Temperature (°C)	30.3	<b>30.8</b>	31.7	29.7	<b>31.4</b>	34.7	
Water column depth	1.8	<b>3.6</b>	5.7	2.6	<b>3.7</b>	4.3	
OD (mg/L)	4.6	<b>6.2</b>	7.4	5.9	<b>7.5</b>	11.7	
Alkalinity (mg/LCaCO <sub>3</sub> )	11.8	<b>17.4</b>	22.5	10.8	<b>13.3</b>	15.1	*
Conductivity (µS/cm)	38	<b>65.3</b>	77	34	<b>44.3</b>	59	*
Secchi disk (m)	0.38	<b>0.7</b>	1.7	0.5	<b>0.7</b>	0.9	
Turbidity (NTU)	4.7	<b>24</b>	73.7	5	<b>17.8</b>	25	
Total suspended solids (mg/L)	32	<b>65.7</b>	138	6.5	<b>21</b>	30	*
Si (mg/L)	1.9	<b>2.41</b>	2.9	2.7	<b>3.01</b>	3.2	*
pH	7.2	<b>7.7</b>	8.5	7.5	<b>8.2</b>	8.9	*
TP (ug/L)	20	<b>83.3</b>	150	30	<b>45</b>	80	
OP (ug/L)	10	<b>75</b>	140	10	<b>30</b>	50	
TN (ug/L)	230	<b>353</b>	430	190	<b>275</b>	370	
TDN (ug/L)	130	<b>255</b>	420	180	<b>253.3</b>	320	
TOC (mg/L)	2.6	<b>5.8</b>	8	2.9	<b>3.9</b>	4.9	
DOC (mg/L)	2.9	<b>3.8</b>	4.9	2.9	<b>3.7</b>	4.9	
POC (mg/L)	0	<b>2.11</b>	4.24	0.01	<b>0.22</b>	0.81	*
DOC/Chlorophyll <i>a</i>	0.01	<b>0.83</b>	1.82	0.05	<b>0.09</b>	0.15	*
Chlorophyll <i>a</i> (µg/L)	1.97	<b>38.2</b>	203.9	19.5	<b>46.2</b>	72.8	
C:N ratio	10.9	<b>19.1</b>	27.5	13	<b>17.2</b>	20.9	
C:P ratio	68.9	<b>227.2</b>	433.9	98.9	<b>270.8</b>	386.8	
N:P ratio	6.31	<b>11.9</b>	22.6	7.55	<b>16</b>	29.7	*

### ***Bacterioplankton community composition***

A total of 2.220.334 high quality reads were retrieved after cleaning, with a minimum of 11.284 reads per sample. Clustering at 97% of similarity resulted in a total of 2.011 OTUs, of which 1.636 remained after rarefaction. Five *Archaea* sequences were found, belonging to phylum *Thaumarchaeota*, and *Euryarchaeota*. However, these sequences were excluded in further analyses, since the primers used are not appropriate for *Archaea* diversity. The most common prokaryotic phyla across sampling sites were *Actinobacteria*, *Cyanobacteria*, *Proteobacteria* and *Planctomycetes*, in both size fractions (Figure I - 2). The phyla *Cyanobacteria* and *Planctomycetes* were proportionally more abundant in the larger fraction (PA, >3µm), with a mean contribution of in average 28.2% and 23.6% of relative abundance respectively. *Actinobacteria* and *Proteobacteria* were more abundant in the smaller fraction (FL, <1.2 and >0.2 µm), contributing with in average 47.6% and 18.6% of relative abundance respectively. Interestingly, we also found a higher relative abundance of *Chloroflexi* in the smaller fraction (in average 5.7%).

We could also observe changes in the main phyla among hydrological periods and influence area (Figure I - 3). In average, the most abundant phylum in rising waters was *Actinobacteria* (34.7%), followed by *Cyanobacteria* (18.3%), *Proteobacteria* (15.9%) and *Planctomycetes* (13.7%). In falling waters, the phylum *Actinobacteria* (34.8%) was also the most abundant, followed by *Cyanobacteria* (18.6%), *Planctomycetes* (16.3%), and *Proteobacteria* (12.2%). For the influence area, we found that the community of lake sites were composed mainly by *Actinobacteria* (36.4%), followed by *Planctomycetes* (16.0%), *Cyanobacteria* (14.5%) and *Proteobacteria* (12.4%). For ATTZ sites the main phyla were *Actinobacteria* (33.2%), *Cyanobacteria* (22.4%), *Proteobacteria* (15.7%) and *Planctomycetes* (14.0%).



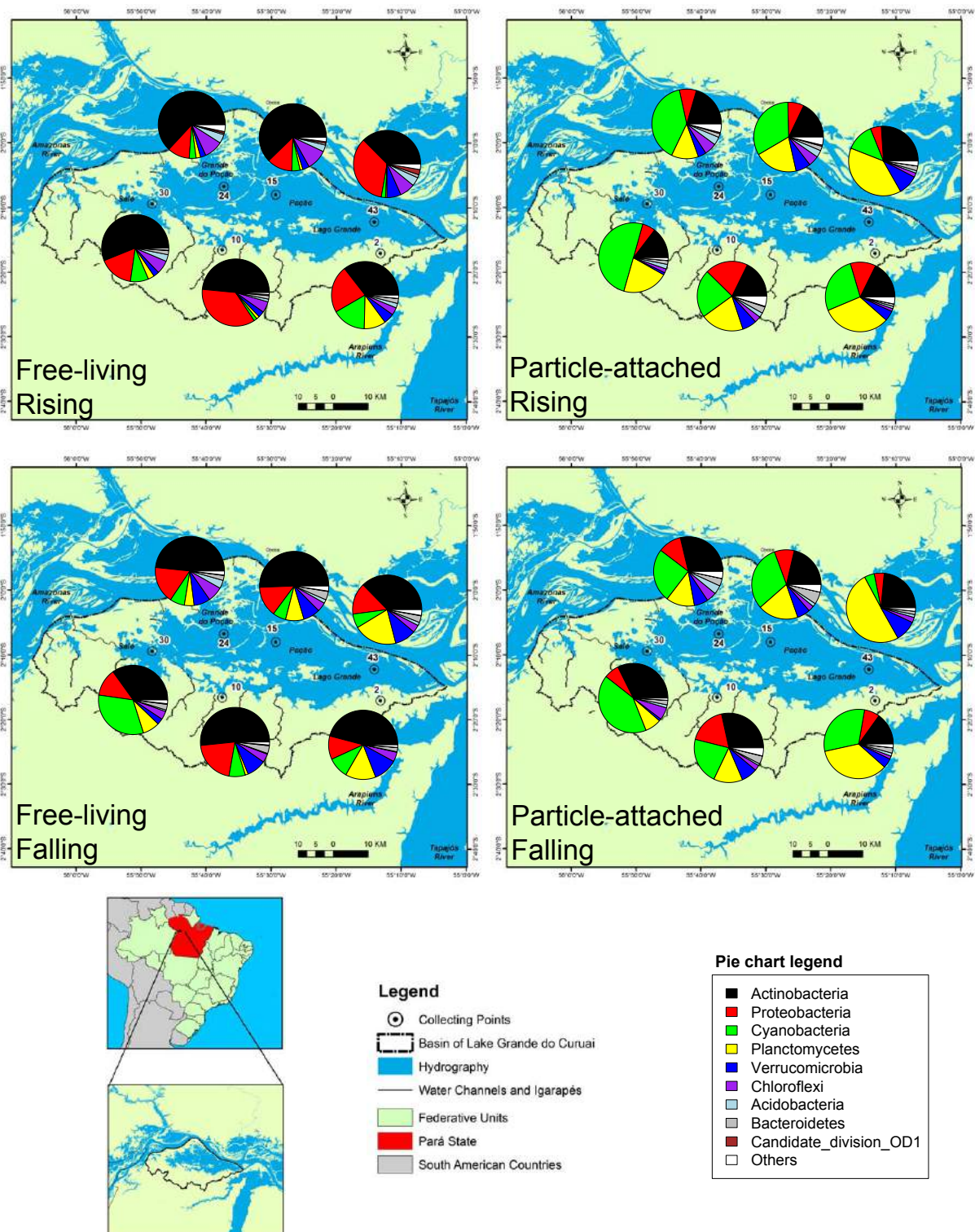
**Figure I - 2:** Phylum – level taxonomic composition of bacterioplankton across sampling sites (free – living (FL) and particle – attached (PA)).

A total of 103 OTUs classified as abundant (contributing with > 1%), while 1607 were classified as rare (contributing with < 0.1%). The detailed composition (at genus-level) of the four main phyla can be found in Supplementary Material II (Figures II - 1 to II - 4). The taxonomic distribution of abundant and rare OTUs between the main phyla were: *Actinobacteria* (in average, 26.2% in abundant and 1.1% in rare), *Cyanobacteria* (in average, 16.5% in abundant and 0.3% in rare), *Planctomycetes* (in average, 10.4% in abundant and 0.6% in rare) and *Proteobacteria* (in average, 4.0% in abundant and 3.1% in rare). Other phyla of abundant OTUs were identified as belonging to *Verrucomicrobia*, *Chloroflexi*, *Acidobacteria*, *Bacteroidetes*, *Armatimonadetes* and *Candidate\_division\_WS3*. The most abundant OTUs were uncultured members of the genus *Synechococcus* (*Cyanobacteria*), two members of *hgcI\_clade* (*Actinobacteria*), and a member of *Planctomycetaceae* (Supplementary Material II, Figures II - 1 to II - 4). They presented relative abundance variation among hydrological phases: *Synechococcus* (rising 13.5% and falling 10.2%), *hgcI\_clade* (rising 19.0% and falling 6.5% - two OTUs) and *Planctomycetaceae* (rising 2.5%

and falling 3.3%).

Some OTUs occurred only in the rare group. The main were affiliated to the phyla *Candidate\_division\_TM7*, *Candidate\_division\_OD1*, *Gemmatimonadetes*, *Chlamydiae*, *Candidate\_division\_WS3*, *Candidate\_division\_BRC1*, *BD1-5*, *Candidate\_division\_SRI*, *Deinococcus-Thermus*, *Firmicutes*, *SHA-109* and *Spirochaetae*. Regarding the percentage of unclassified OTUs in this work we found that 40.3% of abundant OTUs were not classified at genus level and 88.4% at species level. These values were higher among rare OTUs of which 59.6% were not classified at genus level and 92.3% at species level.





**Figure I - 3:** Distribution of free – living (FL) and particle – attached (PA) bacterioplankton phyla in each sampling site, in the two hydrological periods. Points 2, 10 and 30 are aquatic/terrestrial transition zone influenced, 15, 24 and 43 are typical lake sites.

### ***Spatiotemporal patterns of bacterioplankton***

PERMANOVA analyses of all OTUs with Bray-Curtis distance indicated significant differences between FL and PA fractions ( $R^2 = 0.24$  and  $p = 9.999 \times 10^{-5}$ ), influence area ( $R^2 = 0.10$  and  $p = 0.001$ ) and hydrological periods ( $R^2 = 0.07$  and  $p = 0.01$ ) (Figure I – 4).

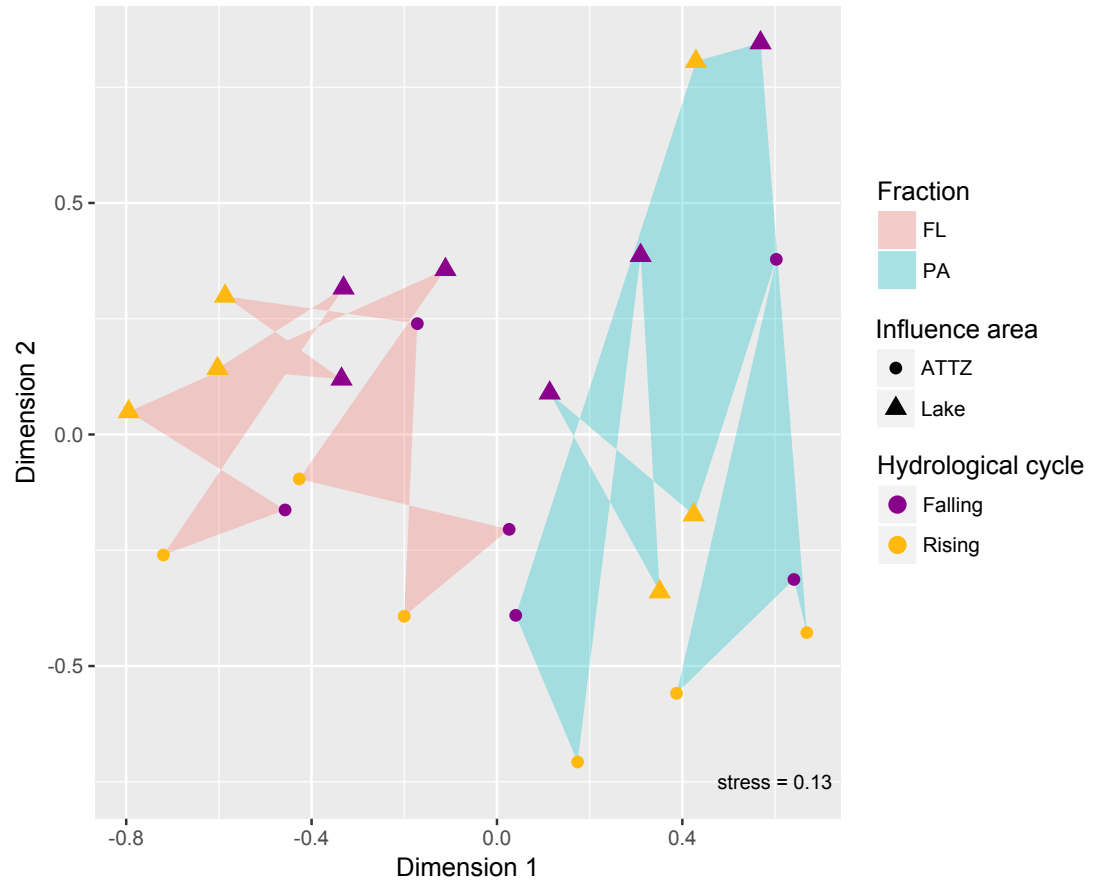
For abundant OTUs PERMANOVA analyses evidenced differences between fractions ( $R^2 = 0.25$  and  $p = 9.999 \times 10^{-5}$ ) and influence area ( $R^2 = 0.09$  and  $p = 0.03$ ). For rare OTUs we found significant differences between fractions ( $R^2 = 0.07$  and  $p = 9.999 \times 10^{-5}$ ), hydrological cycle ( $R^2 = 0.07$  and  $p = 9.999 \times 10^{-5}$ ) and influence area ( $R^2 = 0.07$  and  $p = 0.0002$ ). Despite the statistically significant differences, NMDS ordination plots for influence area in all diversity layers and the NMDS plots of rare OTUs have shown an overlap between groups of samples (Figures I – 5 and I - 6).

We found significant values of betadispersion for fraction in all OTUs and abundant OTUs ( $p = 0.047$  and  $p = 0.001$ , respectively). Although we did not find significant differences in the betadispersion of all OTUs and rare OTUs between hydrological periods, we could observe that samples in falling waters had higher dissimilarity than rising waters in both layers of diversity (Supplementary figure II – 5). The same happened for samples of the ATTZ and lake sites in rare OTUs (Supplementary figure II – 6). Samples of the ATTZ sites presented a lower dissimilarity, and samples of lake sites presented a higher dissimilarity. For all OTUs and abundant OTUs we could not observe these patterns.

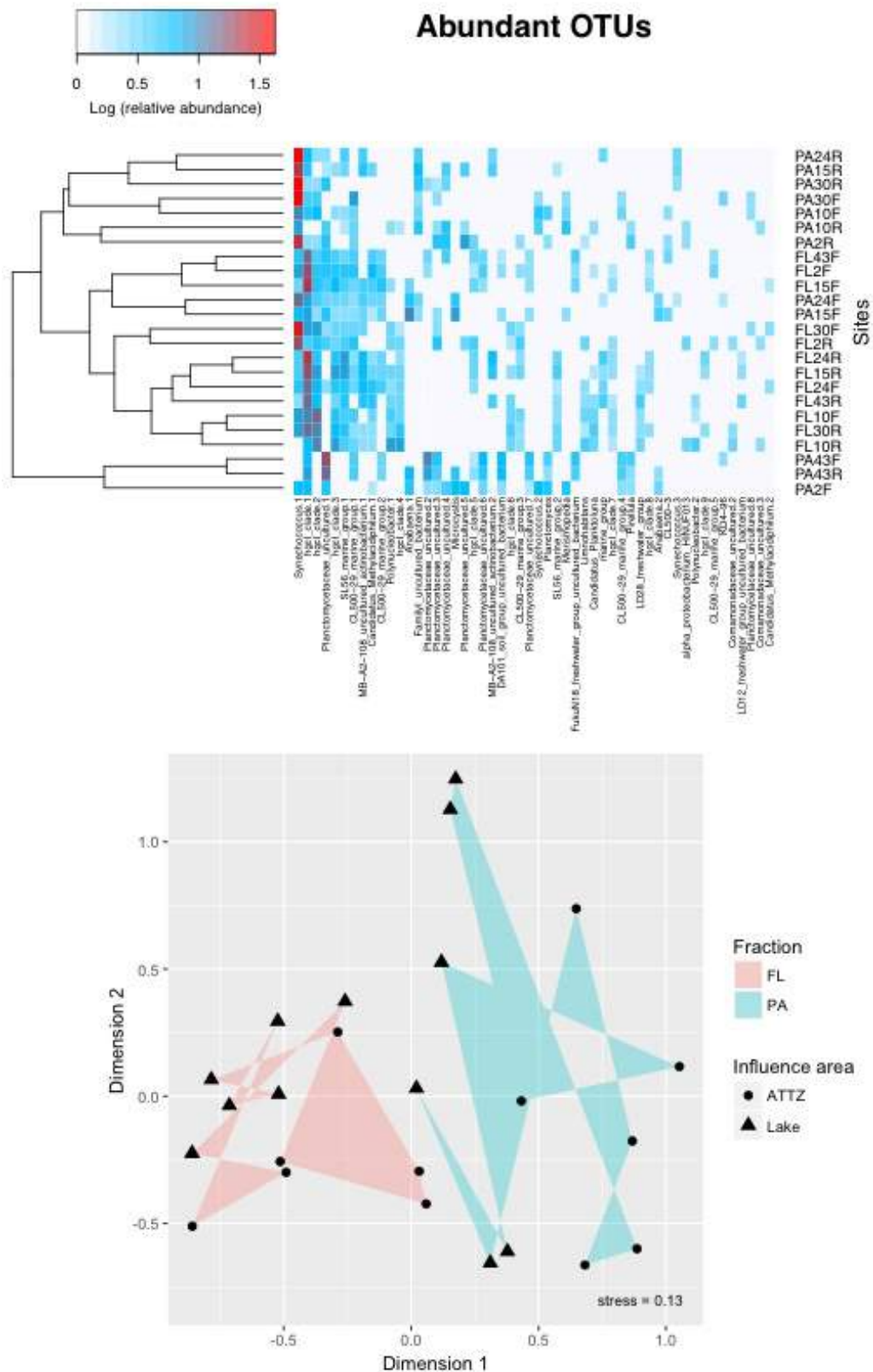
In cluster analyses we observed that within the PA fraction of abundant OTUs there was a separation of samples with higher abundances of *Synechococcus.1* and lower abundance of *hgcI\_clade.1*, sites with an intermediate abundance of *Synechococcus.1* and higher abundance of *hgcI\_clade.1*, and sites with low abundance or absence of *Synechococcus.1* and higher abundance of *Planctomycetes\_uncultured.1* (Figure I - 5). In the FL fraction, we could observe the separation of two groups of samples, one with the presence

of *Planctomyces\_uncultured.1* and one with absence of this OTU (Figure I - 5).

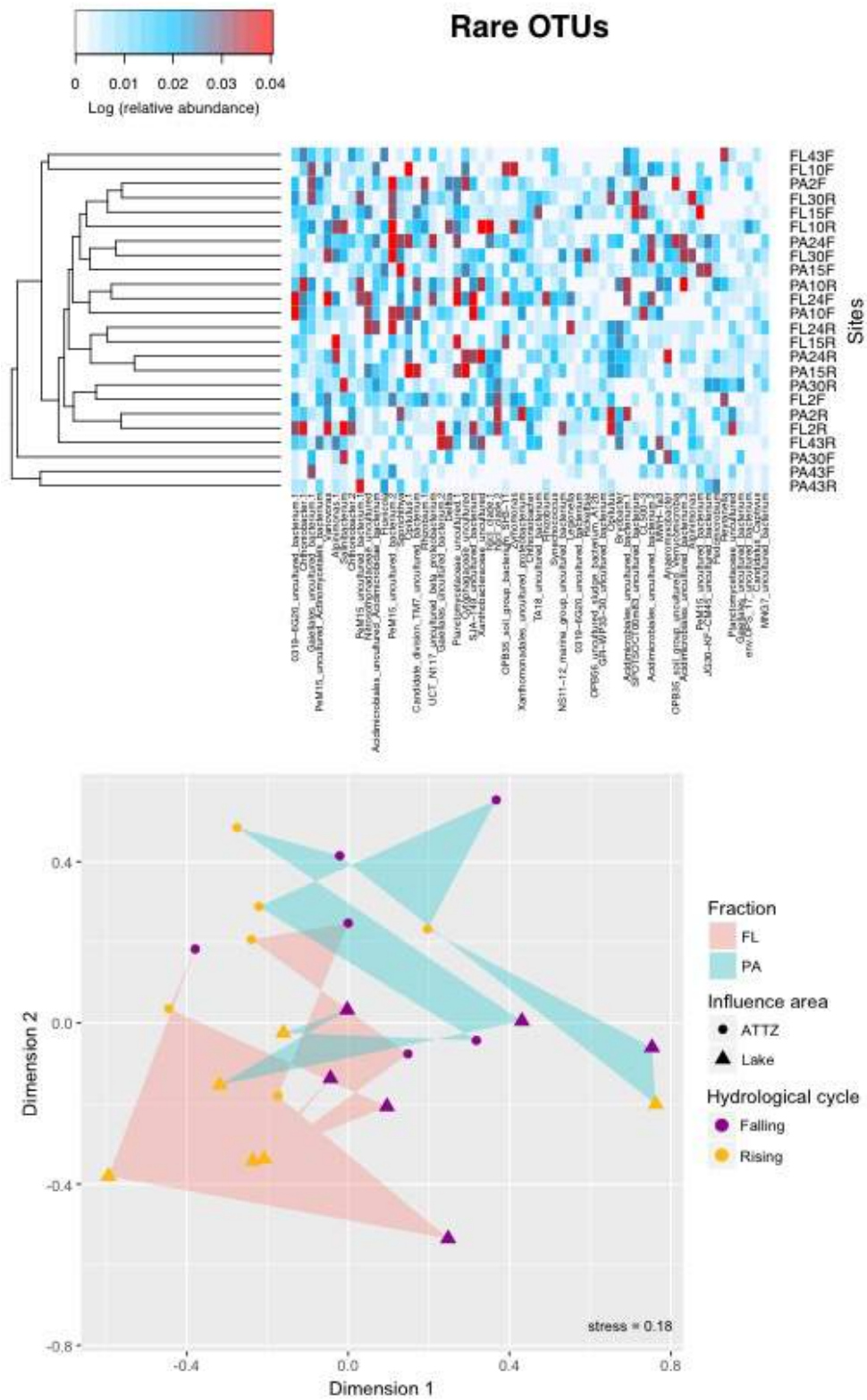
Among rare OTUs, cluster analysis did not reveal a clear trend of distribution (Figure I - 6). In both cases, cluster analysis did not separate samples by area or hydrological period.



**Figure I - 4:** Non-metric multidimensional scaling (NMDS) ordinations with Bray-Curtis distances of all OTUs. Sampling points grouped based on size fractions ( $R^2 = 0.24$  and  $p = 9.999 \text{ e-}05$ ). Shapes indicate influence area ( $R^2 = 0.10$  and  $p = 0.001$ ) and colors indicate the hydrological periods ( $R^2 = 0.07$  and  $p = 0.01$ ).



**Figure I - 5:** Heat map of abundant OTUs with sites grouped by cluster analyses, and non-metric multidimensional scaling (NMDS) ordinations with Bray-Curtis distance of abundant OTUs, sampling points grouped based on size fractions ( $R^2 = 0.25$  and  $p = 9.999 \times 10^{-5}$ ). Shapes indicate influence area ( $R^2 = 0.09$  and  $p = 0.03$ ). For graphical representation purposes, we displayed only OTUs that were present in at least 3 samples.



**Figure I - 6:** Heat map of rare OTUs with sites grouped by cluster analyses, and non-metric multidimensional scaling (NMDS) ordinations with Bray-Curtis distance of rare OTUs, Sampling points grouped based on size fractions ( $R^2 = 0.07$  and  $p = 9.999 \times 10^{-5}$ ). Shapes indicate influence area ( $R^2 = 0.07$  and  $p = 0.0002$ ) and colors indicate the hydrological periods ( $R^2 = 0.07$  and  $p = 9.999 \times 10^{-5}$ ). For graphical representation purposes, we displayed only OTUs that were present in at least 14 samples.

### ***Indicative OTUs of hydrologic period and influence area***

We performed IndVal analyses to find indicative OTUs of fractions, hydrologic periods and of influence area. The detailed information on the taxonomy of indicative OTUs may be found in Supplementary Material II (Tables II – 1 and II – 2). We found 47 indicative OTUs of FL fraction and 73 of PA fraction. Among them OTUs belonging to *Proteobacteria* phyla (mostly *Alphaproteobacteria* and *Betaproteobacteria*) were the most representative in FL fraction, followed by *Actinobacteria*, *Acidobacteria* and *Chloroflexi*. Interestingly, the most representative OTU was an *Acidobacteria* (*Subgroup\_6*). For the PA fraction, most of the representatives belonged to *Proteobacteria* (mostly *Betaproteobacteria* and *Gammaproteobacteria*).

Regarding to indicative OTUs of hydrologic periods, we found 103 indicatives of falling waters and 72 of rising waters. Most falling waters representatives belonged to *Planctomycetes*, *Proteobacteria* (mostly *Alphaproteobacteria*) and *Cyanobacteria*. Rising waters representatives were mainly *Proteobacteria* (mostly *Betaproteobacteria*) and *Actinobacteria*.

For influence area, we just found eight indicative OTUs. ATTZ representatives were *Cyanobacteria*, *Planctomycetes*, *Proteobacteria* and *Actinobacteria*. Lake representatives were three OTUs belonging to *Proteobacteria* and one *Bacteroidetes*.

### ***Environmental drivers of bacterioplankton community composition***

In order to understand which factors explain beta diversity (dissimilarity) in BCC we performed a Mantel test between community matrix and environmental factors. Each size fraction was influenced by different environmental factors. For all OTUs, the FL fraction had a significant relationship with water transparency (Secchi disk depth), TDN, pH, TN and water column depth, while the PA fraction had Si concentrations and distance from the

Amazon river as the driver factors (Table I - 2). Considering the abundant OTUs, the FL fraction was related to TDN, Secchi disk depth, pH and TN, while the PA fraction was related to distance from the Amazon river, Si and Chlorophyll *a* concentrations. For rare OTUs we found significant relationships with conductivity, POC and TOC in FL fraction, and distance from the Amazon river, Si and TP concentrations in the PA fraction (Table I - 2).

**Table I - 2:** Mantel test significant relationships between dissimilarity matrices (Bray Curtis distance) in different diversity layers (all OTUs, abundant OTUs and rare OTUs), with environmental distance matrices (Euclidean).  $r$  = Pearson product-moment correlation coefficient except for those marked with “\*” which are Spearman’s correlation coefficient (non-parametric).

<b>Taxonomic resolution</b>		<b>r</b>	<b>p</b>	
All OTUs	FL*Secchi	0.38	<b>0.007</b>	
	FL*TDN	0.35	<b>0.023</b>	
	FL*pH	0.29	<b>0.019</b>	
	FL*TN	0.27	<b>0.031</b>	
	FL*Depth	0.27	<b>0.042</b>	
	PA*Si	0.37	<b>0.009</b>	
	PA*Distance river	0.33	<b>0.015</b>	
Abundant OTUs	FL*TDN	0.45	<b>0.009</b>	
	FL*Secchi	0.39	<b>0.011</b>	
	FL*pH	0.32	<b>0.009</b>	
	FL*TN	0.27	<b>0.042</b>	
	PA*Distance river	0.35	<b>0.006</b>	
	PA*Si	0.34	<b>0.016</b>	
	PA*Chlorophyll a	0.31	<b>0.025</b>	*
Rare OTUs	FL*Alkalinity	0.39	<b>0.013</b>	
	FL*Conductivity	0.38	<b>0.004</b>	*
	FL*POC	0.35	<b>0.012</b>	
	FL*TOC	0.26	<b>0.04</b>	*
	PA*Distance river	0.35	<b>0.015</b>	
	PA*Si	0.33	<b>0.025</b>	
	PA*TP	0.35	<b>0.015</b>	

## Discussion

This is the first detailed study on bacterioplankton community that accounts for spatial and temporal variations from a flood pulse perspective in an Amazon floodplain lake. A previous metagenome study in one sample of the Solimões river found a lack of close related sequences in the databases (Ghai *et al.*, 2011). In our samples, we found a higher number of OTUs that were not classified at genus (40.3% of abundant and 59.6% of rare) and species level (88.4% of abundant and 92.3% of rare). This high degree of novelty was also found in another recent work in Brazilian floodplain lakes, including Amazon samples (Tessler *et al.*, 2016). They found that 22% of the sequences could not be identified at genus level, and 5% at class level. The Amazon biome, is known by its mega diversity of plants and animals (Wiens *et al.*, 2011), together these results call attention to the high degree of novelty also among bacterioplankton samples, which deserves further exploration.

Across the 103 OTUs with high relative abundance found in this work, the most abundant, uncultured *Synechococcus*, had a variable distribution among sites. Members of *Synechococcus* genus are commonly found in freshwaters and are expected to dominate and persist throughout the year in tropical regions (Sarmiento *et al.*, 2008; Sarmiento, 2012). The dominance of *Synechococcus* can be associated to high growth rates at elevated temperatures, and high surface:volume ratio that maximizes their light and nutrient uptake (Lewis, 1976; Reynolds, 1984; Dvořák *et al.*, 2014). In our work, although *Synechococcus* had a higher relative abundance in rising period (13.5%) than in falling one (10.2%), they dominated the bacterioplankton in both hydrological periods. The uniqueness of Brazilian floodplain systems when compared with global locations has been attributed mainly to the higher abundance of *Cyanobacteria* members (Family I and Family II mostly) (Tessler *et al.*, 2016). They assigned the higher abundance of these organisms to the ephemeral nature of floodplains lakes, which favor resistant organisms. This is a reasonable explanation for the



high abundance of *Synechococcus* in this system, since they are capable to resist the drastic changes in environmental conditions across hydrological phases.

The second and third most abundant OTUs were members of *hgcI*\_clade (also known as acI lineage). These two OTUs presented higher relative abundance in rising period (accounting for 19.1% shared between two OTUs). The *hgcI*\_clade is a group typically found in freshwater systems such as reservoirs (Llirós *et al.*, 2014), estuaries (Liu, J. *et al.*, 2015) and lakes (Newton *et al.*, 2011; Ghylin *et al.*, 2014). The metagenome from a Solimões sample revealed a high abundance of this group, corresponding to 73% of actinobacterial sequences (Ghai *et al.*, 2011). Because of their abundance and high metabolic activity, this group has an important role in the carbon cycle, and has been considered as a carbon sink since they are capable to escape predators because of their small size (Salcher, 2013). A recent single cell genomic study demonstrated that members of this lineage has a streamlined genome with higher gene content related to the degradation of carbohydrate and organic nitrogen compounds than other typical freshwater bacteria (*Polinucleobacter* and *LD12*) (Ghylin *et al.*, 2014). Members of the *hgcI*\_clade are also capable to supplement their heterotrophic lifestyle with an anaplerotic carbon fixation metabolism, which is an alternative pathway to photoheterotrophic carbon fixation, thanks to machinery associated with actinorhodopsin genes. Altogether these characteristics explain the success of these organisms and their higher abundance in the dynamic period of rising waters in this Amazon floodplain system.

Another abundant OTU was an uncultured member of the family *Planctomycetaceae*, which had similar relative abundance in rising (2.5%) and falling period (3.3%). *Planctomyces* are normally found in low abundance in freshwater systems (Newton *et al.*, 2011; Liu, L. *et al.*, 2015). However, some studies reported high abundance associated with cyanobacterial blooms (Cai *et al.*, 2013; Woodhouse *et al.*, 2016). Interestingly, the relative

abundance of *Cyanobacteria* members was also similar between both hydrologic periods (rising – 18.3% and falling – 18.6%).

The “rare biosphere” is a term coined to define the “long tail” of species with a relative abundance usually lower than 0.1% that comprises the majority of diversity (Pedrós-Alió, 2006). Besides low abundance, these species present low growth rates, high capacity to escape to predator and viruses (small cell size) and can play significant roles in nutrient cycles (Pedrós-Alió, 2012). Recent studies have done efforts in understand the distribution patterns of the rare biosphere in different ecosystems (Galand *et al.*, 2009; Logares *et al.*, 2013; Shade *et al.*, 2014; Aanderud *et al.*, 2015; Liu *et al.*, 2015; Lynch e Neufeld, 2015). They have shown that rare OTUs are subject to environmental filtering and can display specific and sometimes unique distribution patterns, different from the abundant OTUs. Moreover, the rare biosphere can contribute up to 97% in the variability of the temporal community dissimilarity (Shade *et al.*, 2014). In our study, we found that the rare biosphere was significantly different between fractions, hydrologic period and influence area (PERMANOVA results), although these patterns are not clear in the NMDS ordination and cluster results (Figure I - 6). These results are similar to all OTUs together, however with a lower explanation. Meanwhile, analyses of abundant OTUs separately showed significant differences only for fraction and influence area. Together, our results point to an important influence of the rare biosphere for the distribution patterns of the role community in this system.

Based on Bray-Curtis dissimilarity index we could observe that the community of this Amazon floodplain system displayed differences between FL and PA fractions (PERMANOVA results) in all diversity layers. Although, the result was more pronounced among all OTUs and abundant OTUs ( $R^2 = 0.24$  and  $R^2 = 0.25$ , respectively). These changes were also perceptible in the distribution of the main phyla. The phyla *Actinobacteria* and *Proteobacteria* were the most abundant in FL fraction and we also found a higher abundance

of *Chloroflexi* in the FL fraction. For PA fraction, *Cyanobacteria* and *Planctomycetes* were the most abundant. We could see an accordance also in the results of indicator taxa. The main indicator taxa in the FL fraction were members of *Proteobacteria* (mainly *Alphaproteobacteria*) and *Actinobacteria*. We also found four indicative OTUs belonging to *Chloroflexi*. For the PA fraction the main indicative OTUs were *Proteobacteria* (mainly *Betaproteobacteria*) and *Bacteroidetes* but we also found *Cyanobacteria* and *Planctomycetes* as important indicative OTUs. Although *Proteobacteria* members are usually recovered in both size fractions, the class *Betaproteobacteria* is often more abundant in the particle-associated fraction (Newton *et al.*, 2011). Members of *Bacteroidetes* phyla also may compose a high proportion of particle-associated bacteria (Newton *et al.*, 2011). *Planctomycetes* members can degrade phytoplankton-derived carbohydrates and are found in association with algal blooms, including cyanobacterial blooms (Newton *et al.*, 2011; Cai *et al.*, 2013; Woodhouse *et al.*, 2016). They are also abundant in sediments from white and black waters of Amazon (Ji *et al.*, 2016), which may explain why they are indicative of the PA fraction. A recent study in 11 north – temperate freshwater systems, also found that *Actinobacteria* was overrepresented in the FL fraction and *Cyanobacteria* and *Planctomycetes* in PA fraction (Schmidt *et al.*, 2016). They attribute it mainly to the cell size. *Planctomycetes* usually have larger cell sizes due to the presence of cellular structures and budding cell division and *Cyanobacteria* presents filamentous forms and cells that forms microcolonies, such as *Synechococcus* (Schmidt *et al.*, 2016). As abovementioned *Actinobacteria* members are known by their small size, being mentioned as ultramicrobacteria and for this reason they are more recovered in the FL fraction (Salcher, 2013).

The significant differences between rising and falling waters (PERMANOVA results) are also consistent with the distribution of the main phyla and the temporal indicative OTUs. We found that the main difference between both hydrological periods was in the abundance of

*Proteobacteria* (rising – 15.9% and falling – 12.2%) and *Planctomycetes* (rising – 13.7% and falling – 16.3%). When we look for the indicative OTUs we can see that the main indicative OTUs of rising waters were *Proteobacteria* (mainly *Betaproteobacteria*), *Actinobacteria* and *Chlamydiae*. For the falling waters the main indicative OTUs were *Planctomycetes*, *Proteobacteria* (mainly *Alphaproteobacteria*) and *Cyanobacteria*. Another study in Amazon waters found similar patterns of indicative OTUs (Doherty *et al.*, 2017). They also found a higher proportion of *Betaproteobacteria* during the high discharge period (rising waters) and higher proportions of *Actinobacteria*, *Cyanobacteria* and *Alphaproteobacteria* in the low discharge period (falling waters) (Doherty *et al.*, 2017). The changes between *Betaproteobacteria* and *Alphaproteobacteria* were mainly attributed to the characteristics of the system between rising and falling waters. The authors suggest that *Betaproteobacteria* organisms are favored by the dynamic conditions associated with high rainfall and high river discharge (Doherty *et al.*, 2017). These results are also consistent with a study in Hunter river that evaluated the effects of a high flooding event on the bacterioplankton community composition (Carney *et al.*, 2015). They also found that the importance of *Proteobacteria* decrease from flooding conditions to low inflow conditions and the classes composition changed. The *Proteobacteria* community in the flooding period was mainly composed by *Betaproteobacteria* and in the low inflow period the contribution of *Alphaproteobacteria* and *Gammaproteobacteria* increased. They attributed this result to the change between high allochthonous input in high inflow period and high autochthonous nutrients in low inflow period (Carney *et al.*, 2015).

Interestingly, the distribution of the main phyla in ATTZ sites was similar to the rising water phase *Proteobacteria* (15.7%) and *Planctomycetes* (14.0%). The same happened for lake sites and falling waters phase *Proteobacteria* (12.4%) and *Planctomycetes* (16.0%). We also could see resemblance in the dissimilarity patterns based on Bray-Curtis index of

samples between rising and falling waters and ATTZ and lake systems (among rare OTUs). Samples from rising waters and ATTZ had a lower dissimilarity (lower dispersion of points) than falling waters and lake samples, probably caused by a mass effect (Logue e Lindström, 2008). Water rising probably carries microorganisms from terrestrial ecosystem into aquatic systems and causes an homogenization of the community, this effect is more pronounced in the sites more subject to lateral influence such as ATTZ sites. On the contrary, during falling waters, when the inflow is low, there was sufficient time for the species sorting to select typical aquatic taxa (Logue e Lindström, 2008). The same happens in lake sites, that are more isolated from lateral influence. This result is accordance with the study in the Hunter river (Carney *et al.*, 2015). They found that samples from flooding conditions had a higher similarity than samples during the low inflow period, which they attributed to the mass effect causing homogenization of samples in the flooding and the specie sorting selecting species in the low inflow period (Carney *et al.*, 2015). It is also interesting highlight that these differences in the dissimilarity distribution between ATTZ and lake was noted just among rare OTUs, which leads us to think that the spatial complexity has an effect more pronounced over the rare OTUs (Supplementary figure II - 6).

Since we found these composition patterns, we were interested in understanding which factors drove those patterns. The Curuai, as most Amazonian floodplain system, has a marked seasonality driven by the local rainfall and the Amazon river flooding (Bourgoin *et al.*, 2007). Usually water inflow into the floodplain starts between December and February and lasts until June (Bonnet *et al.*, 2008). The water outflow from the floodplain into the river main channel starts in June and lasts until December. During the rising phase the river water enters the system and carry high amounts of sediment particles (peak between January and March) and the main source of organic matter in the floodplain is the Amazon river (Bourgoin *et al.*, 2007; Moreira-Turcq *et al.*, 2013). In the exportation phase water and sediments flow out of

the system (peak between July and October) carrying an important pool of labile dissolved and particulate organic matter (Bourgoin *et al.*, 2007; Moreira-Turcq *et al.*, 2013). We found a similar pattern in the period studied. The rising waters phase was marked by significant higher values of conductivity, TSS and POC that indicates the high amount of particles in this period, and higher DOC/Chlorophyll *a* ratio, which corroborates that the main source of organic matter in this phase was from allochthonous source. On the other hand, the falling waters phase was marked by higher N/P ratio and pH and low DOC/Chlorophyll *a* ratio, which together indicates the predominance of phytoplankton primary productivity in this period (Table I - 1). To investigate the influence of this seasonality in the bacterioplankton composition patterns, we performed simple Mantel test in all diversity layers and in both fractions. We found that FL community was influenced mainly by the nutrient concentrations (TDN, TN, TOC and POC) and by physicochemical factors (pH, secchi, alkalinity and conductivity). With exception of Secchi disk depth and water column depth that presented same values in both periods, all these factors presented differences between rising and falling waters (pH, alkalinity, conductivity and POC were significantly different). These results points to a strong influence of the flood pulse over the composition of the FL community. On the other hand, the PA fraction was influenced mainly by Si and distance from the Amazon river in all diversity layers. A study in Amazon waters found a significant increase in bacterial abundance as distance from Amazon river increase (Almeida *et al.*, 2015). They attributed this latitudinal gradient to a decrease of the effect of the Amazon river waters as one moves upriver, consequently the quality and quantity of DOC increase as the bacterial abundance (Almeida *et al.*, 2015). Although not significant, the distance from the Amazon river was different for ATTZ (in average 19.5 km) and lake (in average 10.8 km) sites, which points to a more pronounced influence of the characteristics of the groups of sites in the PA fraction. Water physical and chemical properties in Amazon floodplain systems are extremely

heterogeneous in space and time (Sioli, 1984). These two layers of complexity were reflected and shaped the community composition differentially between size fraction and diversity layers (abundant OTUs were influenced only by size fraction and influence area) in the Curuai floodplain system.

Based on the found results we may conclude that, the community composition patterns were better explained by size fraction in all diversity layers. The rare OTUs had an important contribution to composition patterns of hole community and the abundant OTUs did not present significant difference between rising and falling waters. The FL fraction was mainly influenced by the changes in the water chemistry due to the hydrologic pulse (inorganic and organic nutrients, alkalinity, conductivity and pH) and the PA fraction by the distance from the Amazon river. The dissimilarity of the samples in river and ATTZ sites as well as falling waters and lakes sites points to different forces acting in shaping the bacterioplankton community regionally and temporally in this system. Being the mass effect an important shaping factor in rising waters and ATTZ sites, and the environmental filters more active in the falling waters and lake sites. The dissimilarity results also point to a more pronounced effect of the spatial complexity over the rare biosphere. The spatial and temporal complexity of this system were reflected in BCC. Finally, the Amazon has an elevated degree of uniqueness among bacterioplankton, which deserves further exploration.

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## **Conclusões**

**1** – Os padrões espaço-temporais do bacterioplâncton foram melhor explicados pelas diferenças entre frações FL e PA em todas as OTUs, OTUs abundantes e raras.

**2** – As OTUs raras apresentaram uma importante contribuição para os padrões de composição de toda a comunidade e as OTUs abundantes não apresentaram diferenças significativas entre enchente e vazante.

**3** – A fração FL foi influenciada principalmente pelas mudanças nas características químicas da água devido ao pulso de inundação (nutrientes inorgânicos e orgânicos, alcalinidade, condutividade e pH) e a fração PA pela distância do rio Amazonas.

**4** – A dissimilaridade das amostras de enchente e ATTZ, assim como das amostras de vazante e lago apontam para diferentes forças atuando regionalmente e temporalmente neste sistema. Sendo o efeito de massa um importante fator modulador na enchente e pontos de ATTZ e os filtros ambientais mais ativos na vazante e pontos de lago.

**5** – Os resultados de dissimilaridade também apontam para um efeito mais pronunciado da complexidade espacial sobre a biosfera rara.

**6** – A complexidade espacial e temporal deste sistema foram refletidas na composição da comunidade do bacterioplâncton.

**7** – A Amazônia apresenta um elevado grau de novidade na comunidade do bacterioplâncton, o que merece futura exploração para desvendar a biodiversidade dos microrganismos nesse sistema.

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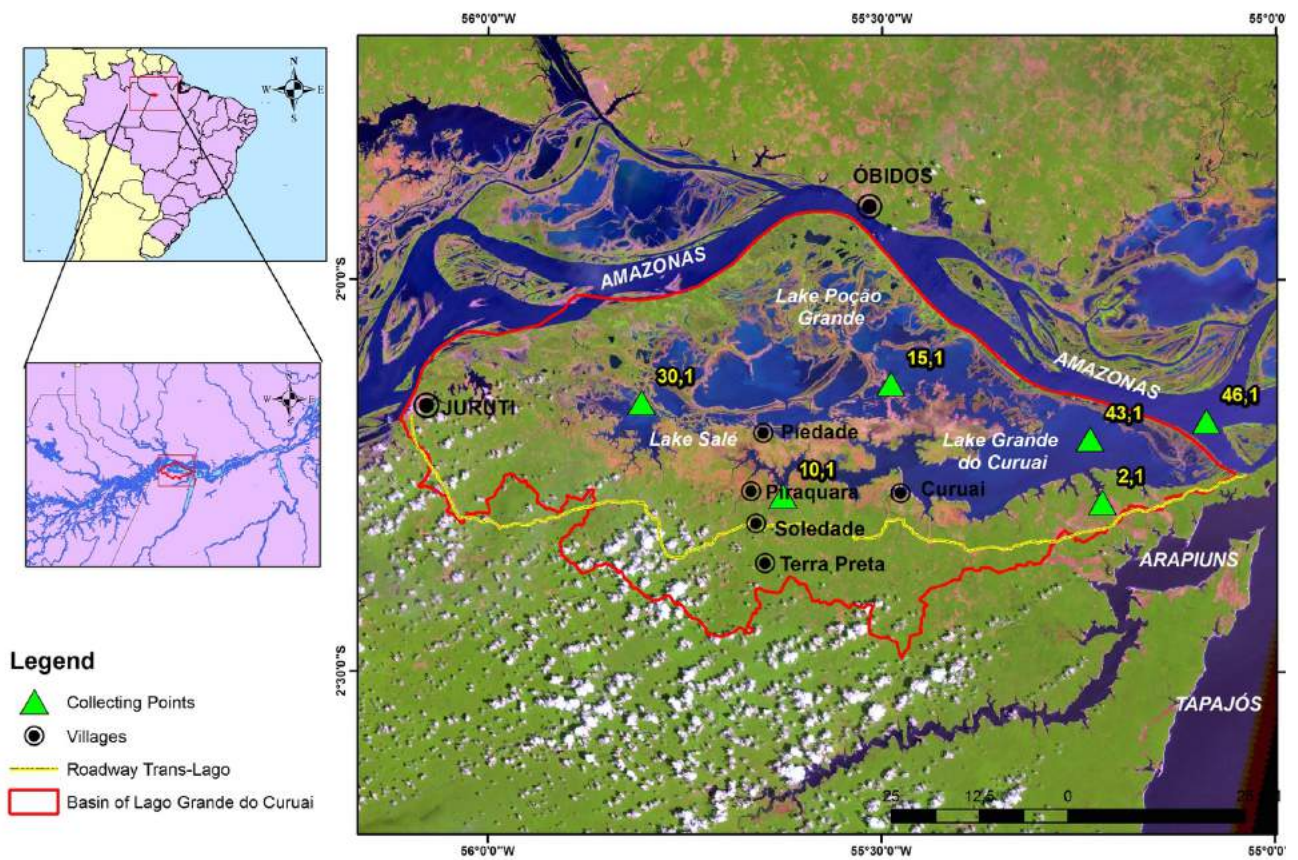
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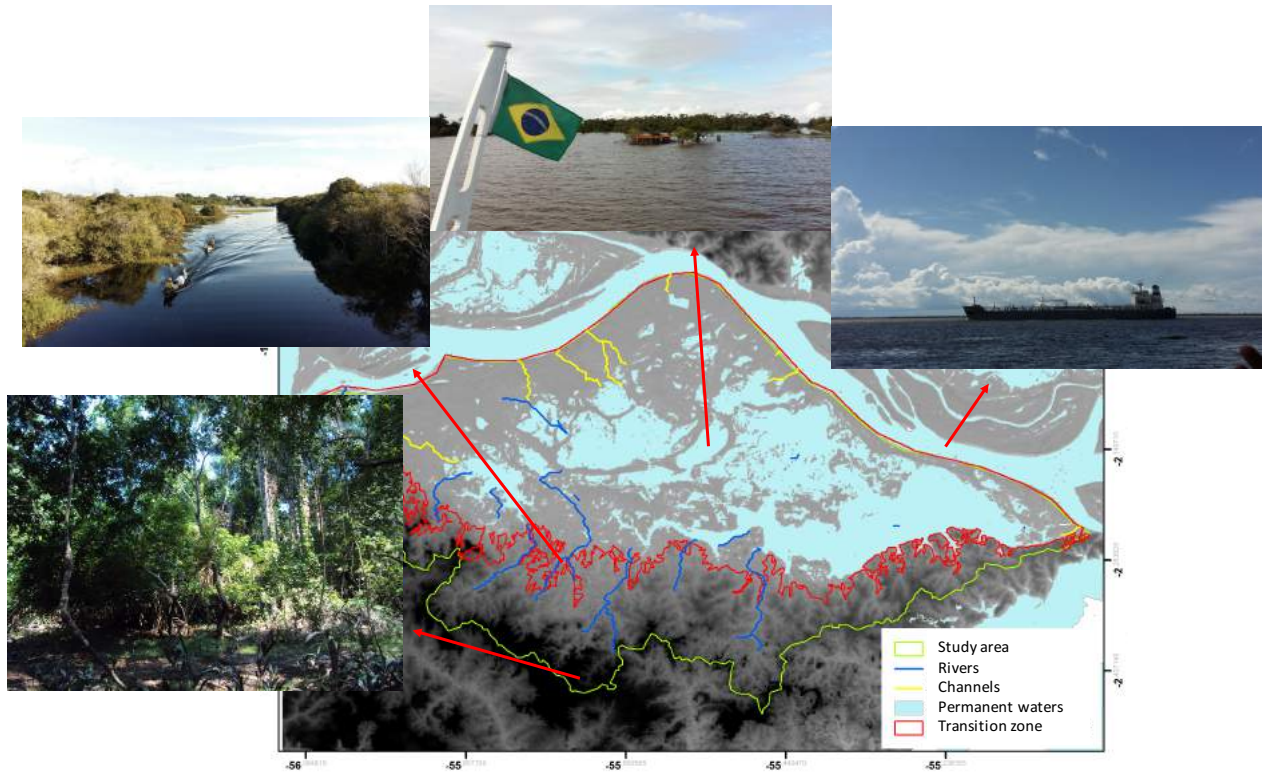
## Supplementary Material I

### Detailed information about study area

The following figures describe the main characteristics of study area, surrounding communities, different types of habitats, changes in water level during the year and sampling procedures.



**Supplementary figure I - 1:** Study area with emphasis on sampling points and surrounding communities. Source: Lucas Garcia.



**Supplementary figure I - 2:** Study area highlighting the main ecosystems of Curuai basin (*Igarapés*, Open waters (lake and Amazon River), and land). Source: Lucas Garcia.





**Supplementary figure I - 3:** Study area at low waters period. Source: Lucas Garcia.



**Supplementary figure I - 4:** Study area at high waters period. Source: Lucas Garcia.

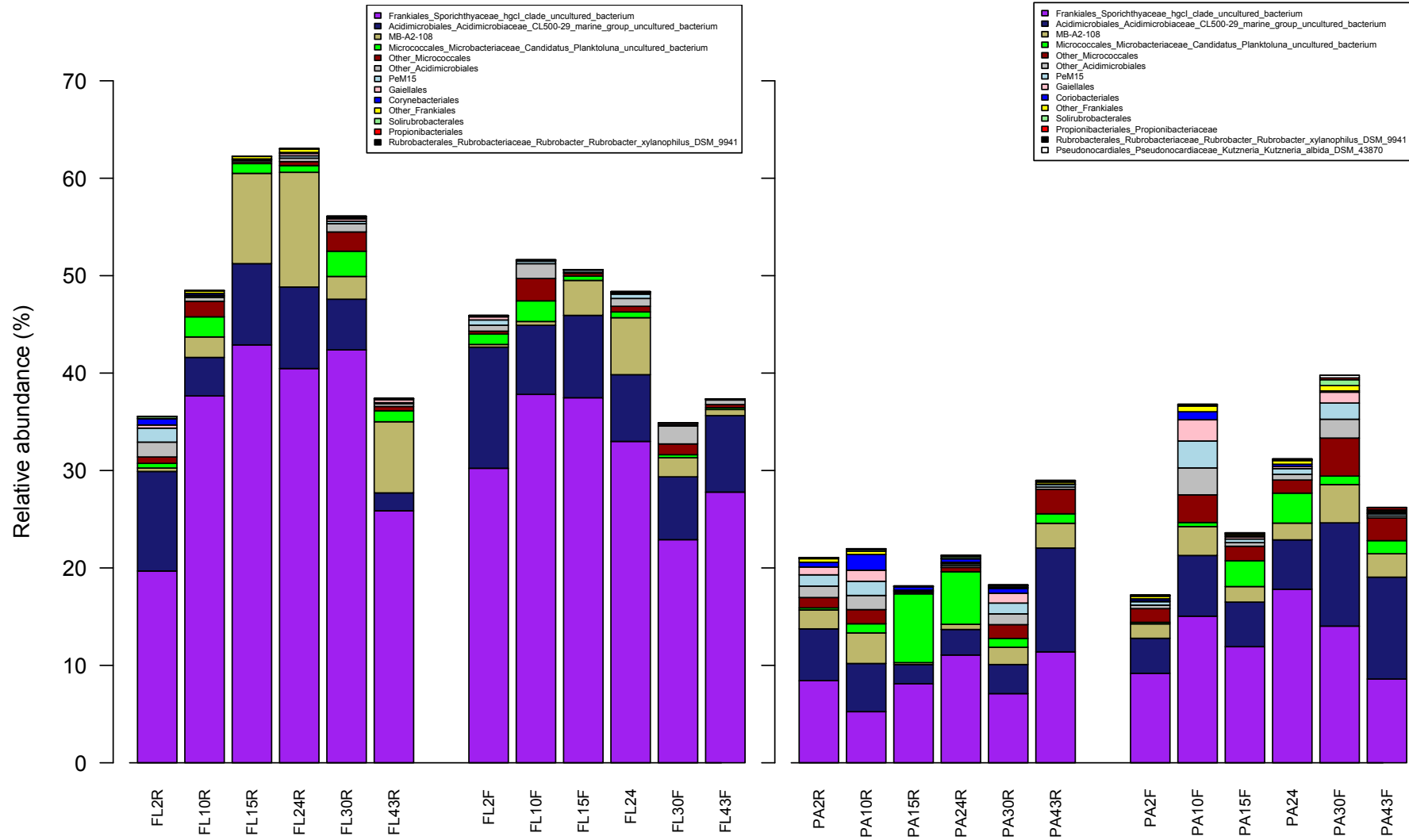


**Supplementary figure I - 5:** Sampling procedures (water collect with Van-Dorn bottle and filtration) and field workstation. Source: Leonardo Gomes.

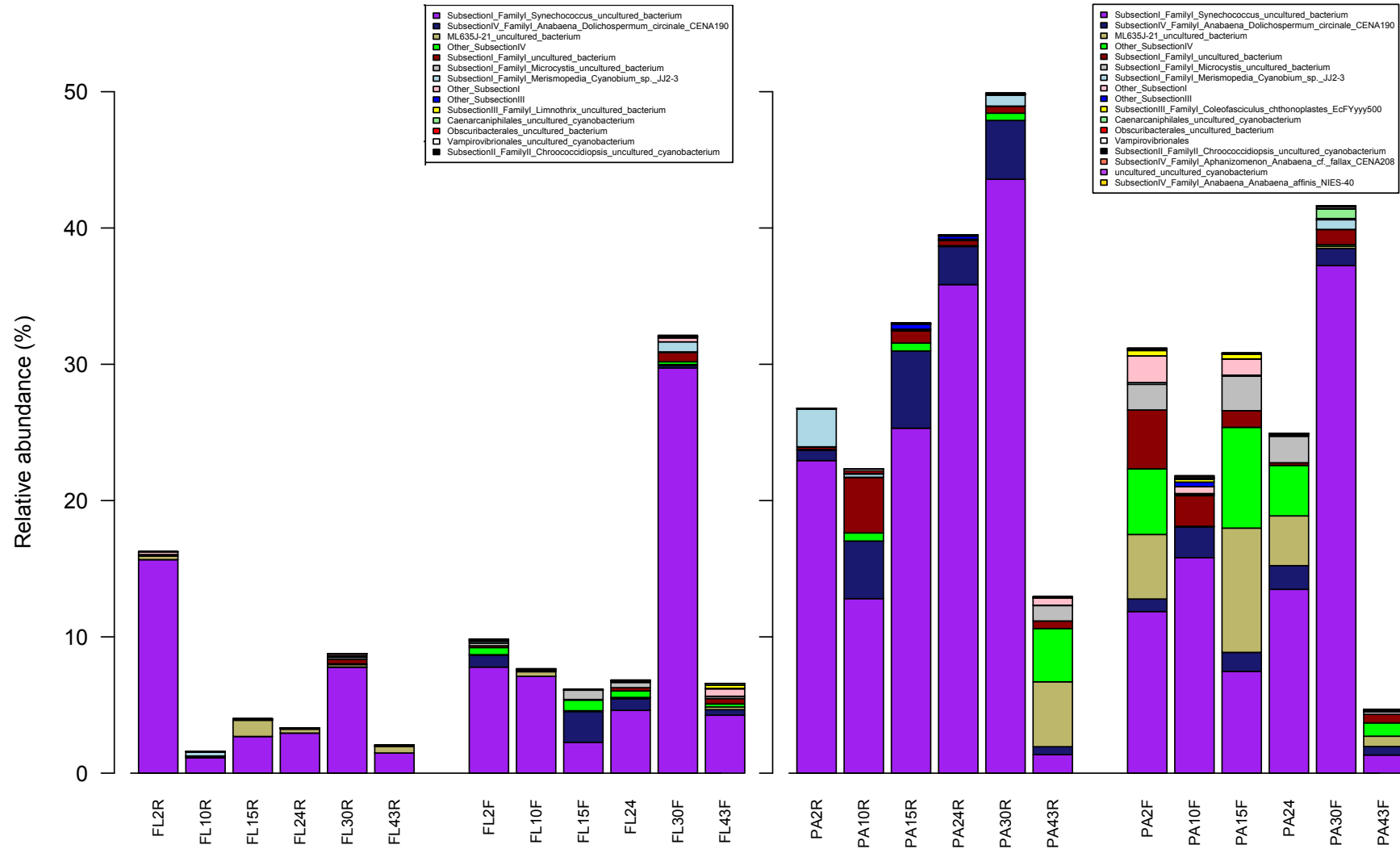
## **Supplementary Material II**

### **Detailed composition of four abundant phyla, betadispersion of samples and detailed information of indicative OTUs**

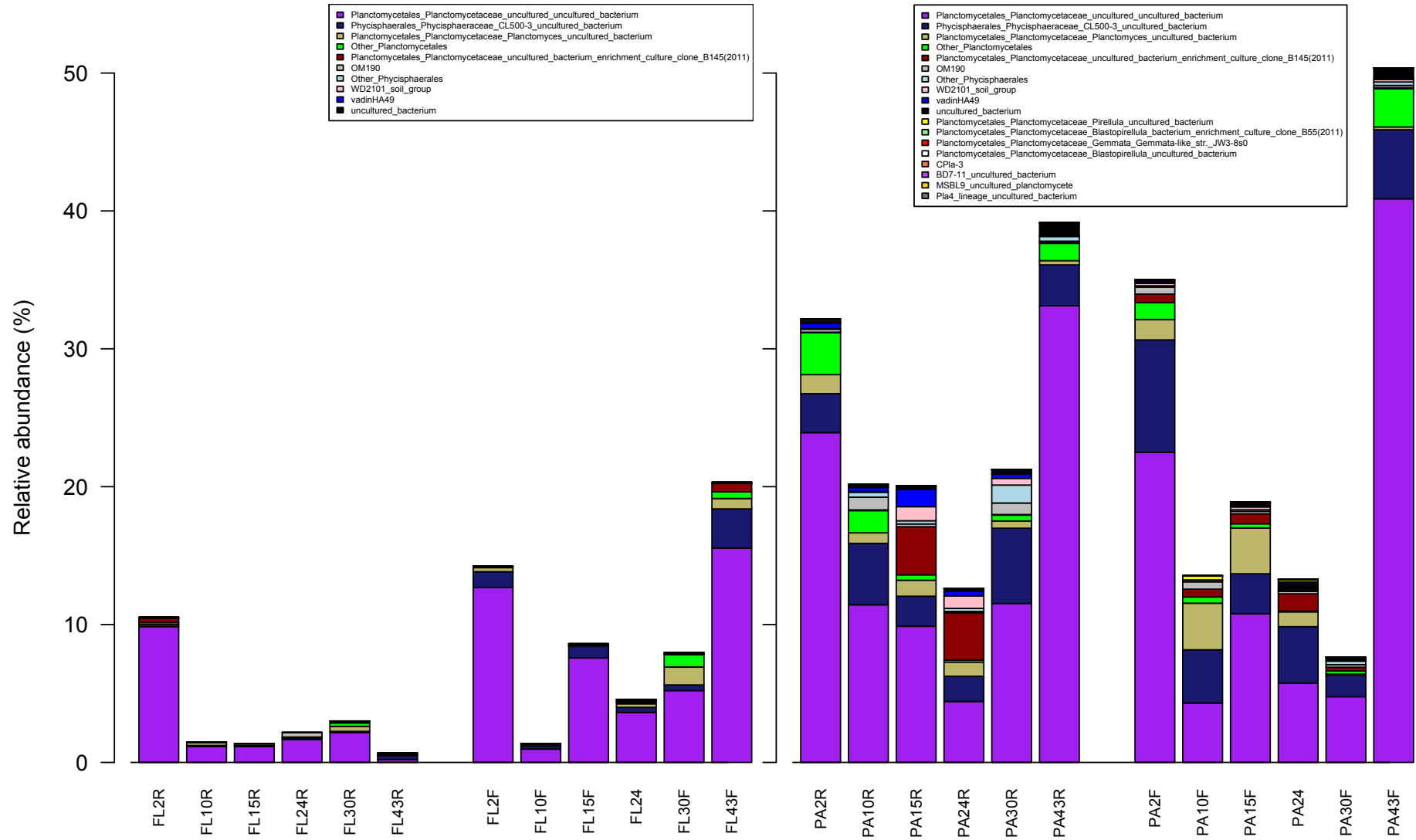
The following figures describe the relative abundance at genus level of the four major phyla in free living fraction (FL) and particle attached fraction (PA) at different sampling sites.



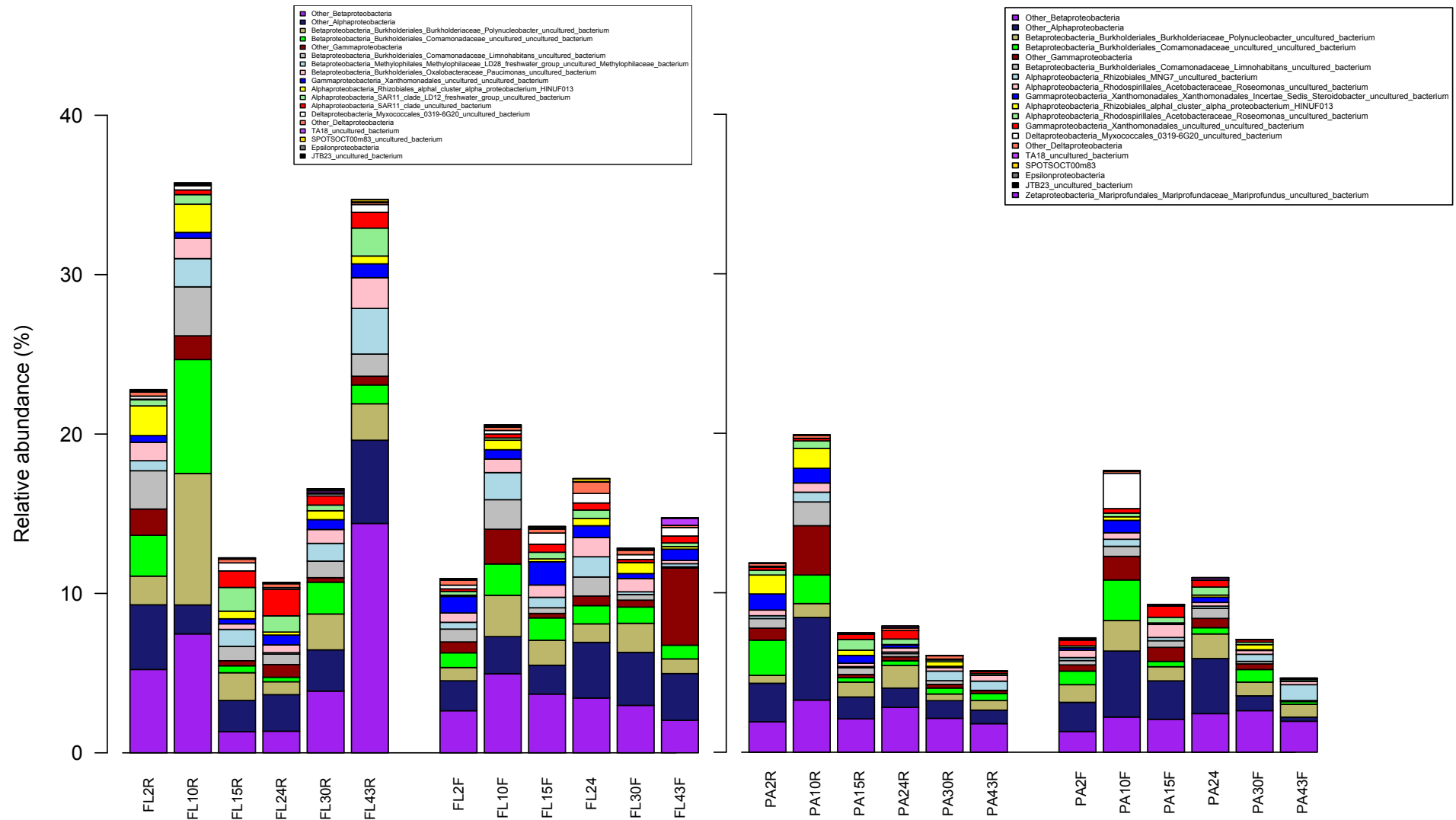
Supplementary figure II - 1: Detailed composition of phylum *Actinobacteria*.



Supplementary figure II - 2: Detailed composition of phylum *Cyanobacteria*.

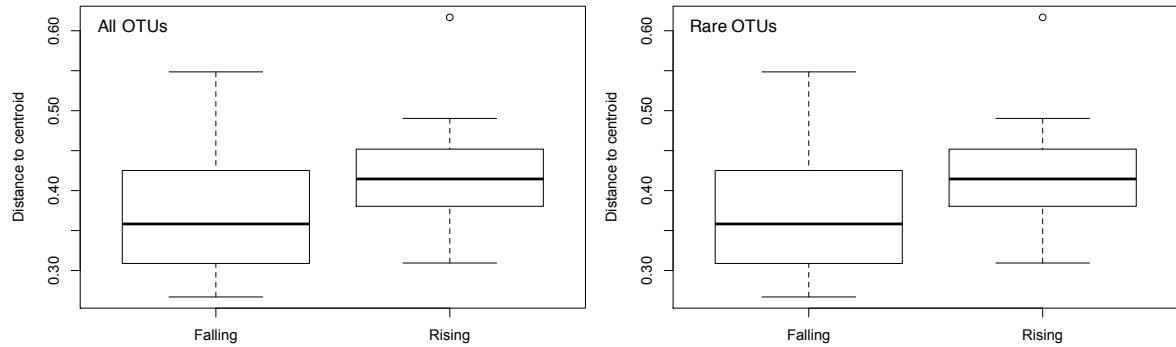


Supplementary figure II - 3: Detailed composition of phylum *Planctomycetes*

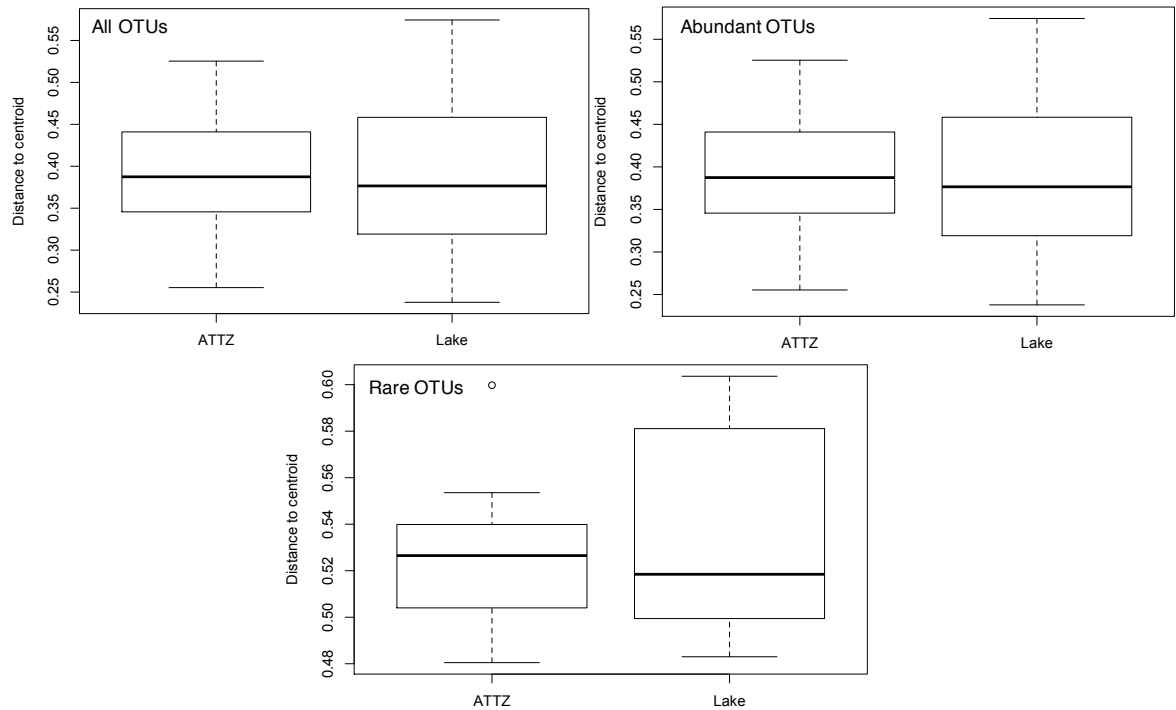


Supplementary figure II - 4: Detailed composition of phylum *Proteobacteria*.

The following figures describe the betadispersion of samples based in dissimilarity index with Bray-Curtis distance between hydrologic periods and influence area



**Supplementary figure II - 5:** Boxplot of betadispersion of samples (all OTUs and rare OTUs) for hydrologic periods based in dissimilarity index with Bray-Curtis distance.



**Supplementary figure II - 6:** Boxplot of betadispersion of samples (all OTUs, abundant OTUs and rare OTUs) for influence area based on dissimilarity index with Bray-Curtis distance.



The following tables describe the detailed information of indicative OTUs of fraction, hydrologic period and influence area.  
**Supplementary table II - 1:** Indicator value, p value and detailed taxonomy of fractions indicative OTUs.

NCBI Sequence	Fraction	Indicator value	P value	Frequency	Phyla	Class	Order	Family	Genus	Strain/Clone/Species
HQ114121	FL	0.92	0.001	9	Acidobacteria	Acidobacteria	Subgroup 6	uncultured bacterium		
KC554233	FL	0.90	0.001	14	Actinobacteria	Actinobacteria	Frankiales	Nakamurellaceae	Nakamurella	uncultured bacterium
KC554339	FL	0.88	0.002	19	Actinobacteria	MB-A2-108	uncultured bacterium			
JN656789	FL	0.87	0.002	21	Acidobacteria	Acidobacteria	Subgroup 6	uncultured Acidobacteria bacterium		
KC554912	FL	0.86	0.008	24	Actinobacteria	MB-A2-108	uncultured bacterium			
JF174954	FL	0.82	0.017	13	Actinobacteria	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	uncultured bacterium
JN869224	FL	0.80	0.049	20	Verrucomicrobia	Spartobacteria	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter	uncultured bacterium
JN869029	FL	0.79	0.001	16	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Prostheco bacter	uncultured bacterium
HM128683	FL	0.77	0.01	16	Actinobacteria	Acidimicrobiia	Acidimicrobiales	Acidimicrobiaceae	CL500-29 marine group	uncultured bacterium
JN672135	FL	0.77	0.01	15	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobium	uncultured bacterium
EU803802	FL	0.77	0.003	3	Proteobacteria	Alphaproteobacteria	SAR11 clade	uncultured bacterium		
EU133219	FL	0.76	0.009	14	Actinobacteria	Thermoleophilia	Gaiellales	uncultured		uncultured bacterium
EU592508	FL	0.76	0.028	17	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcl clade	uncultured bacterium
EU804053	FL	0.76	0.017	23	Proteobacteria	Alphaproteobacteria	SAR11 clade	LD12 freshwater group		uncultured bacterium
AM935724	FL	0.75	0.004	15	Chloroflexi	JG30-KF-CM66	uncultured Chloroflexi bacterium			
HM275988	FL	0.75	0.001	8	Actinobacteria	Thermoleophilia	Solirubrobacterales	480-2		uncultured bacterium
EU804028	FL	0.75	0.001	9	Actinobacteria	Acidimicrobiia	Acidimicrobiales	Acidimicrobiaceae	CL500-29 marine group	uncultured bacterium
FN668062	FL	0.74	0.003	17	Proteobacteria	TA18	uncultured delta proteobacterium			
AB672185	FL	0.71	0.023	4	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	Pseudospirillum	uncultured bacterium
AF523901	FL	0.70	0.033	18	Cyanobacteria	ML635J-21	uncultured bacterium			
AM935269	FL	0.67	0.019	18	Chloroflexi	JG30-KF-CM66	uncultured Chloroflexi bacterium			
GU305700	FL	0.67	0.001	6	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	Legionella	uncultured bacterium
HQ114054	FL	0.66	0.016	20	Planctomycetes	OM190	uncultured bacterium			
JF800690	FL	0.66	0.005	23	SHA-109	uncultured bacterium				
JN941791	FL	0.65	0.013	5	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	uncultured	uncultured bacterium
JN656931	FL	0.65	0.042	18	Chloroflexi	SL56 marine group	uncultured bacterium			
EU133918	FL	0.61	0.017	10	Chlamydiae	Chlamydiae	Chlamydiales	Simkaniaceae	Candidatus Rhabdochlamydia	uncultured bacterium
KC172329	FL	0.59	0.01	9	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales Incertae Sedis	Rhizomicrobium	uncultured alpha proteobacterium
DQ520173	FL	0.59	0.014	12	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcl clade	uncultured bacterium
AJ565420	FL	0.58	0.007	5	Proteobacteria	Alphaproteobacteria	Sphingomonadales	7B-8	Sphingomonadaceae bacterium MWH-CaK2	
JN391738	FL	0.57	0.016	14	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Planctomyces	uncultured bacterium
EF018643	FL	0.51	0.043	18	Acidobacteria	Acidobacteria	Subgroup 6	uncultured bacterium		
AB682299	FL	0.50	0.013	16	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Limnobacter	Limnobacter litoralis
DQ676318	FL	0.50	0.014	2	Verrucomicrobia	OPB33 soil group	uncultured Verrucomicrobia bacterium			
GU940751	FL	0.50	0.019	13	Cyanobacteria	ML635J-21	uncultured bacterium			
AY922093	FL	0.49	0.023	13	Candidate division OD1	uncultured Parcubacteria bacterium				
GQ007402	FL	0.48	0.044	5	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	12up	uncultured bacterium
GQ397007	FL	0.47	0.034	6	Chloroflexi	Thermomicrobia	JG30-KF-CM45	uncultured bacterium		
HQ860608	FL	0.42	0.034	4	Actinobacteria	Acidimicrobiia	Acidimicrobiales	Acidimicrobiaceae	CL500-29 marine group	uncultured bacterium
JQ428024	FL	0.42	0.031	5	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	uncultured	uncultured bacterium
HQ597523	FL	0.42	0.039	6	Acidobacteria	Acidobacteria	Subgroup 6	uncultured Acidobacteria bacterium		
GU731298	FL	0.42	0.042	15	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Devosia	bacterium enrichment culture clone heteroB88 4W
GU563747	FL	0.42	0.031	11	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Curvibacter	uncultured Curvibacter sp.
KF697487	FL	0.42	0.036	6	Acidobacteria	Acidobacteria	Subgroup 6	uncultured bacterium		

HM267237	FL	0.42	0.033	6	<i>Candidate division TM7</i>	<i>uncultured bacterium</i>				
JF904874	FL	0.42	0.037	3	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Neisseriales</i>	<i>Neisseriaceae</i>	<i>Aquitalea</i>	<i>Aquitalea sp. KJ011</i>
KC836077	FL	0.41	0.048	16	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Alcaligenaceae</i>	<i>MWH-UniP1 aquatic group</i>	<i>uncultured bacterium</i>
KC836049	PA	0.91	0.001	17	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Comamonadaceae</i>	<i>Sphaerotilus</i>	<i>uncultured bacterium</i>
JN032909	PA	0.91	0.001	24	<i>Planctomycetes</i>	<i>Phycisphaerae</i>	<i>Phycisphaerales</i>	<i>Phycisphaeraeaceae</i>	<i>CL500-3</i>	<i>uncultured bacterium</i>
JN869043	PA	0.86	0.011	21	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>uncultured</i>	<i>uncultured bacterium</i>
FJ916091	PA	0.86	0.001	17	<i>Bacteroidetes</i>	<i>Flavobacteriia</i>	<i>Flavobacteriales</i>	<i>NS9 marine group</i>	<i>uncultured Bacteroidetes bacterium</i>	
EU801389	PA	0.84	0.003	22	<i>Actinobacteria</i>	<i>Actinobacteria</i>	<i>Frankiales</i>	<i>Sporichthyaceae</i>	<i>hgcl clade</i>	<i>uncultured bacterium</i>
FJ830570	PA	0.83	0.003	23	<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>SubsectionIV</i>	<i>FamilyI</i>	<i>Anabaena</i>	<i>Dolichospermum circinale CENA190</i>
KC253357	PA	0.83	0.002	17	<i>Verrucomicrobia</i>	<i>Verrucomicrobia Incertae Sedis</i>	<i>Unknown Order</i>	<i>Unknown Family</i>	<i>Candidatus Methylocidiphilum</i>	<i>uncultured bacterium</i>
FJ612337	PA	0.81	0.009	24	<i>Verrucomicrobia</i>	<i>Spartobacteria</i>	<i>Chthoniobacterales</i>	<i>FukuN18 freshwater group</i>	<i>uncultured bacterium</i>	
AF247591	PA	0.80	0.012	22	<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>SubsectionIV</i>	<i>FamilyI</i>	<i>Anabaena</i>	<i>Anabaena affinis NIES-40</i>
GU305807	PA	0.79	0.004	11	<i>Planctomycetes</i>	<i>Phycisphaerae</i>	<i>Phycisphaerales</i>	<i>Phycisphaeraeaceae</i>	<i>CL500-3</i>	<i>uncultured bacterium</i>
HQ661200	PA	0.78	0.008	23	<i>Actinobacteria</i>	<i>Actinobacteria</i>	<i>Frankiales</i>	<i>Sporichthyaceae</i>	<i>hgcl clade</i>	<i>uncultured bacterium</i>
EU803688	PA	0.78	0.001	23	<i>Actinobacteria</i>	<i>Actinobacteria</i>	<i>Frankiales</i>	<i>Sporichthyaceae</i>	<i>hgcl clade</i>	<i>uncultured bacterium</i>
JF922442	PA	0.77	0.022	16	<i>Chloroflexi</i>	<i>KD4-96</i>	<i>uncultured bacterium</i>			
KC253307	PA	0.77	0.007	21	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Burkholderiaceae</i>	<i>Limnobacter</i>	<i>uncultured bacterium</i>
CU918341	PA	0.75	0.004	15	<i>Actinobacteria</i>	<i>Actinobacteria</i>	<i>PeM15</i>	<i>uncultured bacterium</i>		
JN868894	PA	0.75	0.003	18	<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Alteromonadales</i>	<i>Alteromonadaceae</i>	<i>BD1-7 clade</i>	<i>uncultured bacterium</i>
FJ830579	PA	0.74	0.005	22	<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>SubsectionIV</i>	<i>FamilyI</i>	<i>Aphanizomenon</i>	<i>Anabaena cf. fallax CENA208</i>
EU803321	PA	0.73	0.011	19	<i>Bacteroidetes</i>	<i>Cytophagia</i>	<i>Cytophagales</i>	<i>Cytophagaceae</i>	<i>uncultured</i>	<i>uncultured bacterium</i>
EU803667	PA	0.72	0.019	10	<i>Bacteroidetes</i>	<i>Sphingobacteriia</i>	<i>Sphingobacteriales</i>	<i>env.OPS 17</i>	<i>uncultured bacterium</i>	
AY509523	PA	0.72	0.005	19	<i>Acidobacteria</i>	<i>Acidobacteria</i>	<i>Subgroup 4</i>	<i>24-Nov</i>	<i>uncultured Acidobacteria bacterium</i>	
AB753965	PA	0.70	0.012	17	<i>Verrucomicrobia</i>	<i>Spartobacteria</i>	<i>Chthoniobacterales</i>	<i>Chthoniobacteraceae</i>	<i>Chthoniobacter</i>	<i>uncultured bacterium</i>
HM446115	PA	0.69	0.015	11	<i>Actinobacteria</i>	<i>Actinobacteria</i>	<i>Frankiales</i>	<i>Sporichthyaceae</i>	<i>Sporichthya</i>	<i>uncultured bacterium</i>
KC189665	PA	0.69	0.006	15	<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Myxococcales</i>	<i>0319-6G20</i>	<i>uncultured bacterium</i>	
GU305750	PA	0.69	0.029	20	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>uncultured</i>	<i>uncultured bacterium</i>
JN626564	PA	0.69	0.018	17	<i>Actinobacteria</i>	<i>Actinobacteria</i>	<i>Micrococcales</i>	<i>Microbacteriaceae</i>	<i>Alpinimonas</i>	<i>uncultured bacterium</i>
EU803309	PA	0.69	0.046	23	<i>Actinobacteria</i>	<i>Acidimicrobiia</i>	<i>Acidimicrobiales</i>	<i>Acidimicrobiaceae</i>	<i>CL500-29 marine group</i>	<i>uncultured bacterium</i>
AY792234	PA	0.65	0.01	14	<i>Actinobacteria</i>	<i>Acidimicrobiia</i>	<i>Acidimicrobiales</i>	<i>uncultured</i>	<i>uncultured actinobacterium</i>	
AY947972	PA	0.65	0.011	4	<i>Bacteroidetes</i>	<i>Flavobacteriia</i>	<i>Flavobacteriales</i>	<i>Cryomorphaceae</i>	<i>Fluviicola</i>	<i>uncultured Bacteroidetes bacterium</i>
JN868810	PA	0.65	0.01	5	<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Pseudomonadales</i>	<i>Pseudomonadaceae</i>	<i>Pseudomonas</i>	<i>uncultured bacterium</i>
JN606076	PA	0.65	0.001	5	<i>Chlamydiae</i>	<i>Chlamydiae</i>	<i>Chlamydiales</i>	<i>Simkaniaceae</i>	<i>uncultured</i>	<i>Chlamydiales bacterium NS16</i>
HQ730087	PA	0.65	0.012	11	<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>SubsectionIV</i>	<i>FamilyI</i>	<i>Sphaerospermopsis torques-reginae IIEP-026</i>	
EU803797	PA	0.65	0.049	24	<i>Bacteroidetes</i>	<i>Sphingobacteriia</i>	<i>Sphingobacteriales</i>	<i>Chitinophagaceae</i>	<i>Ferruginibacter</i>	<i>uncultured bacterium</i>
GU127190	PA	0.64	0.002	12	<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Xanthomonadales</i>	<i>Xanthomonadales Incertae Sedis</i>	<i>uncultured</i>	<i>uncultured bacterium</i>
KC682950	PA	0.64	0.029	15	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>alpha cluster</i>	<i>uncultured bacterium</i>	
HM270195	PA	0.64	0.016	5	<i>Actinobacteria</i>	<i>Acidimicrobiia</i>	<i>Acidimicrobiales</i>	<i>uncultured</i>	<i>uncultured bacterium</i>	
FJ612232	PA	0.63	0.016	2	<i>Bacteroidetes</i>	<i>Flavobacteriia</i>	<i>Flavobacteriales</i>	<i>Cryomorphaceae</i>	<i>Fluviicola</i>	<i>uncultured bacterium</i>
GU305698	PA	0.63	0.008	8	<i>Verrucomicrobia</i>	<i>Spartobacteria</i>	<i>Chthoniobacterales</i>	<i>DA101 soil group</i>	<i>uncultured bacterium</i>	
JX505108	PA	0.62	0.026	20	<i>Actinobacteria</i>	<i>Acidimicrobiia</i>	<i>Acidimicrobiales</i>	<i>uncultured</i>	<i>uncultured Ferrimicrobium sp.</i>	
GQ859644	PA	0.62	0.011	9	<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>SubsectionIII</i>	<i>FamilyI</i>	<i>Pseudanabaena mucicola PMC279.06</i>	
JN941777	PA	0.62	0.018	13	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>uncultured</i>	<i>uncultured bacterium</i>	
KC683058	PA	0.62	0.019	5	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>JG35-K1-AG5</i>	<i>uncultured bacterium</i>	
AB757748	PA	0.61	0.029	7	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>uncultured</i>	<i>uncultured bacterium</i>
JN626564	PA	0.59	0.05	20	<i>Actinobacteria</i>	<i>Actinobacteria</i>	<i>Micrococcales</i>	<i>Microbacteriaceae</i>	<i>Alpinimonas</i>	<i>uncultured bacterium</i>
HQ661200	PA	0.59	0.016	9	<i>Actinobacteria</i>	<i>Actinobacteria</i>	<i>Frankiales</i>	<i>Sporichthyaceae</i>	<i>hgcl clade</i>	<i>uncultured bacterium</i>
EU134497	PA	0.58	0.006	5	<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Bdellovibrionales</i>	<i>Bdellovibrionaceae</i>	<i>OM27 clade</i>	<i>uncultured bacterium</i>

GU118331	PA	0.58	0.006	4	<i>TM6</i>	<i>uncultured bacterium</i>				
HQ827891	PA	0.58	0.009	13	<i>Bacteroidetes</i>	<i>Cytophagia</i>	<i>Cytophagales</i>	<i>Cytophagaceae</i>	<i>uncultured</i>	<i>uncultured bacterium</i>
GQ859616	PA	0.56	0.015	14	<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>SubsectionIV</i>	<i>FamilyI</i>	<i>Anabaena sphaerica UTEX 'B 1616'</i>	
KC253272	PA	0.56	0.004	6	<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Myxococcales</i>	<i>0319-6G20</i>	<i>uncultured bacterium</i>	
KF287766	PA	0.54	0.042	8	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>I-10</i>	<i>uncultured Oceanibaculum sp.</i>	
EF540408	PA	0.51	0.022	7	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Nitrosomonadales</i>	<i>Nitrosomonadaceae</i>	<i>uncultured</i>	<i>uncultured soil bacterium</i>
KC836016	PA	0.51	0.021	7	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Comamonadaceae</i>	<i>uncultured</i>	<i>uncultured bacterium</i>
HQ386626	PA	0.50	0.017	7	<i>Cyanobacteria</i>	<i>Melainobacteria</i>	<i>Caenarcniphilales</i>	<i>uncultured cyanobacterium</i>		
AF316773	PA	0.50	0.012	8	<i>Planctomycetes</i>	<i>OM190</i>	<i>uncultured Crater Lake bacterium CL500-15</i>			
AF418968	PA	0.50	0.014	2	<i>Planctomycetes</i>	<i>OM190</i>	<i>uncultured bacterium</i>			
HM799060	PA	0.49	0.034	17	<i>Cyanobacteria</i>	<i>Melainobacteria</i>	<i>Vampirovibrionales</i>	<i>uncultured cyanobacterium</i>		
DQ450182	PA	0.48	0.037	1	<i>Bacteroidetes</i>	<i>Flavobacteriia</i>	<i>Flavobacteriales</i>	<i>Cryomorphaceae</i>	<i>Fluviicola</i>	<i>uncultured proteobacterium</i>
AF207074	PA	0.47	0.039	2	<i>Bacteroidetes</i>	<i>Flavobacteriia</i>	<i>Flavobacteriales</i>	<i>Flavobacteriaceae</i>	<i>Elizabethkingia</i>	<i>Elizabethkingia meningoseptica</i>
DQ463716	PA	0.47	0.037	5	<i>Bacteroidetes</i>	<i>Sphingobacteriia</i>	<i>Sphingobacteriales</i>	<i>ST-12K33</i>	<i>uncultured Bacteroidetes bacterium</i>	
JN868942	PA	0.46	0.03	3	<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Legionellales</i>	<i>Legionellaceae</i>	<i>Legionella</i>	<i>uncultured bacterium</i>
JQ814737	PA	0.45	0.047	5	<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>SubsectionIII</i>	<i>FamilyI</i>	<i>Phormidium</i>	<i>uncultured bacterium</i>
DQ856516	PA	0.45	0.044	6	<i>Bacteroidetes</i>	<i>Sphingobacteriia</i>	<i>Sphingobacteriales</i>	<i>Chitinophagaceae</i>	<i>uncultured</i>	<i>uncultured bacterium</i>
AB661545	PA	0.42	0.032	3	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Rhodospirillaceae</i>	<i>Roseospirillum</i>	<i>uncultured bacterium</i>
KF697437	PA	0.42	0.042	6	<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Alteromonadales</i>	<i>Alteromonadaceae</i>	<i>BD1-7 clade</i>	<i>uncultured bacterium</i>
GQ340071	PA	0.42	0.036	6	<i>Bacteroidetes</i>	<i>Sphingobacteriia</i>	<i>Sphingobacteriales</i>	<i>env.OPS 17</i>	<i>uncultured bacterium</i>	
EU135413	PA	0.42	0.032	3	<i>Verrucomicrobia</i>	<i>Spartobacteria</i>	<i>Chthoniobacteriales</i>	<i>Chthoniobacteraceae</i>	<i>Chthoniobacter</i>	<i>uncultured bacterium</i>
FJ208823	PA	0.42	0.04	14	<i>Planctomycetes</i>	<i>OM190</i>	<i>uncultured bacterium</i>			
DQ520168	PA	0.42	0.032	2	<i>Bacteroidetes</i>	<i>Sphingobacteriia</i>	<i>Sphingobacteriales</i>	<i>ST-12K33</i>	<i>uncultured bacterium</i>	
AF236012	PA	0.42	0.046	6	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Nitrosomonadales</i>	<i>Nitrosomonadaceae</i>	<i>uncultured</i>	<i>beta proteobacterium A0837</i>
AB753965	PA	0.42	0.044	4	<i>Verrucomicrobia</i>	<i>Spartobacteria</i>	<i>Chthoniobacteriales</i>	<i>Chthoniobacteraceae</i>	<i>Chthoniobacter</i>	<i>uncultured bacterium</i>
KF460029	PA	0.42	0.042	6	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Burkholderiaceae</i>	<i>Cupriavidus</i>	<i>Cupriavidus sp. USMAA2-4</i>
AACY020170993	PA	0.42	0.029	2	<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Myxococcales</i>	<i>0319-6G20</i>	<i>marine metagenome</i>	
AY945863	PA	0.39	0.047	8	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Rhodocyclales</i>	<i>Rhodocyclaceae</i>	<i>Thauera</i>	<i>uncultured bacterium</i>

Supplementary table II - 2: Indicator value, p value and detailed taxonomy of hydrologic periods indicative OTUs.

NCBI Sequence	Period	Indicator value	P value	Frequency	Phyla	Class	Order	Family	Genus	Strain/Clone/Species
EU592508	Rising	0.87	0.002	17	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcl clade	uncultured bacterium
DQ520189	Rising	0.85	0.001	20	Spirochaetae	Spirochaetes	Spirochaetales	Leptosiraceae	Leptospira	uncultured bacterium
EU804053	Rising	0.85	0.001	23	Proteobacteria	Alphaproteobacteria	SAR11 clade	LD12 freshwater group	uncultured bacterium	
D84522	Rising	0.84	0.003	8	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	Sphingomonas sp. MK347
EU803894	Rising	0.84	0.001	24	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcl clade	uncultured bacterium
HM856377	Rising	0.84	0.003	22	Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	LD28 freshwater group	uncultured Methylophilaceae bacterium
EU801940	Rising	0.84	0.001	23	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Polynucleobacter	uncultured bacterium
EU801613	Rising	0.82	0.001	18	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Paucimonas	uncultured bacterium
HM322101	Rising	0.82	0.001	23	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcl clade	uncultured bacterium
JN656882	Rising	0.82	0.005	24	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	Sedimibacterium	uncultured Bacteroidetes bacterium
KC253296	Rising	0.80	0.001	24	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcl clade	uncultured bacterium
GU127184	Rising	0.79	0.003	16	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Variovorax	uncultured bacterium
GU127278	Rising	0.79	0.001	24	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcl clade	uncultured bacterium
EU803802	Rising	0.79	0.002	24	Proteobacteria	Alphaproteobacteria	SAR11 clade	uncultured bacterium		
EU803573	Rising	0.78	0.003	20	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Limnohabitans	uncultured bacterium
KC189777	Rising	0.78	0.018	15	Proteobacteria	Betaproteobacteria	TRA3-20	uncultured bacterium		
JN868868	Rising	0.77	0.027	14	Candidate division TM7	uncultured bacterium				
EU801319	Rising	0.77	0.002	20	Proteobacteria	Betaproteobacteria	Nitrosomonadales	Nitrosomonadaceae	uncultured	uncultured bacterium
AB672185	Rising	0.77	0.01	4	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	Pseudospirillum	uncultured bacterium
EU803847	Rising	0.76	0.001	22	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcl clade	uncultured bacterium
AACY023437746	Rising	0.76	0.008	19	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	uncultured	marine metagenome
JN869093	Rising	0.76	0.001	18	Proteobacteria	Gammaproteobacteria	Xanthomonadales	uncultured	uncultured bacterium	
KC253357	Rising	0.76	0.011	17	Verrucomicrobia	Verrucomicrobia Incertae Sedis	Unknown Order	Unknown Family	Candidatus Methylophilum	uncultured bacterium
KC253357	Rising	0.75	0.002	24	Verrucomicrobia	Verrucomicrobia Incertae Sedis	Unknown Order	Unknown Family	Candidatus Methylophilum	uncultured bacterium
KC253327	Rising	0.75	0.017	12	Proteobacteria	Gammaproteobacteria	Xanthomonadales	uncultured	uncultured bacterium	
EU803281	Rising	0.75	0.003	23	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcl clade	uncultured bacterium
EU803951	Rising	0.75	0.001	24	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	uncultured	uncultured bacterium
EU804035	Rising	0.74	0.001	24	Chloroflexi	SL56 marine group	uncultured bacterium			
JF429366	Rising	0.74	0.001	21	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Paucimonas	uncultured bacterium
JN626564	Rising	0.74	0.027	19	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Alpinimonas	uncultured bacterium
EU803917	Rising	0.74	0.001	24	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcl clade	uncultured bacterium
JN656931	Rising	0.73	0.001	18	Chloroflexi	SL56 marine group	uncultured bacterium			
GU183621	Rising	0.72	0.005	16	Verrucomicrobia	OPB35 soil group	uncultured bacterium			
EU803961	Rising	0.72	0.017	21	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Candidatus Planktoluna	uncultured bacterium
EU803227	Rising	0.72	0.017	24	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcl clade	uncultured bacterium
KC253346	Rising	0.71	0.008	23	Actinobacteria	Acidimicrobia	Acidimicrobiales	Acidimicrobiaceae	CL500-29 marine group	uncultured bacterium
EU803741	Rising	0.71	0.001	23	Actinobacteria	Acidimicrobia	Acidimicrobiales	Acidimicrobiaceae	CL500-29 marine group	uncultured bacterium
KC835934	Rising	0.71	0.001	23	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcl clade	uncultured bacterium
EU803321	Rising	0.70	0.016	19	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	uncultured	uncultured bacterium
EU803898	Rising	0.70	0.004	10	Chloroflexi	SL56 marine group	uncultured bacterium			
EU803415	Rising	0.70	0.002	20	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	uncultured	uncultured bacterium
AY328773	Rising	0.69	0.014	14	Proteobacteria	SPOTSOC00m83	uncultured bacterium			
JQ958638	Rising	0.69	0.005	17	Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	uncultured	uncultured bacterium
EU800430	Rising	0.69	0.019	20	Actinobacteria	Acidobacteria	Subgroup 6	uncultured bacterium		
JF830210	Rising	0.69	0.042	22	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	MWH-UniPI aquatic group	bacterium enrichment culture clone B63(2011)
EU803802	Rising	0.69	0.017	3	Proteobacteria	Alphaproteobacteria	SAR11 clade	uncultured bacterium		

JN398062	Rising	0.68	0.021	16	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Comamonadaceae</i>	<i>uncultured</i>	<i>uncultured bacterium</i>
FJ184387	Rising	0.67	0.002	10	<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>SubsectionIII</i>	<i>FamilyI</i>	<i>Planktothrix</i>	<i>Planktothrix pseudagardhii HAB366</i>
EU090709	Rising	0.67	0.002	6	<i>Chlamydiae</i>	<i>Chlamydiae</i>	<i>Chlamydiales</i>	<i>Simkaniaceae</i>	<i>Candidatus Rhabdochlamydia</i>	<i>uncultured Candidatus Rhabdochlamydia sp.</i>
AM935269	Rising	0.65	0.038	18	<i>Chloroflexi</i>	<i>JG30-KF-CM66</i>	<i>uncultured Chloroflexi bacterium</i>			
EU803777	Rising	0.64	0.021	14	<i>Gemmatimonadetes</i>	<i>Gemmatimonadetes</i>	<i>Gemmatimonadales</i>	<i>Gemmatimonadaceae</i>	<i>uncultured</i>	<i>uncultured bacterium</i>
KC253289	Rising	0.62	0.027	10	<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Myxococcales</i>	<i>0319-6G20</i>	<i>uncultured bacterium</i>	
DQ395963	Rising	0.62	0.026	16	<i>Acidobacteria</i>	<i>Acidobacteria</i>	<i>Subgroup 6</i>	<i>uncultured organism</i>		
EU683887	Rising	0.59	0.019	9	<i>Chlamydiae</i>	<i>Chlamydiae</i>	<i>Chlamydiales</i>	<i>Simkaniaceae</i>	<i>Candidatus Rhabdochlamydia</i>	<i>Chlamydiales bacterium CRIB33</i>
FJ894109	Rising	0.58	0.004	5	<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Pseudomonadales</i>	<i>Pseudomonadaceae</i>	<i>Pseudomonas</i>	<i>uncultured bacterium</i>
AY947972	Rising	0.58	0.041	4	<i>Bacteroidetes</i>	<i>Flavobacteriia</i>	<i>Flavobacteriales</i>	<i>Cryomorphaceae</i>	<i>Fluviicola</i>	<i>uncultured Bacteroidetes bacterium</i>
EU133918	Rising	0.55	0.037	10	<i>Chlamydiae</i>	<i>Chlamydiae</i>	<i>Chlamydiales</i>	<i>Simkaniaceae</i>	<i>Candidatus Rhabdochlamydia</i>	<i>uncultured bacterium</i>
AM935541	Rising	0.54	0.014	7	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Nitrosomonadales</i>	<i>Nitrosomonadaceae</i>	<i>uncultured</i>	<i>uncultured beta proteobacterium</i>
EU133526	Rising	0.54	0.05	9	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Rhodospirillaceae</i>	<i>uncultured</i>	<i>uncultured bacterium</i>
JN606076	Rising	0.53	0.016	5	<i>Chlamydiae</i>	<i>Chlamydiae</i>	<i>Chlamydiales</i>	<i>Simkaniaceae</i>	<i>uncultured</i>	<i>Chlamydiales bacterium NS16</i>
KC172329	Rising	0.53	0.027	9	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Rhizobiales Incertae Sedis</i>	<i>Rhizomicrobium</i>	<i>uncultured alpha proteobacterium</i>
DQ316339	Rising	0.53	0.042	8	<i>Actinobacteria</i>	<i>Actinobacteria</i>	<i>Frankiales</i>	<i>Sporichthyaceae</i>	<i>hgcl clade</i>	<i>uncultured actinobacterium</i>
EU683887	Rising	0.51	0.034	8	<i>Chlamydiae</i>	<i>Chlamydiae</i>	<i>Chlamydiales</i>	<i>Simkaniaceae</i>	<i>Candidatus Rhabdochlamydia</i>	<i>Chlamydiales bacterium CRIB33</i>
AB682299	Rising	0.50	0.012	16	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Burkholderiaceae</i>	<i>Limnobacter</i>	<i>Limnobacter litoralis</i>
EF173340	Rising	0.50	0.01	9	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Rhodospirillaceae</i>	<i>uncultured</i>	<i>uncultured bacterium</i>
EU090707	Rising	0.47	0.014	7	<i>Chlamydiae</i>	<i>Chlamydiae</i>	<i>Chlamydiales</i>	<i>Simkaniaceae</i>	<i>Candidatus Rhabdochlamydia</i>	<i>uncultured Candidatus Rhabdochlamydia sp.</i>
FR714402	Rising	0.46	0.037	3	<i>Chlamydiae</i>	<i>Chlamydiae</i>	<i>Chlamydiales</i>	<i>Simkaniaceae</i>	<i>uncultured</i>	<i>uncultured Chlamydia sp.</i>
FJ936764	Rising	0.45	0.036	8	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>DA111</i>	<i>uncultured bacterium</i>	
GQ340273	Rising	0.44	0.034	6	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Rhodospirillaceae</i>	<i>Ferrovibrio</i>	<i>uncultured bacterium</i>
DQ903988	Rising	0.42	0.036	7	<i>Chlamydiae</i>	<i>Chlamydiae</i>	<i>Chlamydiales</i>	<i>Simkaniaceae</i>	<i>Candidatus Rhabdochlamydia</i>	<i>uncultured Chlamydia sp.</i>
DQ903988	Rising	0.42	0.039	4	<i>Chlamydiae</i>	<i>Chlamydiae</i>	<i>Chlamydiales</i>	<i>Simkaniaceae</i>	<i>Candidatus Rhabdochlamydia</i>	<i>uncultured Chlamydia sp.</i>
AM991246	Rising	0.42	0.041	4	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rickettsiales</i>	<i>mitochondria</i>	<i>uncultured bacterium</i>	
GU305732	Falling	0.96	0.001	22	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>Pirellula</i>	<i>uncultured bacterium</i>
GQ340346	Falling	0.94	0.001	22	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>Planctomyces</i>	<i>uncultured bacterium</i>
BX294853	Falling	0.94	0.001	20	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>Planctomyces</i>	<i>uncultured bacterium</i>
AF239694	Falling	0.94	0.002	18	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>Gemmata</i>	<i>Gemmata-like str. JW3-8s0</i>
HQ661209	Falling	0.94	0.001	22	<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>SubsectionI</i>	<i>FamilyI</i>	<i>Synechococcus</i>	<i>uncultured bacterium</i>
DQ444388	Falling	0.93	0.001	24	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>Planctomyces</i>	<i>uncultured bacterium</i>
KC835930	Falling	0.93	0.005	24	<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>SubsectionI</i>	<i>FamilyI</i>	<i>Microcystis</i>	<i>uncultured bacterium</i>
AB661594	Falling	0.93	0.001	23	<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>SubsectionI</i>	<i>FamilyI</i>	<i>uncultured bacterium</i>	
AM710363	Falling	0.92	0.001	24	<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>SubsectionI</i>	<i>FamilyI</i>	<i>Merismopedia</i>	<i>Cyanobium sp. JJ2-3</i>
EU340186	Falling	0.92	0.002	24	<i>Actinobacteria</i>	<i>Acidimicrobiia</i>	<i>Acidimicrobiales</i>	<i>uncultured</i>	<i>uncultured bacterium</i>	
GU305789	Falling	0.92	0.001	19	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>Planctomyces</i>	<i>uncultured bacterium</i>
JN679170	Falling	0.91	0.001	20	<i>Armatimonadetes</i>	<i>Armatimonadia</i>	<i>Armatimonadales</i>	<i>Armatimonadaceae</i>	<i>Armatimonas</i>	<i>uncultured beta proteobacterium</i>
HM127635	Falling	0.90	0.001	24	<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>SubsectionI</i>	<i>FamilyI</i>	<i>Synechococcus</i>	<i>uncultured bacterium</i>
KC620924	Falling	0.90	0.001	10	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Hyphomicrobiaceae</i>	<i>Hyphomicrobium</i>	<i>uncultured bacterium</i>
AF316773	Falling	0.88	0.002	23	<i>Planctomycetes</i>	<i>OM190</i>	<i>uncultured Crater Lake bacterium CL500-15</i>			
JX271902	Falling	0.87	0.01	16	<i>Actinobacteria</i>	<i>Acidimicrobiia</i>	<i>Acidimicrobiales</i>	<i>Acidimicrobiaceae</i>	<i>CL500-29 marine group</i>	<i>uncultured bacterium</i>
JN656817	Falling	0.87	0.001	21	<i>Bacteroidetes</i>	<i>Sphingobacteriia</i>	<i>Sphingobacteriales</i>	<i>Chitinophagaceae</i>	<i>Ferruginibacter</i>	<i>uncultured Bacteroidetes bacterium</i>
JF830148	Falling	0.86	0.017	23	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>uncultured</i>	<i>uncultured bacterium</i>
EU644173	Falling	0.86	0.005	14	<i>Actinobacteria</i>	<i>Acidimicrobiia</i>	<i>Acidimicrobiales</i>	<i>uncultured</i>	<i>uncultured bacterium</i>	
HE648177	Falling	0.85	0.009	22	<i>Verrucomicrobia</i>	<i>OPB35 soil group</i>	<i>uncultured Verrucomicrobia subdivision 3 bacterium</i>			
JF830208	Falling	0.85	0.001	19	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>Blastopirellula</i>	<i>bacterium enrichment culture clone B55(2011)</i>
KC253366	Falling	0.85	0.004	24	<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>SubsectionI</i>	<i>FamilyI</i>	<i>Synechococcus</i>	<i>uncultured bacterium</i>
AY212628	Falling	0.84	0.006	21	<i>Armatimonadetes</i>	<i>Armatimonadia</i>	<i>Armatimonadales</i>	<i>Armatimonadaceae</i>	<i>Armatimonas</i>	<i>uncultured bacterium</i>
JN868815	Falling	0.84	0.013	13	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>uncultured</i>	<i>uncultured bacterium</i>

DQ444452	Falling	0.84	0.001	23	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>uncultured</i>	<i>uncultured bacterium</i>
KC253336	Falling	0.83	0.005	24	<i>Bacteroidetes</i>	<i>Cytophagia</i>	<i>Cytophagales</i>	<i>Cytophagaceae</i>	<i>Hymenobacter</i>	<i>uncultured bacterium</i>
FJ382111	Falling	0.82	0.001	8	<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>SubsectionI</i>	<i>FamilyI</i>	<i>Synechococcus</i>	<i>uncultured bacterium</i>
JN869039	Falling	0.82	0.02	24	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>uncultured</i>	<i>uncultured bacterium</i>
GU305751	Falling	0.82	0.002	21	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>Blastopirellula</i>	<i>uncultured bacterium</i>
CU926364	Falling	0.81	0.001	8	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Hyphomicrobiaceae</i>	<i>Pedomicrobium</i>	<i>uncultured bacterium</i>
JF922442	Falling	0.81	0.003	16	<i>Chloroflexi</i>	<i>KD4-96</i>	<i>uncultured bacterium</i>			
GU305750	Falling	0.81	0.003	20	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>uncultured</i>	<i>uncultured bacterium</i>
HM069053	Falling	0.80	0.004	19	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>MNG7</i>	<i>uncultured bacterium</i>	
KC157044	Falling	0.80	0.006	20	<i>Bacteroidetes</i>	<i>Sphingobacteriia</i>	<i>Sphingobacteriales</i>	<i>Chitinophagaceae</i>	<i>uncultured</i>	<i>Niastella sp. HME8655</i>
JF830203	Falling	0.79	0.001	19	<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>GR-WP33-30</i>	<i>bacterium enrichment culture clone B30(2011)</i>		
JN038791	Falling	0.79	0.013	17	<i>Candidate division WS3</i>	<i>uncultured Latescibacteria bacterium</i>				
JX505108	Falling	0.78	0.005	20	<i>Actinobacteria</i>	<i>Acidimicrobiia</i>	<i>Acidimicrobiales</i>	<i>uncultured</i>	<i>uncultured Ferrimicrobium sp.</i>	
AF418950	Falling	0.78	0.024	20	<i>Planctomycetes</i>	<i>Phycisphaerae</i>	<i>Phycisphaerales</i>	<i>Phycisphaeraceae</i>	<i>CL500-3</i>	<i>uncultured bacterium</i>
KC331285	Falling	0.78	0.013	24	<i>Chloroflexi</i>	<i>KD4-96</i>	<i>uncultured bacterium</i>			
HM263209	Falling	0.77	0.004	16	<i>Actinobacteria</i>	<i>Acidimicrobiia</i>	<i>Acidimicrobiales</i>	<i>Acidimicrobiales Incertae Sedis</i>	<i>Candidatus Microthrix</i>	<i>uncultured bacterium</i>
AB661543	Falling	0.77	0.001	9	<i>Bacteroidetes</i>	<i>Sphingobacteriia</i>	<i>Sphingobacteriales</i>	<i>AKYH767</i>	<i>uncultured bacterium</i>	
AF247591	Falling	0.76	0.015	22	<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>SubsectionIV</i>	<i>FamilyI</i>	<i>Anabaena</i>	<i>Anabaena affinis NIES-40</i>
KF836214	Falling	0.75	0.001	3	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Caulobacterales</i>	<i>Hyphomonadaceae</i>	<i>uncultured</i>	<i>uncultured bacterium</i>
HQ114058	Falling	0.73	0.015	20	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>uncultured</i>	<i>uncultured bacterium</i>
EU135040	Falling	0.72	0.006	7	<i>Planctomycetes</i>	<i>Phycisphaerae</i>	<i>Phycisphaerales</i>	<i>Phycisphaeraceae</i>	<i>SM1A02</i>	<i>uncultured bacterium</i>
JN868894	Falling	0.70	0.008	18	<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Alteromonadales</i>	<i>Alteromonadaceae</i>	<i>BD1-7 clade</i>	<i>uncultured bacterium</i>
GU454869	Falling	0.69	0.02	17	<i>Acidobacteria</i>	<i>Acidobacteria</i>	<i>Subgroup 3</i>	<i>SJA-149</i>	<i>uncultured bacterium</i>	
DQ520172	Falling	0.69	0.017	10	<i>Actinobacteria</i>	<i>Acidimicrobiia</i>	<i>Acidimicrobiales</i>	<i>uncultured</i>	<i>uncultured bacterium</i>	
JF830212	Falling	0.69	0.008	6	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>Pirellula</i>	<i>bacterium enrichment culture clone B70(2011)</i>
JN868746	Falling	0.69	0.026	23	<i>Acidobacteria</i>	<i>Acidobacteria</i>	<i>Subgroup 4</i>	<i>Unknown Family</i>	<i>Blastocatella</i>	<i>uncultured bacterium</i>
HM445220	Falling	0.69	0.018	23	<i>Bacteroidetes</i>	<i>Sphingobacteriia</i>	<i>Sphingobacteriales</i>	<i>AKYH767</i>	<i>uncultured bacterium</i>	
JF922553	Falling	0.68	0.017	13	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Burkholderiaceae</i>	<i>Lautropia</i>	<i>uncultured bacterium</i>
GO397007	Falling	0.67	0.01	3	<i>Chloroflexi</i>	<i>Thermomicrobia</i>	<i>JG30-KF-CM45</i>	<i>uncultured bacterium</i>		
HM446115	Falling	0.67	0.041	11	<i>Actinobacteria</i>	<i>Actinobacteria</i>	<i>Frankiales</i>	<i>Sporichthyaceae</i>	<i>Sporichthya</i>	<i>uncultured bacterium</i>
FJ830579	Falling	0.66	0.027	22	<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>SubsectionIV</i>	<i>FamilyI</i>	<i>Aphanizomenon</i>	<i>Anabaena cf. fallax CENA208</i>
AB757748	Falling	0.65	0.006	7	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>uncultured</i>	<i>uncultured bacterium</i>
AM935786	Falling	0.64	0.039	13	<i>Actinobacteria</i>	<i>Acidimicrobiia</i>	<i>Acidimicrobiales</i>	<i>uncultured</i>	<i>uncultured Acidimicrobiidae bacterium</i>	
AY509523	Falling	0.64	0.03	19	<i>Acidobacteria</i>	<i>Acidobacteria</i>	<i>Subgroup 4</i>		<i>24-Nov</i>	<i>uncultured Acidobacteria bacterium</i>
HQ114142	Falling	0.64	0.023	21	<i>Verrucomicrobia</i>	<i>OPB35 soil group</i>	<i>uncultured bacterium</i>			
EU283346	Falling	0.64	0.005	5	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>A0839</i>	<i>uncultured gamma proteobacterium</i>	
JN038717	Falling	0.63	0.023	15	<i>Candidate division WS3</i>	<i>uncultured Latescibacteria bacterium</i>				
JO941791	Falling	0.62	0.028	14	<i>Verrucomicrobia</i>	<i>OPB35 soil group</i>	<i>uncultured bacterium</i>			
CU918341	Falling	0.61	0.047	15	<i>Actinobacteria</i>	<i>Actinobacteria</i>	<i>PeM15</i>	<i>uncultured bacterium</i>		
KC253276	Falling	0.61	0.04	6	<i>Bacteroidetes</i>	<i>Sphingobacteriia</i>	<i>Sphingobacteriales</i>	<i>Chitinophagaceae</i>	<i>uncultured</i>	<i>uncultured bacterium</i>
GO859644	Falling	0.60	0.02	9	<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>SubsectionIII</i>	<i>FamilyI</i>	<i>Pseudanabaena mucicola PMC279.06</i>	
JF830229	Falling	0.59	0.031	16	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>Pirellula</i>	<i>bacterium enrichment culture clone B176(2011)</i>
AY947978	Falling	0.59	0.034	6	<i>Bacteroidetes</i>	<i>Flavobacteriia</i>	<i>Flavobacteriales</i>	<i>NS9 marine group</i>	<i>uncultured Bacteroidetes bacterium</i>	
DQ640688	Falling	0.58	0.006	7	<i>Bacteroidetes</i>	<i>Sphingobacteriia</i>	<i>Sphingobacteriales</i>	<i>Saprosiraceae</i>	<i>uncultured</i>	<i>uncultured Bacteroidetes bacterium</i>
KF411639	Falling	0.58	0.007	5	<i>Actinobacteria</i>	<i>Acidimicrobiia</i>	<i>Acidimicrobiales</i>	<i>Acidimicrobiales Incertae Sedis</i>	<i>Candidatus Microthrix</i>	<i>uncultured actinobacterium</i>
KC172272	Falling	0.58	0.01	13	<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Myxococcales</i>	<i>Polyangiaceae</i>	<i>Sorangium</i>	<i>uncultured delta proteobacterium</i>
JN023788	Falling	0.58	0.031	12	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Hyphomicrobiaceae</i>	<i>Hyphomicrobium</i>	<i>uncultured bacterium</i>
FM201077	Falling	0.57	0.015	17	<i>Verrucomicrobia</i>	<i>OPB35 soil group</i>	<i>uncultured bacterium</i>			
JN868850	Falling	0.57	0.018	17	<i>Planctomycetes</i>	<i>Phycisphaerae</i>	<i>Phycisphaerales</i>	<i>Phycisphaeraceae</i>	<i>CL500-3</i>	<i>uncultured bacterium</i>
FJ769516	Falling	0.57	0.022	5	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>Planctomyces</i>	<i>uncultured bacterium</i>

FJ936832	Falling	0.57	0.007	19	<i>Planctomycetes</i>	<i>Phycisphaerae</i>	<i>Phycisphaerales</i>	<i>Phycisphaeraeaceae</i>	<i>SM1A02</i>	<i>uncultured bacterium</i>
KC189789	Falling	0.56	0.029	20	<i>Actinobacteria</i>	<i>Acidimicrobiia</i>	<i>Acidimicrobiales</i>	<i>Acidimicrobiaceae</i>	<i>CL500-29 marine group</i>	<i>uncultured bacterium</i>
JQ794638	Falling	0.56	0.008	4	<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Myxococcales</i>	<i>mle1-27</i>	<i>uncultured Myxococcales bacterium</i>	
HQ682028	Falling	0.55	0.006	7	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Comamonadaceae</i>	<i>uncultured</i>	<i>uncultured bacterium</i>
HQ661210	Falling	0.55	0.04	6	<i>Armatimonadetes</i>	<i>uncultured bacterium</i>				
JN391737	Falling	0.53	0.019	13	<i>Verrucomicrobia</i>	<i>Verrucomicrobiae</i>	<i>Verrucomicrobiales</i>	<i>Verrucomicrobiaceae</i>	<i>Prostheco bacter</i>	<i>uncultured bacterium</i>
AY375088	Falling	0.52	0.04	5	<i>Bacteroidetes</i>	<i>Sphingobacteriia</i>	<i>Sphingobacteriales</i>	<i>Chitinophagaceae</i>	<i>Lacibacter</i>	<i>uncultured bacterium</i>
FJ208845	Falling	0.51	0.049	14	<i>Planctomycetes</i>	<i>OM190</i>	<i>uncultured bacterium</i>			
AB274849	Falling	0.51	0.036	10	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Acetobacteraceae</i>	<i>uncultured</i>	<i>uncultured bacterium</i>
FJ828506	Falling	0.50	0.021	7	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>uncultured</i>	<i>bacterium enrichment culture clone KIST-JY167</i>
AF235999	Falling	0.50	0.014	5	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Xanthobacteraceae</i>	<i>Labrys</i>	<i>alpha proteobacterium A0838</i>
GU127204	Falling	0.50	0.016	8	<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Methylococcales</i>	<i>Methylococcaceae</i>	<i>Methylocaldum</i>	<i>uncultured Methylocaldum sp.</i>
FJ612230	Falling	0.50	0.014	7	<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>SubsectionI</i>	<i>FamilyI</i>	<i>Snowella</i>	<i>uncultured bacterium</i>
EF516185	Falling	0.50	0.013	7	<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Myxococcales</i>	<i>mle1-27</i>	<i>uncultured bacterium</i>	
GQ859616	Falling	0.48	0.036	14	<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>SubsectionIV</i>	<i>FamilyI</i>	<i>Anabaena sphaerica UTEX 'B 1616'</i>	
GQ340157	Falling	0.48	0.047	7	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Burkholderiaceae</i>	<i>Lautropia</i>	<i>uncultured bacterium</i>
HM329700	Falling	0.48	0.036	2	<i>Chloroflexi</i>	<i>TK10</i>	<i>uncultured bacterium</i>			
JQ923788	Falling	0.48	0.049	6	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>	<i>Methylobacteriaceae</i>	<i>Meganema</i>	<i>uncultured bacterium</i>
AF448072	Falling	0.47	0.044	6	<i>Cyanobacteria</i>	<i>Cyanobacteria</i>	<i>SubsectionI</i>	<i>FamilyI</i>	<i>Cyanobium sp. PCC 8966</i>	
FJ444644	Falling	0.42	0.034	5	<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Myxococcales</i>	<i>Haliangiaceae</i>	<i>Haliangium</i>	<i>uncultured bacterium</i>
EU135194	Falling	0.42	0.042	2	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>Planctomyces</i>	<i>uncultured bacterium</i>
KC874430	Falling	0.42	0.037	4	<i>Planctomycetes</i>	<i>Phycisphaerae</i>	<i>Phycisphaerales</i>	<i>Phycisphaeraeaceae</i>	<i>SM1A02</i>	<i>uncultured bacterium</i>
KC011149	Falling	0.42	0.037	4	<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Myxococcales</i>	<i>Polyangiaceae</i>	<i>uncultured delta proteobacterium</i>	
JN868880	Falling	0.42	0.039	7	<i>Acidobacteria</i>	<i>Subgroup 22</i>	<i>uncultured bacterium</i>			
FJ502252	Falling	0.42	0.034	6	<i>Bacteroidetes</i>	<i>Sphingobacteriia</i>	<i>Sphingobacteriales</i>	<i>Chitinophagaceae</i>	<i>Filimonas</i>	<i>uncultured bacterium</i>
GQ480086	Falling	0.42	0.035	2	<i>Planctomycetes</i>	<i>OM190</i>	<i>uncultured bacterium</i>			
JX079242	Falling	0.42	0.031	5	<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Myxococcales</i>	<i>Phaselicytidaceae</i>	<i>Phaselicystis</i>	<i>uncultured bacterium</i>
DQ444389	Falling	0.40	0.044	4	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>Planctomyces</i>	<i>uncultured bacterium</i>
AF418968	Falling	0.39	0.038	2	<i>Planctomycetes</i>	<i>OM190</i>	<i>uncultured bacterium</i>			

**Supplementary table II - 3:** Indicator value, p value and detailed taxonomy of influence area indicative OTUs.

NCBI Sequence	Influence area	Indicator value	P value	Frequency	Phyla	Class	Order	Family	Genus	Strain/Clone/Species
GQ350701	ATTZ	0.62	0.043	11	<i>Cyanobacteria</i>	<i>ML635J-21</i>	<i>uncultured bacterium</i>			
JN941791	ATTZ	0.61	0.033	5	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>	<i>Planctomycetaceae</i>	<i>uncultured</i>	<i>uncultured bacterium</i>
GQ472429	ATTZ	0.58	0.028	7	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Acetobacteraceae</i>	<i>Acidocella</i>	<i>uncultured bacterium</i>
AY948005	ATTZ	0.58	0.05	22	<i>Actinobacteria</i>	<i>Actinobacteria</i>	<i>Frankiales</i>	<i>Sporichthyaceae</i>	<i>hgcl clade</i>	<i>uncultured actinobacterium</i>
KC432462	Lake	0.68	0.036	12	<i>Proteobacteria</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Comamonadaceae</i>	<i>Delftia</i>	<i>uncultured bacterium</i>
JN618338	Lake	0.57	0.031	11	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Caulobacteriales</i>	<i>Caulobacteraceae</i>	<i>Phenylobacterium</i>	<i>Phenylobacterium sp. 1.9217</i>
HQ014631	Lake	0.48	0.027	10	<i>Bacteroidetes</i>	<i>Sphingobacteriia</i>	<i>Sphingobacteriales</i>	<i>Saprospiraceae</i>	<i>uncultured</i>	<i>uncultured bacterium</i>
KC502954	Lake	0.47	0.042	3	<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Pseudomonadales</i>	<i>Pseudomonadaceae</i>	<i>Pseudomonas</i>	<i>uncultured Pseudomonas sp.</i>